ACTA AGRICULTURAE SLOVENICA

119 • 1
2023

Acta agriculturae Slovenica • eISSN 1854-1941 • 119 – 1 • Ljubljana, april 2023

Univerza *v Ljubljani*
Biotehniška fakulteta

Acta agriculturae Slovenica **eISSN 1854-1941**

Letnik / Volume 119 · Številka / Number 1 · 2023

Založnik in izdajatelj / *Publisher and Issuer*

Založila / Published by: Založba Univerze v Ljubljani / University of Ljubljana Press
Za založbo / For the Publisher: Gregor MAJDIČ, rektor Univerze v Ljubljani / the Rector of the University of Ljubljana
Izdala / Issued by: Univerza v Ljubljani, Biotehniška fakulteta / University of Ljubljana, Biotehnical Faculty
Za izdajatelja / For the Issuer: Marina PINTAR, dekanja Biotehniške fakultete UL / the Dean of the Biotehnical Faculty UL

Naslov Uredništva / *Editorial Office Address*

Univerza v Ljubljani, Biotehniška fakulteta, Acta agriculturae Slovenica
Jamnikarjeva ulica 101, SI-1000 Ljubljana

E-naslov / *E-mail*: Acta.Agriculturae.Slovenica@bf.uni-lj.si

Spletni naslov / *Web address*: https://journals.uni-lj.si/aas/

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Acta agriculturae Slovenica izhaja s finančno pomočjo / *is published with the financial support:* Javne agencije za raziskovalno dejavnost Republike Slovenije / *Slovenian Research Agency*.

Acta agriculturae Slovenica je vključena v / *is included into*: Scopus (SJR, SNIP), DOAJ, WOS Zoological Records, CrossRef, CAB Abstracts, FSTA, Google Scholar, dLib, COBISS.

Ovitek: Samec bakrenega podkrilca (*Amphipyra pyramidea*) na feromonski pasti za načrtno spremljanje gobarja (*Lymantria dispar*) v gozdu Ginjevec (SV Slovenija), 17. avgust 2022 (Foto: Stanislav Trdan, 1–8)
Cover: Adult male of Amphipyra pyramidea *on the pheromone trap for the monitoring of* Lymantria dispar *in the forest Ginjevec (NE Slovenia), August 17 2022 (Photo: Stanislav Trdan, 1–8)*

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doi:10.14720/aas.2023.119.1.2425 Original research article / izvirni znanstveni članek

Comparative assessment of ISSR, DAMD and RAPD markers for evaluation of genetic diversity of gerbera (*Gerbera jamesonii* Bolus ex Hooker f.) cultivars

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Received November 15, 2021; accepted December 06, 2022.
Delo je prispelo 15. november 2021, sprejeto 6. decembra 2022

Comparative assessment of ISSR, DAMD and RAPD markers for evaluation of genetic diversity of gerbera (*Gerbera jamesonii* Bolus ex Hooker f.) cultivars

Abstract: Genetic diversity is the best way to improve available genetic resources for breeding programs in gerbera. In present study, genetic diversity of 22 gerbera cultivars was investigated using inter-simple sequence repeat (ISSR), directly amplified minisatellite DNA (DAMD), and Random Amplified Polymorphic DNA (RAPD) markers. Average polymorphism information content (PIC) for ISSR, DAMD and RAPD markers was 0.40, 0.41 and 0.40, respectively. Cluster analysis for ISSR, DAMD and RAPD divided the cultivars into three distinct clusters. The comparative analysis of the three markers (ISSR, DAMD and RAPD) showed that DAMD had superiority over RAPD and ISSR in characterization of genetic diversity in *Gerbera*. To our knowledge, this is the first report of a comparison of performance among DAMD, ISSR and RAPD techniques on a set of gerbera genotypes. Overall, our results showed that DAMD markers well represented different genotypes of gerbera diversity.

Key words: *Gerbera*; DAMD; genetic diversity; ISSR; DAMD

Primerjalno preverjanje ISSR, DAMD in RAPD markerjev za vrednotenje genetske raznolikosti sort gerbere (*Gerbera jamesonii* Bolus ex Hooker f.)

Izvleček: Genetska raznolikost je izhodišče razpoložljivih genetskih virov v žlahtniteljskih programih gerbere. V študiji smo genetsko raznolikost 22 sort gerbere preučevali z označevalci ISSR (inter-simple sequence repeat, regije med mikrosateliti), DAMD (direct amplified minisatellite DNA, pomnoženi minisateliti) in RAPD (Random Amplified Polymorphic DNA, naključno pomnožena polimorfna DNA). Popreprečna informacijska vrednost polimorfizma (PIC) je bila za ISSR, DAMD in RAPD markerje 0,40; 0,41 in 0,40. Klasterska analiza ISSR, DAMD in RAPD markerjev je sorte razdelila v tri različne skupine. Primerjalna analiza treh markerjev (ISSR, DAMD in RAPD) je pokazala, da so imajo DAMD markerji prednost pred RAPD in ISSR za določitev genetske raznolikosti pri gerberi. Pričujoča raziskava je ena prvih, ki primerja učinkovitost DAMD, ISSR in RAPD markerjev na izbranem naboru genotipov gerber. Naši rezultati so pokazali, da so markerji DAMD najbolje prikazali raznolikost genotipov gerbere.

Ključne besede: *Gerbera*; DAMD; genetska raznolikost; ISSR; DAMD

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1 INTRODUCTION

Gerbera (*Gerbera jamesonii* Bolus ex Hooker f.)) belongs to the Compositae family and is one of the top five cut flowers in the world in terms of production and consumption, which has a great economic value in the international flowering industry (Aghdam et al., 2019). *Gerbera* (Compositae), is native to tropical regions of South America, Africa and Asia (Danaee et al., 2011). It is a diploid species with somatic chromosome number of 2n = 50. *Gerbera* is commercially important and it is considered a tender perennial plant. Gerbera was introduced to China in the 1980s and has been used as a model organism in surveying flower formation (Yang, 2012). Evaluation of genetic diversity and genetic relationships among cultivars are necessary tools for future breeding and improvement programs. Selection is essential for germplasm breeding and is effective only when the visible variation in the population is highly heritable (Kumari et al., 2011). Detection of genetic diversity and classification of genetic resources (germplasm) are important and essential strategies in breeding and preservation of plant genetic resources. Breeding of gerbera is based on crossing among cultivars and thereby phenotypic selection of new and improving progeny, followed by producing new cultivars. Molecular markers are an appropriate tool for measuring the diversity of plant species. Important factors such as low assay cost, affordable hardware, throughput, convenience and ease of assay development, make this technique an important aid to breeding and cultivar development (Rafalski, 2002). ISSRs can be targeted towards sequences, which are reported to be abundant in the genome and can overcome the technical difficulties of RFLP. In recent years, many new alternative and promising marker techniques have been developed in line with the rapid growth of genomic research. Minisatellite DNAs are tandemly repeated regions of genomes and are used in a procedure denoted directed amplification of minisatellite-region DNA (DAMD). This technique utilized help effectively in genetic diversity analysis in gerbera. Genetic diversity in the gerbera has been reported using diverse molecular markers such as RAPD (Prajapati et al., 2014), and EST-SSR (Gong et al., 2010), ISSR (Li et al., 2004). In the present study, RAPD, ISSR and DAMD markers were used as useful tools for genetic variability analysis to provide more comprehensive insight regarding the genetic relationships and germplasm management of the cultivars utilized in this study (Kumar et al., 2011). Here, we report for the first time the use of the DAMD marker-based technique for surveying genetic diversity in gerbera cultivars. The objectives of the present research were to evaluate genetic diversity of gerbera using three markers namely ISSR, DAMD, and RAPD. The efficiencies of these markers were also evaluated and compared for diagnostic fingerprinting of the gerbera cultivars.

2 MATERIALS AND METHODS

The plant material used in this study included twenty-two cultivars of *G. jamesonii* were collected from the Research Centre for Plants and Flowers (RCPF), Mahallat, Iran. Names of the cultivars are given in Table 1.

Genomic DNA was extracted from young leaves of plants according to the modified CTAB method as described by Lassner et al. (1989). The purified total DNA was quantified by agarose-gel electrophoresis using a known concentration of uncut λ DNA as a standard. A set of 30 RAPD primers were procured of which 17 primers gave clear and polymorphic patterns. The polymorphic primers were then used for further analysis of 22 cultivars (Table 2). PCR amplifications were performed in 25 µl reactions containing 30 ng sample DNA, 2.5 µM primer, 200 µM of each dNTP, 1.5–2.5 mM $MgCl_2$ (Magnesium chloride) and 1.5 unit of Taq DNA polymerase (Cinnagene, Iran). Thermal cycling included 3 min at 94 °C followed by 35 cycles of denaturation at 93 °C for 45 s, annealing at optimum temperature for 45 s, extension at 72 °C for 90 s, and a final extension cycle at 72 °C for 10 min. PCR products were separated on 1.5 % agarose gels, stained with ethidium bromide and scored for the presence or absence of bands.

Six ISSR and eight DAMD primers were selected for final amplification (Table 2). The amplification was performed in a thermal cycler (Eppendorf, Germany) with the following conditions: initial denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 1 min, an-

Table 1: Names of the studied cultivars in this research

Sample NO.	Genotype	Sample NO.	Genotype
1	Rosalin	12	Cacharlle
2	Sorbet	13	Hooper
3	Souvenir	14	Nuance
4	Dune	15	Quote
5	Intense	16	Esmara
6	Aquamelone	17	Sazo
7	Edelweiss	18	Pink Elegance
8	Carambole	19	Essendre
9	Balance	20	Cabana
10	Stanza	21	Klimanjaro
11	Double Dutch	22	Red-417

Table 2: RAPD, ISSR, and DAMD primers used for amplification

No.	Name primer	Seq (5'-3')	No.	Name primer	Seq (5'-3')
ISSR			RAPD		
1	UBC828	TGTGTGTGTGTGTGTGA	17	OPE-10	CACCAGGTGC
2	UBC822	TCTCTCTCTCTCTCTCA	18	OPF-02	GAGGATCCCT
3	UBC801	ATATATATATATATATT	19	OPG-19	GTCAGGGCAA
4	UBC874	CCCTCCCTCCCTCCCT	20	OPK-17	CCCAGCTGTG
5	UBC816	CACACACACACACACAT	21	OPL-08	AGCAGGAGGA
DAMD			22	OPM-03	GGGGGATGAG
7	URP2F	GTGTGCGATCAGTTGCTGGG	23	OPN-04	GACCGACCCA
8	URP9R	AGGACTCGATAACAGGCTCC	24	OPQ-11	TCTCCGCAAC
9	URP25F	GGCAAGCTGGTGGGAGGTAC	25	OPR-10	CCATTCCCCA
10	URP30F	TACATCGCAAGTGACACAGG	26	OPR-17	CCGTACGTAG
11	URP13R	AATGTGGGCAAGCTGGTGGT	27	OPAA-04	AGGACTGCTC
12	URP17R	GATGTGTTCTTGGAGCCTGT	28	OPAF-10	GGTTGGAGAC
13	URP 6R	GGACAAGAAGAGGATGTGGA	29	OPAD-06	AAGTGCACGG
14	URP2F	AAGAGGCATTCTACCACCAC	30	OPAE-05	CCTGTCAGTG
RAPD					
15	OPB-07	GGTGACGCAG			
16	OPD-06	ACCTGAACGG			

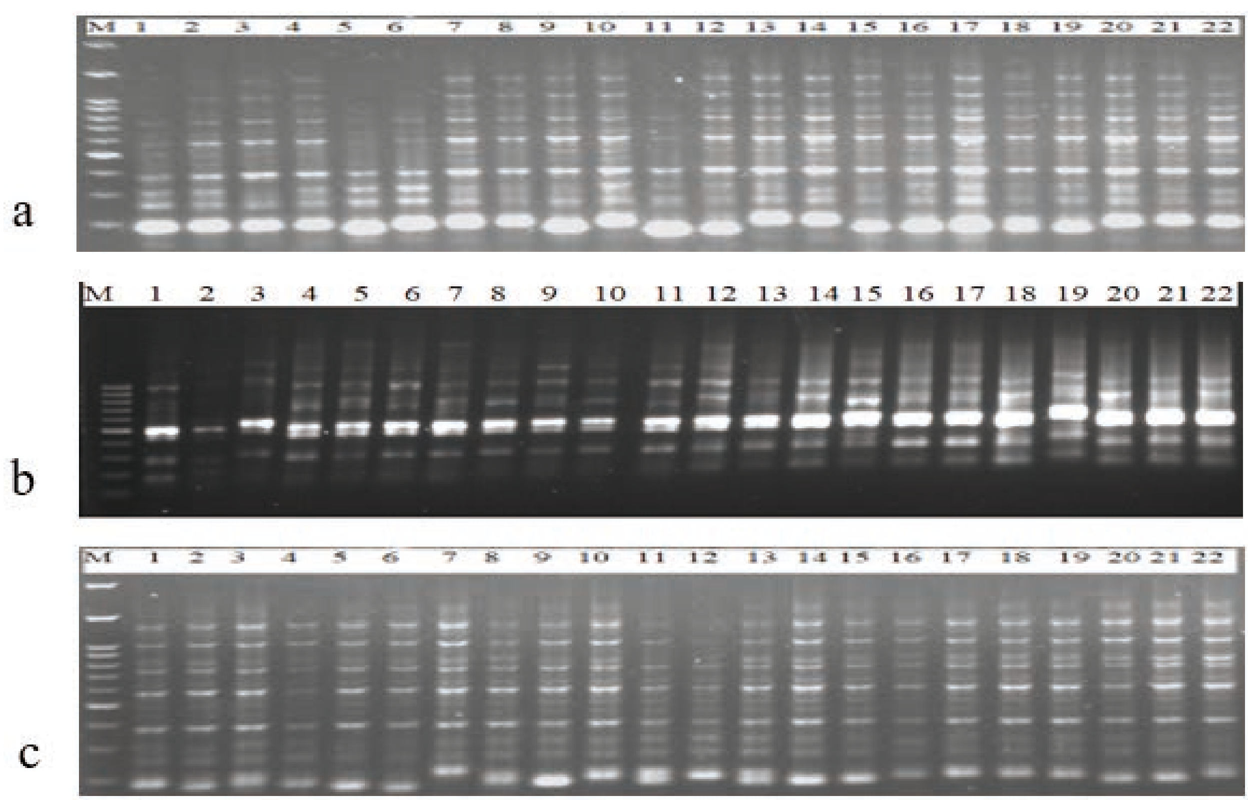

Fig. 1: The RAPD pattern obtained with OPZ-17 primer (a), the ISSR pattern obtained with UBC822 primer (b), and the DAMD pattern obtained with URP17R (c)

Table 3: DAMD, ISSR, and RAPD markers used for genetic diversity analysis

primer	No. of polymorphic bands	No. of monomorphic bands	No. of amplified bands	PIC value	Polymorphism (%)	MI
ISSR						
UBC828	4	0	4	0.33	100	1.34
UBC822	9	0	9	0.44	100	3.96
UBC801	5	2	7	0.38	100	1.93
UBC874	10	0	10	0.44	100	4.94
UBC816	7	0	7	0.42	71	2.97
DAMD						
URP2F	7	0	7	0.39	100	2.76
URP9R	10	0	10	0.43	100	4.39
URP25F	5	0	5	0.38	100	1.93
URP30F	7	0	7	0.41	100	2.93
URP13R	10	1	11	0.43	90	4.32
URP17R	9	0	9	0.43	100	3.92
URP 6R	6	0	6	0.40	100	2.41
RAPD						
OPB-07	9	0	9	0.42	100	3.86
OPD-06	8	0	8	0.35	100	2.87
OPE-10	9	1	10	0.39	90	3.56
OPF-02	11	1	12	0.43	91	4.8
OPG-19	10	0	10	0.44	100	4.47
OPL-08	7	1	8	0.44	87	3.01
OPZ-17	13	0	17	0.48	100	6.71
OPM-03	7	1	8	0.39	87	2.75
OPN-04	7	0	7	0.41	100	2.87
OPQ-11	6	1	7	0.39	85	2.38
OPR-10	5	0	5	0.37	100	1.86
OPR-17	7	1	8	0.44	87	3.01
OPAA-04	7	0	7	0.41	100	2.87
OPAF-10	8	0	8	0.42	100	3.42
OPAD-06	6	0	6	0.36	100	2.18
OPAE-05	2	0	2	0.24	100	0.48

nealing at 48 °C (for ISSR analysis) and 50 °C (for DAMD analysis) for 1min each, extension at 72 °C for 2 min and final extension at 72 °C for 7 min. The PCR products obtained were separated on 2 % agarose gel using 1 × TBE buffer at a constant voltage of 100 V for one hour. The gel stained with ethidium bromide and visualized using a gel documentation.

The amplified RAPD, ISSR and DAMD fragments obtained were scored for presence (1) or absence (0) of bands. Tree construction following an NJ tree using a similarity matrix was performed through Splits Tree. The dissimilarity matrix thus obtained was subjected to cluster analysis using the un-weighted neighbor-joining analyses (UNJ), followed by bootstrap analysis with 1,000 permutations to obtain a dendrograme for all 22 genotypes. Mantel statistic was used to compare the similarity

Fig. 2: Dendrogram of the 22 gerbera genotypes based on the dissimilarity matrix developed using ISSR markers

matrices as well as the dendrogrames produced by the ISSR, DAMD, and RAPD techniques. All these procedures were performed by appropriate routines in NTSYS-pc version 2.0. Polymorphic information content (PIC) values were calculated for each ISSR, DAMD, and RAPD primers according to the formula: *PIC = 1 – ∑(Pij)2*; where Pij is the frequency of the i^{th} (frequency of the ith pattern) pattern revealed by the j^{th} (primer summed across all patterns) primer summed across all patterns revealed by the primers. The Mantel test of significance (Mantel 1967) was also used to compare each pair of similarity matrices produced.

3 RESULT AND DISCUSSION

The 17 primers produced a total of 143 reliable fragments of which 138 were polymorphic. The number of fragments generated by these RAPD primers was found to range from 2 to 15 bands. OPZ-17 primer produced the maximum number of polymorphic bands and OPAE-05 primer generated the minimum number of polymorphic bands. The RAPD pattern obtained with OPZ-17 primer is shown in Fig1a. The percentage of polymorphism ranged from 85 % for OPQ-11 to 100 % for OPN-04, OPR-10, OPAF-10, OPZ-17, OPAE-05, OPA-02, OPK-07, OPL-08, OPG-19, OPB-07, OPAD-06, and OPD-06 with an average of 96 % polymorphism per primer. The polymorphism information content ranged from a 0.44 (OPG-19, OPL-08, OPZ-17, and OPR-17) to 0.24 (OPAE-05) with an average of 0.40, indicating hypervariability among the individuals studied. The marker index (MI) for RAPD was the highest for the primer OPZ-17 (6.71) and the lowest for the primer OPZ-17 (0.48). An average MI of 2.52 per primer was observed. Cluster analysis showed similar grouping pattern.

A total 10 ISSR primers were screened of which five primers showed polymorphic bands. A total of 57 polymorphic bands were detected amongst 22 gerbera cultivars using 5 ISSR markers. Average polymorphism percentage was 94 % across all cultivars. The number of products generated per primer was found to range from 4 (UBC 828) to 10 (UBC 874) bands. The ISSR pattern obtained with UBC822primer is shown in Fig1b. Polymorphism information content (PIC) values ranged from 0.33 to 0.44, with an average value of 0.40 per prim-

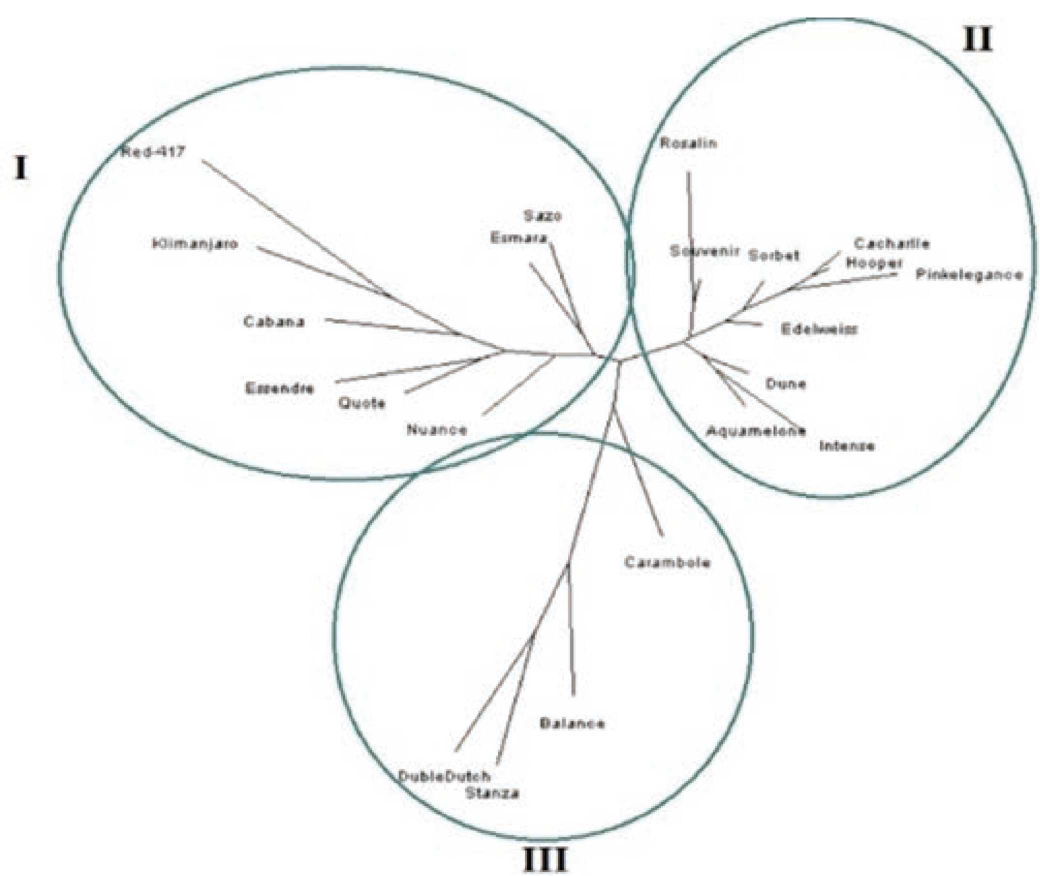

Fig. 3: Dendrogram of the 22 gerbera genotypes based on the dissimilarity matrix developed using RAPD markers

er (Table 3). Cluster analysis grouped *Gerbera* cultivars into three distinct clusters (Fig. 2). Cluster I and II each contained seven cultivars. The marker index was found to be the highest for primer UBC874 (4.94) and the lowest for the primer UBC828 (1.34) with an average MI of 3.03 per primer. Cluster III showed similar grouping patterns with those obtained by RAPD data.

Cluster I and II included 8 and 7 cultivars, respectively. Cluster III contained 3 cultivars (Fig 3). Seven primers generated a total of 55 bands of which 54 bands were polymorphic. The number of amplified products generated by DAMD primers were in the range of 5–10 bands. Primers URP13R and URP9R generated the maximum (10 bands) and primer URP25F generated the minimum (5 bands) number of amplicons. Average polymorphism percentage was 99 % across all cultivars. PIC values ranged from 0.38 to 0.43, with an average value of 0.41 per primer (Table 4). The DAMD pattern obtained with URP17R primer is shown in Fig. 1c. Based on un-weighted neighbour-joining method, all 22 gerbera varieties were grouped into three distinct clusters (Fig. 3). The estimates of MI were found to be the highest for primer URP9R (4.39) and the lowest with the primer URP25F (1.93) with an average MI of 3.24 per primer. Cluster I contained seven cultivars, cluster II included 11 cultivars, and cluster III included four cultivars showing relatively a similar grouping patterns with clusters III and IV of ISSR and cluster I of DAMD markers (Fig 4).

Our study using three marker systems suggested the presence of a significant polymorphism and revealed high level of variability in surveyed gerbera genotypes which agrees with those reported by Prajapati (2014). This is the first research to utilize RAPD, ISSR and DAMD molecular markers to examine the extent of genetic variability among germplasm of gerbera. In the current study, three different molecular markers, ISSR, DAMD, and RAPD were employed for the analysis of the genetic variability of a set of 22 gerbera cultivars. The results of our study suggests that these markers can be used in breeding programmes to reliably distinguish studied gerbera varieties. A comparison of level of polymorphism and effective performance of ISSR, DAMD, and RAPD showed that each of the three markers can be detected genetic relationships and discriminating efficacy within cultivars. All of the three markers were used to show high level of polymorphism. Previous studies also revealed that DAMD

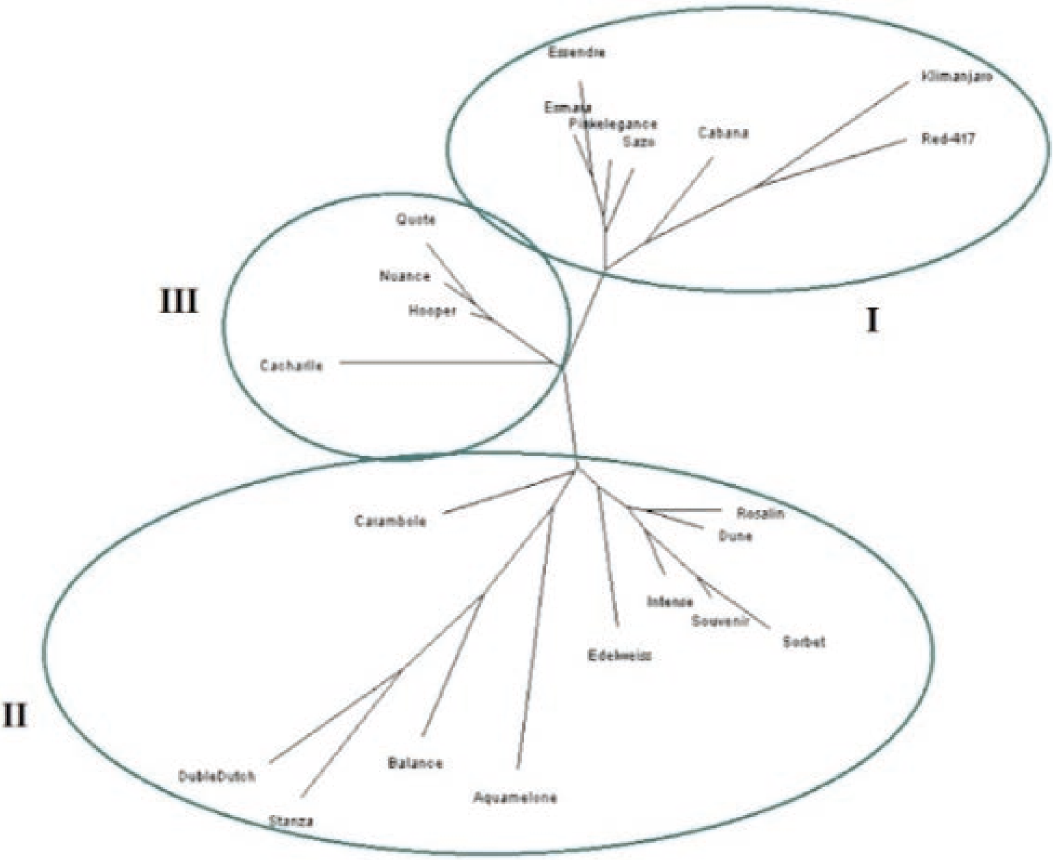

Fig. 4: Dendrogram of the 22 gerbera genotypes based on the dissimilarity matrix developed using DAMD markers

techniques were able to provide more reliable diversity information compared to RAPD or ISSR techniques (Pakseresht et al., 2013) and are useful as tools for studying the genetic diversity of different plant germplasm. In comparison with those studies, we observed that the average polymorphism rate for RAPD, ISSR, and DAMD markers in gerbera cultivars were relatively high. ISSR and DAMD showed higher polymorphism percentage than RAPD but PIC in three markers equal. These results suggested the presence of a considerable polymorphism in two markers at studied and revealed a high potential of genetic diversity in the existing gerbera germplasm. Mantel coefficient correlation test showed higher positive correlation between ISSR and DAMD metrices, indicating a consistent relationship between genetic distances from both marker systems. The correlation coefficient (r) was 0.73 between ISSR and RAPD, 0.80 between DAMD and ISSR, and 0.77 (significant $p > 0.01$) between RAPD and DAMD. All three molecular marker types showed positive but significant correlation with each other. Based on Mantel test results, the rate of genetic diversity for genotypes was in ISSR and DAMD approximately equal but RAPD was less than two markers. We predict that the source of detected diversity is various, as each technique targets different regions of the genome. Results of mantel coefficient correlation test showed higher positive correlation between ISSR and DMAD metrices, indicating a consistent relationship between genetic distances from both marker systems. This higher correlation may have been attributable to similarity in DNA sequence variation at primer binding sites between the ISSR and DAMD techniques. It is interesting to note that all three (ISSR, DAMD, and RAPD) datasets showed high levels of correlation. This is not surprising as these markers are known to target different genomic fractions involving repeat and/or unique sequences, which may have been differentially evolved or preserved in due course of natural or human selection. These techniques were more informative and effective than the previously used molecular methods to study variation in gerbera, such as SSR (Hajibarat et al., 2014), and RAPD markers (Feghhi et al., 2014, Prajapati et al., 2014, Saidi et al., 2018). Discordance between dendograms obtained by ISSR and DAMD with RAPD could be explained by the different nature of each technique, region coverage of genome by each marker, level of polymorphism and the number of loci.

Our results substantiate the previous reports by clustering genotypes using different marker systems in chickpea (Pakseresht et al., 2013), and Lenti (Seyedimoradi and Talebi 2014). Although the level of diversity for the three marker techniques was approximately equal, we offered that there are several possible explanations for such results: some of them linked with stricter of different molecular markers that designed from different regions of genome. Comparison with MI between ISSR, DAMD, and RAPD shows that DAMD has higher marker rate than RAPD and ISSR. The MI, which can be a resolving power to distinguish among different accessions (Khodadadi et al., 2011), was different in three marker markers (Table 3). Our study revealed that the resolving power of DAMD markers is higher than ISSR primers. Information about current genetic diversity permits the classification of our available germplasm into various/heterotic groups, which is particularly important to hybrid/cross-breeding programs in gerbera. DAMD markers are usually reproducible, while primer length and annealing temperature are not the sole factors determining reproducibility (Saidi et al., 2017).

In conclusion, DAMD marker analysis was successfully developed to evaluate the genetic relationships among the gerbera cultivars. Polymorphism percentage revealed by DAMD was so abundant and could be used for molecular genetics study of the gerbera cultivars, providing high-valued information for the management of germplasm, improvement of the current breeding strategies, and conservation of the genetic resources of *Gerbera* species.

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doi:10.14720/aas.2023.119.1.2259 Original research article / izvirni znanstveni članek

Heat tolerance in Mashona beef cows in semi-arid rangelands: does conformation matter?

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Received June 23, 2021; accepted Avgust 23, 2022.
Delo je prispelo 23. junija 2021, sprejeto 23. avgusta 2022.

Heat tolerance in Mashona beef cows in semi-arid rangelands: does conformation matter?

Abstract: High temperatures and frequent heat waves raise concerns about heat stress in cattle in grass-based systems, especially in arid and semiarid areas. This study analysed the relationship between conformation traits and physiological parameters associated with heat stress in Mashona cattle. A total of 200 records from fifty cows were used to study the relationships between seven conformation traits and physiological parameters associated with heat stress. Body conformation traits were categorised into three principal components related to body capacity (body depth, flank circumference, chest girth), frame size (stature and body length), and loose skin fold (navel height and dewlap size). As the size of abdominopelvic and thoracic cavities increased, respiratory rate, heart rate, and rectal temperature decreased significantly, while blood triiodothyronine concentration increased. Cattle with deeper bodies, larger flanks, and larger chest girths had significantly lower heart rate, respiratory rate, and rectal temperature but higher blood triiodothyronine concentration than cattle with shallower bodies, smaller flanks, and smaller chest girths. Respiratory rate increased with increasing frame size. Large-framed cattle had significantly higher respiratory rate and lower blood thyroxine concentration. Small-framed cattle with larger chest girth, larger dewlap, and navel farther from the ground surface are better adapted to higher ambient temperatures.

Key words: beef cattle; breeds; Mashona; grazing; heat stress; physiological parameters; conformation traits; body frame; arid rangelands; semi-arid rangelands

Vpliv telesnih lastnosti na dovzetnost za vročinski stres pri kravah mesne pasme mashona v polsušnih pašnih pogojih

Izvleček: Visoke temperature in pogosti vročinski valovi povečujejo nevarnost vročinskega stresa pri govedu na paši, zlasti v sušnih in polsušnih okoljih. Raziskava je analizirala povezave med telesnimi lastnostmi goveda pasme mashona in fiziološkimi parametri, povezanimi z vročinskim stresom. Skupno 200 meritev, pridobljenih na petdesetih kravah, je bilo uporabljenih za preučevanje povezave med sedmimi telesnimi lastnostmi in različnimi fiziološkimi parametri, povezanimi z vročinskim stresom. Telesne lastnosti so bile razvrščene v tri kategorije, ki se nanašajo na obseg telesa (globina telesa, obseg trebuha, prsni obseg), velikost okvira (višina križa in dolžina telesa) in viseči kožni gubi (višina popka in velikost podgrline). S povečevanjem velikosti prsne in trebušne votline se je zmanjševala frekvenca dihanja, srčni utrip in rektalna temperatura, koncentracija trijodtironina v krvi pa se je povečevala. Govedo z večjo globino telesa, in večjim obsegom trebuha ter prsnega koša je imelo nižji srčni utrip, frekvenco dihanja in rektalno temperaturo, vendar višjo koncentracijo trijodotironina v krvi kot govedo s plitvejšim telesom, in manjšim obsegom trebuha ter prsnega koša. S povečevanjem velikosti okvira se je povečevala frekvenca dihanja. Živali z velikim okvirom so imele znatno manjšo frekvenco dihanja in nižjo koncentracijo tiroksina v krvi. Živali manjšega okvira z večjim prsnim obsegom, večjo podgrlino in popkom, bolj oddaljenim od površine tal, so bolje prilagojene na višje temperature okolja.

Ključne besede: govedo; mesne pasme; mashona; paša; vročinski stres; fiziološki parametri; telesne lastnosti; okvir; sušno okolje; polsušno okolje

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1 INTRODUCTION

The effects of global warming on cattle are well-documented (Rust and Rust, 2013). Cattle are vulnerable to high temperatures due to fermentation in the rumen, which generates additional heat and low surface to body mass ratio (Alfonzo et al., 2016). Furthermore, cattle do not sweat effectively and mostly rely on respiration to cool themselves (Baumgard and Rhoads, 2012). The sub-Saharan region has been subjected to extremely hot and dry conditions in the past decade (Descheemaeker et al., 2018). This has resulted in low productivity, poor growth performance and increased mortality of cattle (Dzavo et al., 2019). As a result, there has been wide exploration on management and genetic strategies to address the effects of heat stress in dairy cattle because the effects of heat stress are more pronounced in highly productive cattle due to the close relationship between metabolic heat production and production levels (Bernabucci and Mele, 2014). Little, if any, has been done on countering heat stress in beef cattle.

Beef cattle grazing on natural rangelands are more susceptible to heat stress than confined cattle because most natural rangelands do not have confinement resources (Scasta et al., 2016). Due to physiological differences, beef cows are reportedly more vulnerable to heat stress than steers, heifers and bulls (Brown-Brandl, 2018). Heat stress can have a tremendous impact on the profitability of grassland-based beef farms as their productivity largely depends on female performance (Mulliniks et al., 2020). Managing heat stress of beef cattle in grass-based beef farms is, therefore, very important. While strategies like providing shade and spraying animals with water to reduce excessive heat loads are used in dairy production (Marcillac-Embertson et al., 2009), it is difficult to provide such measures in extensive production systems. Although cattle grazing on natural rangelands have the ability to seek natural shade, water and air movement to cool down, natural rangelands in semiarid regions lose their natural shade during dry periods (Scasta et al., 2016). Animals are therefore exposed to warm air and direct radiant heat during grazing.

The use of more robust cattle that can thrive in hot climates is a possible solution to counteract the effects of heat stress in semi-arid regions. Some beef breeds, usually *Bos indicus,* have thermoregulatory ability as an adaptation to harsh environmental conditions (Mwai et al., 2015). One of such breeds is the Mashona breed, a popular Sanga type beef breed in the sub-Sahara, which is native to Zimbabwe. Dilution of the adaptation genes through crossbreeding has, however, reduced their tolerance to heat stress (Scholtz and Theunissen, 2010). Thus, there is a need to improve the heat tolerance of the increasingly popular crossbred or nondescript beef populations in semi-arid areas. Traits such as sweating rate, heart rate, rectal temperature, thyroxine (T4) and triiodothyronine (T3) concentration in blood have been suggested as indicators of heat stress in dairy cows (De Rensis and Scaramuzzi, 2003; Levente et al., 2012; Alfonzo et al., 2016). However, the use of these indicator traits is limited because their measuring is expensive and time consuming. Considering that thermoregulation in cows is also determined by anatomical factors (McManus et al., 2009a), use of conformation traits as indirect indicators of heat stress susceptibility in cows may be an option.

Conformation traits, described by measurements of a range of visual characteristics are related to cattle anatomical and skeletal appearance (Zindove et al., 2015). Phenotypic and genetic relationships between the conformation traits such as body depth, body length, stature, flank circumference, and chest girth and reproductive performance of cows under harsh environmental conditions have been reported (Zindove et al., 2015). There is limited data on the relationship between conformation traits with heat tolerance and/or heat stress in cows. Katiyatiya et al. (2017) suggested that dark pigmentation in Nguni cows results in absorption of solar radiation resulting in high rectal temperature and breathing rate. There are suggestions that dewlap size is associated with heat tolerance in cattle (Hansen, 2004), but there is no empirical data to support these suggestions. There is need to ascertain the relationships between conformation traits and heat tolerance before using conformation traits as indicators of heat tolerance in beef cows. This will help cattle producers to understand and improve the conformation traits that have a significant impact on beef cattle tolerance to extreme temperatures. Conformation traits can be observed and recorded at an early stage and, thus, heat tolerant animals can be identified early in their lifetime. Farmers who do not keep records can also identify heat tolerant cows using conformation traits through visual appraisal (Zindove et al., 2015). Even communal farmers, who do not keep records, can use mere inspection to identify cows that are more heat tolerant.

There is a wide range of conformation traits which could be used to identify heat tolerant cows and most of the traits are highly correlated (Zindove et al., 2015). If conformation traits are to be used as indicators of heat tolerance, there is a need to combine them into a small number of variables or constructs to reduce redundancy and collinearity. The objectives of this study were to assess the reduction in dimensionality of seven conformation traits and to determine the relationships between extracted constructs and physiological parameters associated with heat stress traits in beef cattle.

2 MATERIALS AND METHODS

Animal handling adhered to guidelines by the Zimbabwe Scientific Animal Act, 1963, subsection 2 of section 4, License Number L624.

2.1 STUDY SITE

The study was conducted in Muzarabani district which is located in Zimbabwe's Mashonaland Central province. The district is in agro-ecological region IV. Zimbabwe's agro ecological region IV is a semi-arid region characterised by erratic rainfall of 600 mm per annum, highest mean monthly temperature of 37 °C in February and a mean annual temperature of 23 °C (Mugandani et al., 2012). The area is usually hit by frequent droughts and is characterised by mopane woodlands and vast grasslands of the *Eragrostis* species (Mugandani et al., 2012).

2.2 CONFORMATION TRAITS

Ten farmers with at least 20 multiparous Mashona cows grazing on communal rangelands were selected using the snow-ball technique. Breed score for each of the cows in the selected herds was determined by trained personnel using a 1–9 scale score based on nine physical characteristics of Mashona cows as described by Dzavo et al. (2020). A cow was considered to be a Mashona breed only if it met at least five of the nine characteristics. Rectal palpation was performed by an experienced veterinarian to determine the pregnancy status of the cows. Pregnant cows were excluded from the study. For each of the ten herds, a sampling frame was created by entering the identification numbers of the cattle in an excel sheet. Five cows were then selected from each of the ten sampling frames using Microsoft excel random function. The cows were then ear tagged for identification. Measurements were taken during November 2017 and June 2018, once in the cold-dry season (June 2018) and once in the hot-wet season (November 2017) by trained personnel. The cows were held in a race for measurement and before taking the measurements each farmer was interviewed on a number of calvings (parity) of each cow. Measurements for conformation traits were taken by the same individual throughout the trial between 8:00 am and 10:00 am. The five-point European system was used to determine the body condition score (BCS) of the cows, where a score of 1 is emaciated and a score of 5 is very fat (Edmonson et al., 1989). Coat colour for each cow used in the experiment was recorded.

The stature, body depth, chest girth, flank circumference, navel height and body length were measured using a plastic tape measure. Stature was measured from top of the spine in between hips to the ground. Body depth was measured as distance between the top of spine and bottom of barrel at last rib measured from the left side of the cow. Chest girth was defined as circumference of the body taken just behind the shoulders. Flank circumference was defined as circumference of the body taken just in front of the hook bones. Navel height was measured as the distance from the ground to the navel. Body length was measured as the horizontal distance from withers to pin bone (Zindove et al., 2015). Dewlap size was measured as the maximum width of skin folds in the ventral neck region (Khan et al., 2018). Dentition was used to determine the age of each cow. Cows with visible incisors were categorised as young whilst those with broken or gummy mouth were categorised as old (Raines et al., 2008).

2.3 PHYSIOLOGICAL PARAMETERS AND BLOOD COLLECTION

Heart rate, respiratory rate and rectal temperature were measured on a single day during the hot-wet and cold-dry season by a veterinarian. For each cow, the parameters were measured in the morning around 8:00 am and the measurements repeated in the afternoon around 2:00 pm. The animals were kept in an open paddock without shade for three hours before measurement of physiological parameters (Brown-Brandl, 2018). Heart rate was determined using a stethoscope. Respiratory rates were determined by observing and counting the number of flank movements per minute (da Costa et al., 2015). A digital thermometer with a sensitivity of 0.1 °C was used to determine rectal temperature. The thermometer was inserted into the rectum at a depth of about 10 cm. After every measurement, the thermometer was washed in cold water, cleaned with cotton wool soaked in alcohol, and then rinsed to remove the alcohol. After collecting the physiological parameters, 10 ml of blood were taken from the jugular vein of each of the cows using EDTA coated vacutainer tubes and temporarily stored in chilled cooler boxes before the analyses.

2.4 BLOOD SAMPLE ANALYSES

Blood T3 and T4 concentrations were determined using an automated MAGLUMI 800 chemiluminescence immunoassay system (Snibe Co. Ltd, Shenzhen 518000, China) at Diagnopath Medical Laboratories, Harare, Zimbabwe. Competitive immunoluminometric assay was performed to determine T3 and T4. Serum, ABEI labeled anti-T4 monoclonal antibody and T4 antigen coated magnetic microbeads were added to microwells. Unbound liquid fractions were then aspirated from the immune complex formed with the labeled antibody and precipitation in the magnetic field. Signal reagents were added to the wells to initiate a light which was then measured as relative light unit (RLU) by a photomultiplier. The RLU was proportional to T4 concentration in the samples. For T3 concentration, the same process was repeated using serum, ABEI labeled anti-T3 monoclonal antibody and T3 antigen coated magnetic microbeads.

2.5 STATISTICAL ANALYSES

All data were analysed using SAS 9.4 (SAS, 2012). The effects of parity, season, coat colour, age, time of the day, BCS on respiratory rate, heart rate, and T3 and T4 concentration in blood were determined using PROC GLM for repeated measures assuming fixed models with all possible first-order interactions. First-order autoregressive correlation (AR (1)) was fitted to the model. The model for the final analysis was obtained after eliminating interactions that were not significant ($p > 0.05$). Preliminary analyses showed that BCS, the age and parity of cows had no effect on any of the response variables and were, thus, excluded from the final models.

Principal component analysis (PCA) was used to organise conformation traits into more homogeneous groups called principal components. The matrix of partial correlations, Kaiser Statistic for sampling adequacy (MSA) was used to determine the degree of interrelations between variables and adequacy for use in principal component analyses. Principal components were chosen based on Kaiser's eigenvalue rule which states that only principal components with eigenvalues greater than one should be considered (Kong et al., 2017). The principal components were rotated using varimax rotation. Principal components weights greater than 0.55 were considered to indicate a significant correlation between traits and principal components (Kong et al., 2017). The PROC REG (SAS, 2012) was then used to test whether the relationships between physiological characteristics and principal components extracted from conformation traits were linear, quadratic or exponential.

3 RESULTS

Table 1 shows summary statistics for the traits analyzed. Amongst the linear measurements, flank circumference showed the largest variation while dewlap size showed the least variation. The standard deviation for the physiological traits ranged from 0.51 for rectal tempertaure to 7.7 for heart rate. Heart and respiratory rate had more variability as compared to rectal temperature and concentarion of thyroid hormones. There was little variation in body fat of the animals as indicated by low standard deviation for BCS.

Breed score and BCS had no significant effect on

Table 1: Summary statistics for conformation traits, breed score, body condition score (BCS), respiratory rate, heart rate, rectal temperature, blood thyroxine (T4) and triiodothyronine (T3) concentration of Mashona cows used in the study

Variable	N	Mean	SD	Minimum	Maximum
Body depth (cm)	100	101.9	6.91	83	119
Body Stature (cm)	100	124.2	6.15	110	141
Body length (cm)	100	135.2	9.39	114	156
Flank circumference (cm)	100	173.5	13.44	141	206
Chest girth (cm)	100	164.1	10.11	142	186
Navel height (cm)	100	52.4	7.03	41	61
Dewlap size (cm)	100	41.4	4.87	29	52
Breed score	50	8.3	1.05	5	9
BCS	100	3.4	0.53	2.5	4.5
Heart rate (b/min)	200	51.1	7.70	33	68
Respiratory rate (br/min)	200	30.1	7.39	13	48
Rectal temperate (T°C)	200	38.8	0.51	37	40
T3 (nM/L)	200	3.9	1.63	1.2	13.7
T4 (nM/L)	200	17	4.20	3.5	28.6

Table 2: Least square means (±SE) of the effect of season, time of the day and coat colour on respiratory rate, heart rate, respiratory rate, blood thyroxine (T4) and triiodothyronine (T3) concentration of Mashona cows (n = 200)

	Physiological Parameters				
Parameter	Respiratory Rate (br/min)	Heart Rate (b/min)	Rectal Temperature (T°C)	T3 (nM/L)	T4 (nM/L)
Season					
Cold-dry	25.2 ± 1.16^{b}	47.1 ± 1.23^{b}	38.2 ± 0.09^{b}	4.0 ± 0.29^{a}	17.5 ± 0.72
Hot-wet	30.7 ± 1.16^{a}	52.6 ± 1.23^{a}	38.7 ± 0.09^{a}	3.4 ± 0.29^{b}	16.4 ± 0.72
Time					
Morning	26.7 ± 1.15^{b}	47.9 ± 1.23^{b}	38.6 ± 0.09	4.0 ± 0.29^{a}	16.9 ± 0.71
Afternoon	29.1 ± 1.14^{a}	51.8 ± 1.22^{a}	38.6 ± 0.09	3.3 ± 0.29^{b}	16.0 ± 0.72
Coat colour					
Black	26.5 ± 1.25^{b}	48.2 ± 1.33^{b}	38.5 ± 0.09	3.8 ± 0.31	17.0 ± 0.78
Brown	29.4 ± 1.14^{a}	51.5 ± 1.2^{a}	38.6 ± 0.09	3.6 ± 0.28	19.0 ± 0.71

br/min – breaths per minute; b/min – beats per minute; ^{ab} Values of each parameter in a column with different superscripts differ ($p < 0.05$)

respiratory rate, heart rate, and T3 and T4 concentration. Table 2 shows the effect of season, time of the day, and coat colour of the cows on respiratory rate, heart rate, rectal temperature, and T3 and T4 concentration. Heart rate was higher during the hot-wet season than the cold-dry season ($p < 0.05$). The concentration of T4 in blood during the hot-wet season in the afternoon was lower than during the cold-dry season in the morning ($p < 0.05$). Black-colored cows had higher heart rates than brown cows ($p < 0.05$). Rectal temperature and T4 concentration in blood were the same in black and brown cows ($p > 0.05$). Variation of rectal temperatures with breed score during the cold-dry and hot-wet season is shown in Figure 1. During the cold-dry season, rectal temperature was the same for all the cows ($p > 0.05$). Cows with low breed scores (5 and 6) had higher rectal temperatures during the hot-wet season than those with high breed scores ($p < 0.05$). Respiratory rates and T3 concentration in the blood of cows during the cold-dry and hot-wet seasons are shown in Figure 2 and 3, respectively. Season did not affect respiratory rate and blood T3 concentration in brown-colored cows ($p > 0.05$). Black-colored cows had higher respiratory rate and lower blood T3 concentration during the hot-wet season as compared to the cold-dry season ($p < 0.05$).

Principal component pattern coefficients of the varimax rotated components and aggregated groups of conformation traits, grouped using principal component analysis, are shown in Table 3. The conformation traits had three principal components, which contributed

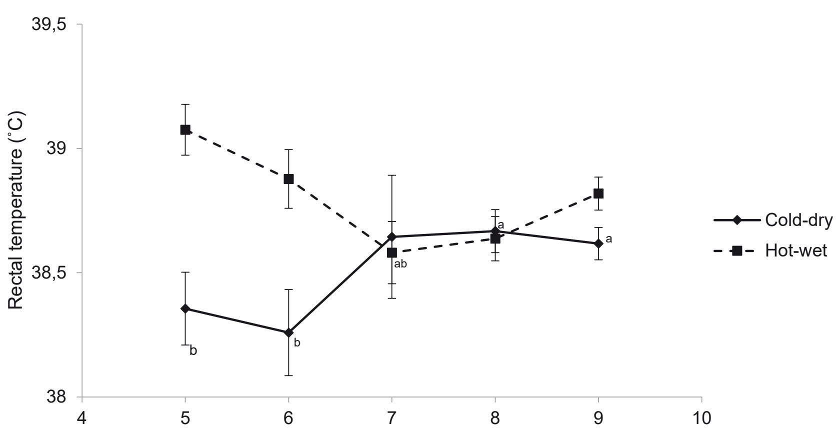

Figure 1: Effect of breed score of Mashona cows on rectal temperature during cold-dry and hot-wet seasons. ^{ab} Different letters indicate significant difference ($p < 0.05$)

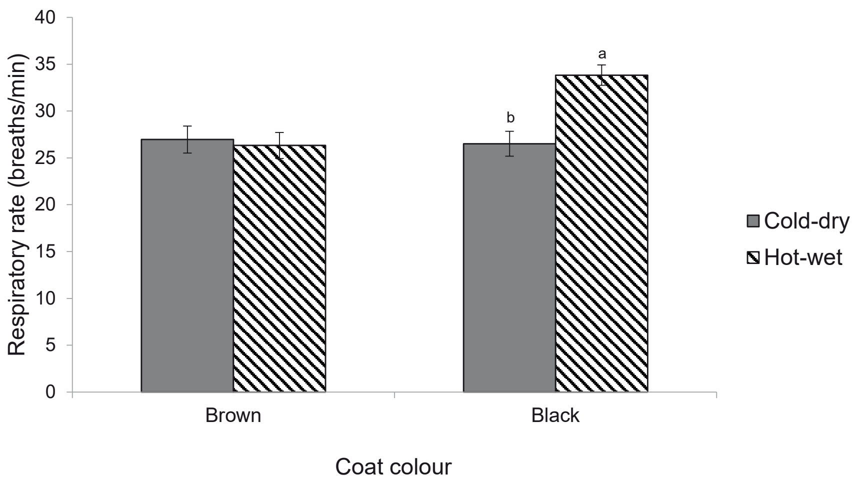

Figure 2: Respiratory rate of black and brown Mashona cows during the cold-dry and hot-wet season. [ab] Different letters indicate significant difference ($p < 0.05$)

53.76 % of the total variability of the original seven traits. Principal component 1, 2 and 3 accounted for 28.03 %, 14.20 % and 11.53 % of the total variance, respectively. Body depth, flank circumference and chest girth had significant principal component weights in principal component 1 (principal component weights > 0.55), whilst stature and body length had significant principal component weights in principal component 2. Principal component 3 was comprised of navel height and dewlap size (principal component weights > 0.55).

Relationships between principal components extracted from conformation traits and physiological parameters associated with heat stress did not vary with season ($p > 0.05$). Relationships between principal components extracted from conformation traits with heart rate, respiratory rate, rectal temperature, T3 and T4 concentration in blood are shown in Table 4. As principal component one increased respiratory rate, heart rate and rectal temperature decreased whilst T3 concentration in blood increased ($p < 0.05$). Cows characterised by deep bodies, large flanks and chest girths tended to show lower heart rate, respiratory rate and rectal temperature but higher T3 concentration in blood. Principal component two had significant negative linear and quadratic relationships with blood T4 concentration. As principal component two increased, respiratory rate increased quadratically ($p < 0.05$). Large-framed cows with long bodies had high respiratory rates and low T4 concentra-

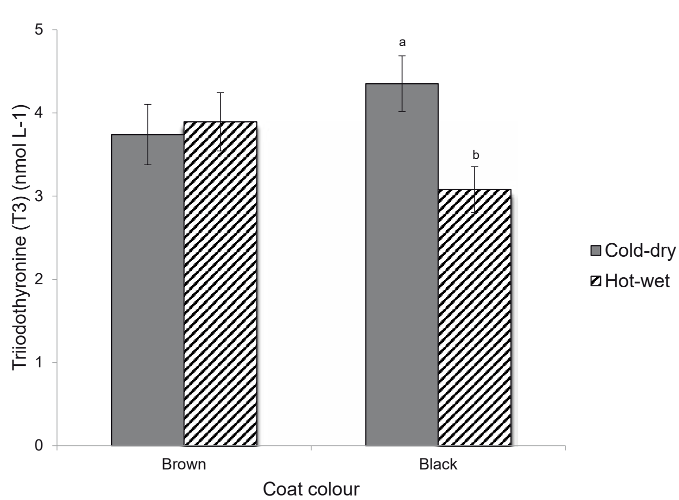

Figure 3: Concentration of serum triiodothyronine (T3) in black and brown Mashona cows during the cold-dry and hot-wet season. [ab] Different letters indicate significant difference ($p < 0.05$)

Table 3: Eigenvalues and share of total variance and principal component (PC) weights after rotation of conformation traits of Mashona cows

Traits	PC 1	PC 2	PC 3	Communality
Body depth	0.89*	0.15	−0.18	0.85
Stature	0.07	0.76*	−0.24	0.65
Body length	0.26	0.77*	0.19	0.70
Flank circumference	0.89*	0.05	−0.04	0.80
Chest girth	0.87*	0.18	−0.19	0.84
Navel height	−0.29	0.12	0.63*	0.50
Dewlap size	−0.02	0.37	−0.65*	0.57
Eigen Values	3.11	1.22	1.04	
Percent of total variance	28.03	14.20	11.53	

* Principal component weights equal to or > 0.58 were significant

tion in blood. As principal component three increased, respiratory rate increased quadratically ($p < 0.05$).

4 DISCUSSION

Cattle cannot dissipate their heat load effectively due to their large frames and ineffective sweating (Veissier et al., 2018). Ineffective heat dissipation, coupled with fermentation, which generates additional heat, results in heat stress and, consequently, reduced performance in cows (Dzavo et al., 2019). Conformation traits are becoming increasingly important to cow-calf farms due to their high heritability, ease of recording and close relationship with feed efficiency and energy balance in cows (Zindove et al., 2015). As a result, the conformation traits are being used as indicators for traits which are difficult to measure such as nutritional status, disease resistance and longevity (Larroque and Ducrocq, 2001; Zindove et al., 2015). Conformation traits such as body depth, body length, stature, flank circumference, chest girth, and BCS are, thus, expected to be related to physiological parameters associated to heat stress in cows.

The range of BCS was within reported values for Sanga and Zebu cattle in similar environments (Kanuya et al., 2006; Ndlovu et al., 2009; Zindove et al., 2015). To our knowledge, there is limited selection for conformation traits in beef cattle, so high variation in traits such as flank circumference and body length might be an indication that the traits respond more to the environment than those with low variation. The findings that heart rate and rectal temperature were higher during the hot-wet season than the cold-dry season confirmed assertions by Kadzere et al. (2002) and De Rensis and Scaramuzzi (2003) that it is common for cows to have relatively higher heart rates and rectal temperatures during hot seasons than cold seasons. An increase in heart rate of cows above normal ranges indicates heat intolerance (Scharf et al., 2010). Normal heart rates in beef cows ranges between 48 and 84 beats per minute (Rashamol et al., 2018). Although heart rates for the cows were higher during the hot-wet season than the cold-dry season, they were within the normal range. Observed high respiratory rate during the hot-wet season concurs with the findings of Svotwa et al. (2007) who reported higher respiratory rates in Mashona cows during hot periods compared to cold periods. This might be attributed to the fact that during hot periods, cows counter high temperatures through evaporative cooling by releasing moisture from respiratory tract (Veissier et al., 2018). Although rectal temperature was higher during the hot-wet season, it was within the normal range of 38.5 to 39.5 °C as suggested by Da Costa et al. (2015). Katiyatiya et al. (2017) measured rectal temperature in Nguni cows, a Sanga breed similar to Mashona cows, during the hot-wet season and found similar results. This could be an indication that Sanga cows are able to regulate their body temperatures during the hot-wet season without experiencing heat stress. Farmers raising cows on natural pastures in warm climates can thus be encouraged to adopt Mashona breed as they seem to tolerate high temperatures well. This postulation is supported by the findings herein that cattle carrying more Mashona breed genes, as indicated by high breed scores, had lower rectal temperatures during the hot-wet season.

The observation that black coloured cows had higher heart rates during the hot-wet season than brown coloured is in line with the findings of Katiyatiya et al. (2017) who reported higher heart rates in dark coloured cows. Since black cattle feel the effects of heat stress earlier and more than brown and white-coloured (Scasta et al., 2016), it can be inferred that black-coloured cows

Table 4: Regression coefficients (±SE) of principal components (PC) extracted from conformation traits on heart rate (HR), respiratory rate (RR), rectal temperature, blood triiodothyronine (T3) and thyroxine (T4) concentration in Mashona cows

Parameter	PC 1	PC 2	PC 3
Traits	BD; FC; CG	BL; ST	SH; DL
Linear			
RR ± SE	−0.4 ± 0.15**	0.1 ± 0.18^{ns}	0.3 ± 0.16^{ns}
HR ± SE	−0.5 ± 0.16**	−0.2 ± 0.19^{ns}	0.2 ± 0.17^{ns}
Rectal Temperature	−0.03 ± 0.011*T*	−0.003 ± 0.0013^{ns}	−0.02 ± 0.011^{ns}
T3	0.08 ± 0.034*	0.04 ± 0.020^{ns}	−0.02 ± 0.016^{ns}
T4	−0.05 ± 0.008^{ns}	−0.2 ± 0.10*	−0.08 ± 0.009^{ns}
Quadratic			
RR ± SE	−0.04 ± 0.014**	0.008 ± 0.0017*	0.03 ± 0.015**
HR ± SE	−0.03 ± 0.014**	−0.03 ± 0.018^{ns}	0.02 ± 0.016^{ns}
Rectal Temperature	−0.003 ± 0.00095**	−0.0005 ± 0.00012^{ns}	−0.002 ± 0.0011^{ns}
T3	0.007 ± 0.0031*	0.002 ± 0.0039^{ns}	−0.002 ± 0.0014^{ns}
T4	−0.001 ± 0.0019^{ns}	−0.02 ± 0.010*	−0.005 ± 0.0018^{ns}

* BD = body depth; ST = stature; BL = body length; FC = flank circumference; CG = chest girth; SH = navel height; DL = dewlap size. ** $p < 0.01$; * $p < 0.05$.; ^{ns} $p > 0.05$

had higher heart rates during the hot-wet season than brown-coloured to increase cooling rate. Low T3 concentration in the blood of black cows during the hot-wet season could be a result of heat stress. Although, to our knowledge, there are no comparable studies yet, Baumgard and Rhoads (2012) reported that heat stressed cattle reduces circulating blood T3 concentration. Low blood T3 concentration slows metabolic rates and consequently reduces heat production (Kahl et al., 2015). Lower blood T3 concentration in black cows could, therefore, be a consequence of higher body temperatures during the hot-wet season. High respiratory rates in black cows during the hot-wet season observed in the current study could also be a result of high temperatures. In a similar study on different species, McManus et al. (2009b) found that black sheep had higher respiratory rates indicating they are more susceptible to heat stress. The finding that black cattle are more susceptible to heat stress could be attributed to the fact that cows with darker hair have higher solar absorption as reported by Scasta et al. (2016). Light coat colours are therefore desired for heat stress management in cows. Beef cattle producers in hot climates should consider light coat colours when selecting breeding and replacement animals. Considering that only two coat colours were studied herein, further investigation on the impact of other coat colours on thermal regulations in beef cattle seem important. Some light coat colors such as white have been reported to be susceptible to photo-chemical damage (Lee et al., 2016) and, thus, might be undesirable under sunny conditions.

Low T3 concentration in blood of the cows in the afternoon observed in the current study agrees with previous work by Baek et al. (2019) who found that blood T3 concentration of steers subjected to warmer ambient temperatures was lower than in cooler ambient temperatures. Considering that low T3 concentration in blood is associated with heat stress in cattle (Baumgard and Rhoads, 2012), grazing in the afternoon during hot periods can be considered stressful to cattle. This assertion could be further strengthened by the current finding that respiratory and heart rate of the cows were higher in the afternoon than in the morning. Similarly, Neiva et al. (2004) reported that an increase in the temperature in the afternoon leads to increased heart and respiratory rate of livestock. During the afternoon when ambient temperature is high and air humidity low, there is more evaporative water loss through rapid breathing (Levente et al., 2012; Veissier et al., 2018). To avoid afternoon grazing and exposing cattle to heat stress during hot seasons, beef cattle producers should facilitate early morning and/or late afternoon grazing. Cattle can be released for grazing early in the morning and late in the afternoon whilst cattle handling activities can be done under the shade from noon up to about 4:00 pm. Limiting grazing time might, however, result in reduced feed intake and, consequently, peformance of the cows (Gregorini, 2012). It is, therefore, important in hot environments to come up with strategies, which will counter heat stress for grass-based beef cattle grazing systems throughout the day. Use of conformation traits for prediction of heat tolerance of cattle could be an option. In dairy production, composite indices of conformation traits are commonly used as predictors of traits of economic importance (Wu et al., 2013). The conformation traits used to form these indices are, however, strongly correlated and thus redundant (Olasege et al., 2019). A study by Zindove et al. (2015) showed that individual conformation traits in beef cows have high collinearity and correlations. There is a need to combine conformation traits of cows into smaller number of variables to reduce redundancy and collinearity before using them as indicators of traits of interest.

Principal component analyses showed biological associations underlying the phenotypic relationships for the six conformation traits under the study. In agreement with findings by Zindove et al. (2015), two of the three generated principal components derived in this study describe general size of the cows. The finding that body depth, chest girth and flank circumference, which describe aspects of a cow's body and rumen capacity (Hansen et al., 1999), were grouped into one principal component concurs with Zindove et al. (2015). Zindove et al. (2015) also grouped body length and stature into one principal component like in the current study. Body length and stature are commonly used as measurements for frame size in cows (Dubey et al., 2012). To avoid analysing large numbers of correlated traits, reduce computation burdens associated with analysing large and complex data and improve precision, beef producers recording conformation traits can use the three principal components instead of the individual traits.

The finding that cows characterised by deep bodies, large flanks and chest girths had lower heart rates, respiratory rates, and rectal temperatures and higher T3 concentrations in blood agrees with Abdurehman (2019) who argued that large flank circumference and chest girth of East African Shorthorn Zebu cows are anatomical adaptations, which contribute to lower respiratory rates, rectal temperatures, and heart rates even when the cows are subjected to high temperatures. This could be because of the interactions between body depth, chest girth size, metabolic heat production and heat dissipation by the cattle. Chest girth size influences the respiratory rate by limiting lung expansion (Brown-Brandl, 2018). Livestock with large chests are be able to inhale and exhale large air volumes thereby losing much heat to the environment through the released vapour without increasing their respiratory rate (Marai et al., 2007). Cows with deep bodies coupled with large flanks and chests have large guts resulting in high fermentation rates and metabolic heat production (Hansen et al., 1999). Thus, contrary to our findings that cows characterised by deep bodies, large flanks, and chest girths have low heart rate, respiratory rate, and rectal temperature coupled with high T3 concentration in blood, cows with large flanks, chests and deep bodies are expected to produce high metabolic heat. This implies that heat tolerance in cows with large flanks, chests and deep bodies could be mainly due to greater ability to dissipate heat and not because of reduced heat production (as result of reduced metabolic rates). Cows with large flanks, chests and deep bodies are, therefore, desirable for hot environments since they are able to tolerate high temperatures without reducing their metabolic rates. Based on the findings herein, beef cattle producers can use flank size, chest girth and body depth to come up with a classification system, which better explains variation in heat tolerance among their cattle.

The positive correlation between frame size of the cows (body length and stature) and T4 concentration in blood and negative relationship with respiratory rate reflects on the importance of frame size of cows in hot environments. In a similar study, Srikandakumar and Johnson (2004) found out that Australian Milking Zebu and Jersey cows had lower respiratory rates than Holsten cows and attributed it to differences in frame size. This implies that small-framed cows are more adapted to heat compared to their large-framed counterparts. This can be attributed the fact that the larger the body surface area the higher the amount of heat the cows absorb from the environment when environmental temperatures are high (Alfonzo et al., 2016). Based on the current studies and literature, beef producers in hot climates should be encouraged to prioritize small-framed cows. Although, to our knowledge, there are no comparable studies in cows, the higher respiratory rate was expected in cows with larger navel height and dewlap. The distance between ground and the bottom of belly of cows is critical to resilience against hyperthermia as it helps insulate cattle from the ground when it's hot (Brown-Brandl, 2018). Although the function of the dewlap remains largely unexplored, there are suggestions that excess skin of the dewlap is responsible for dissipating heat in large mammals (Bro-Jørgensen, 2016). Studies in elands and dairy cattle using infrared thermography confirmed the dewlap as a site of high heat loss (Kotrba et al., 2007). When principal component three was used for classification of beef cows, cows with large dewlaps coupled with higher distance between ground and navel should be considered more heat tolerant. It is, however, necessary to conduct more studies on the relationship of indicators of heat tolerance with navel height and dewlap size in beef cattle.

5 CONCLUSIONS

The present evidence suggests that heat production, absorption and heat dissipation in cattle are associated with physical characteristics such as coat colour, dewlap size, frame size, chest size and distance between abdomen and ground surface. Black cows are more prone to heat stress. Small-framed cows with large chest girths, large dewlaps and navel further away from the ground surface are less sensitive to high ambient temperatures. Further studies are required to support the relationship between conformation and heat tolerance in beef cows. It is also necessary to investigate the relationship between conformation and heat tolerance in other classes of beef cattle such as calves, steers, heifers and bulls.

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doi:10.14720/aas.2023.119.1.2764 Original research article / izvirni znanstveni članek

Mitigation of the negative effect of auxinic herbicide by bacterial suspension of *Pseudomonas protegens* DA1.2 in wheat plants under drought conditions

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Received July 07, 2022; accepted October 21, 2022.
Delo je prispelo 7. julija 2022, sprejeto 21. oktobra 2022

Mitigation of the negative effect of auxinic herbicide by bacterial suspension of *Pseudomonas protegens* DA1.2 in wheat plants under drought conditions

Abstract: Effect of auxin-producing bacterial strain (*Pseudomonas protegens* DA1.2) was investigated under conditions of drought and herbicide treatment in wheat plants. Positive effect of the bacterial suspension on wheat plants treated with auxinic herbicide under drought conditions was manifested in reducing the content of malondialdehyde and proline, preventing inhibition of plant growth and normalizing chlorophyll content. Under combined stress, changes in concentrations and redistribution of phytohormones in plants were detected. An imbalance in auxin distribution between shoots and roots could be the reason for the decrease in plant resistance to drought in combination with the herbicide. Treatment of plants with the bacterial suspension restored normal shoot-to-root ratio of auxins in plants. Thus, this bacterial strain showed the properties of synthetic auxin antidotes and can be recommended for optimizing the technology of herbicide application under drought conditions.

Key words: bacterial strain; water deficiency; auxinic herbicide; plant hormones; MDA; proline

Blaženje negativnih učinkov herbicidov na osnovi auksina s suspenzijo bakterije *Pseudomonas protegens* DA1.2 pri pšenici v razmerah suše

Izvleček: Učinek sevov bakterije, ki tvorijo auksin (*Pseudomonas protegens* DA1.2) je bil preučevan pri pšenici v razmerah suše in obravnavanja s herbicidi. Pozitivni učinki suspenzije bakterij na pšenici, ki je bila tretirana s herbicidi na osnovi auksina so se pokazali v zmanjšanju vsebnosti malondialdehida in prolina, kar je preprečilo zaviranje rasti rastlin in normaliziralo vsebnost klorofila. V razmerah kombiniranega stresa so se pojavile spremembe v koncentraciji in redistribuciji rastlinskih hormonov v tretiranih rastlinah. Neravnovesje v porazdelitvi auksina med poganjki in koreninami bi lahko bilo vzrok za zmanjšanje odpornosti rastlin na sušo v kombinaciji s herbicidi. Obravnavanje rastlin s suspenzijo bakterij je ohranilo normalno razmerje v vsebnosti auksinov med poganjki in koreninami. Zaključimo lahko, da je ta bakterijski sev pokazal lastnosti antidotov sintetičnih auksinov in bi ga lahko priporočili za optimiziranje tehnologije uporabe herbicidov v razmerah suše.

Ključne besede: bakterijski sev; pomankanje vode; auksinski herbicidi; rastlinski hormoni; MDA; prolin

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1 INTRODUCTION

Drought is a serious stress that reduces the yield of cultivated plants below their genetic potential (Shao et al., 2008; Asseng et al., 2020). Weeds are serious problem in modern agriculture, causing an average decrease in yield by 35 % (Oerke, 2006). The effectiveness of herbicides in weed control has allowed them to be widely used to prevent crop losses (Varhney et al., 2015). However, the use of preparations for chemical weeding of plants has a negative effect on cultivated plants, causing "herbicidal stress", which becomes most notable under drought conditions (Varshney et al., 2015).

It is known that under herbicidal stresses, photosynthesis is the most sensitive process contributing to the plant biomass production (Kutilkin et al., 2018). There are also evidences of the effects of herbicides on the antioxidant system of plants (Ahemad and Khan, 2010).

The role of plant growth regulators, such as hormones, in stress tolerance allows plants to choose the necessary strategy to counteract the adverse effects of abiotic stress and to develop stress resistance responses (Han et al., 2015). Hormones are necessary for the normal functioning of growth processes throughout the plant life cycle; as a result they influence the yield and quality of agricultural crops.

Recently, bacterial treatments are being given more attention in reducing plant abiotic stress. In the scientific literature, there are two areas of research aimed on reduction of the damage caused by currently used herbicides. The goal of the first of them is to accelerate the process of herbicide destruction thereby reducing the negative impact of herbicides on cultivated plants. Microbiological transformation and detoxification of pesticides is rather fully presented in the literature (Silva et al., 2007; Martins et al., 2011; Kanissery and Sims, 2011). However, the potential of bacteria in mitigating pesticide stress in plants (the other area of research) has not been sufficiently studied. There are few publications addressing this question (Ahemad and Khan, 2010; Bourahla et al., 2018; Chennappa et al., 2018). And we have not found any work devoted to the use of growth-stimulating bacteria under conditions of combined stress caused by drought and herbicide treatment.

The previously isolated strain of *Pseudomonas protegens* DA1.2 have shown to possess useful properties, namely resistance to herbicides, capacity of auxin synthesis, mobilization of inorganic phosphates, antagonism to phytopathogenic fungi, growth in the absence of a nitrogen source in the medium, growth-stimulating and anti-stress effects on wheat plants (Chetverikov et al., 2021).

The aim of the present work was to show the prospects of using the bacterial suspension of *Pseudomonas protegens* DA1.2 as an anti-stress agent when applied to wheat plants against the background of combined "herbicide + drought" stress.

2 MATERIALS AND METHODS

The studies were carried out in laboratory conditions on plants of bread spring wheat (*Triticum aestivum* L.) of a drought-resistant variety, Ekada 109. The bacterial strain *P. protegens* DA1.2 from the collection of microorganisms of the Ufa Institute of Biology, Ufa Federal Research Center, Russian Academy of Sciences and the herbicide (against dicotyledons) based on auxin-like active substances 2,4-D (2-ethylhexyl ester) and dicamba (sodium salts) – "Chistalan" extra (LLC "AHK-AGRO", Ufa), were used in the work. Drought conditions were achieved by reducing irrigation to 30 % of the total water capacity of the soil (this was 60 % in the control) and maintaining it throughout the experiment. Vessels with plants were watered daily with distilled water to the required weight every day.

Wheat seeds were sterilized and germinated for 3 days in a pallet on wet filter paper. Then they were planted in vessels with a volume of 0.5 l filled with a soil-sand mixture in the ratio of 9 : 1 of 6 plants each, the plants were watered only with distilled water. Seedlings were grown at 14 - h photoperiod, day/night temperature regimes of 26 / 22 °C and irradiance of 400 μmol m^{-2} s^{-1} from mercury-arc and sodium vapor lamps. Seven days after seed germination, 1 ml of an aqueous solution of "Chistalan" (the working concentration of the herbicide was 0.5 ml l^{-1}) and / or bacterial suspension of *P. protegens* DA1.2 (108 CFU ml^{-1}) grown in the King B was sprayed on the soil and directly on the plants, using a manual sprayer with a graduated capacity of 20 ml with a division price of 0.1 ml.

Three days after the treatment, shoots and roots of plants were sampled for analysis for hormones (3-indolylacetic acid (IAA), abscisic acid (ABA), and cytokinins), proline, malondialdehyde (MDA), and chlorophylls a, b while growth parameters were evaluated 2 weeks later.

Determination of hormones content was performed using an enzyme immunoassay method. The plant material (shoots and roots) was cut and ground in a porcelain mortar to a homogeneous state, then extracted with 80 % ethanol. Alcohol extract was evaporated to an aqueous residue, centrifuged and the aliquots of the supernatant were taken for analysis. Purification and concentration of ABA and IAA was carried out according to a modified scheme with a decrease in volume (Veselov et al., 1992; Vysotskaya et al., 2009). Cytokinins were concentrated on the C - 18 column and separated using thin-layer chromatography (Kudoyarova et al., 2014).

To determine proline content in wheat shoots, the suspension was homogenized in 10 ml of 3 % sulfosalicylic acid for protein precipitation. Then the homogenate was filtered, 1 ml of filtrate was taken into a clean test tube and 1 ml of acidic ninhydrin reagent (30 ml of glacial acetic acid + 20 ml of 6 M phosphoric acid + 1.25 g of ninhydrin) and 1 ml of glacial acetic acid were added. The mixture was incubated for 1 hour in a water bath at 100 °C, after which it was quickly cooled in ice. Two ml of toluene was added to the mixture and shaken. The colored organic phase was separated and its optical density measured against pure toluene at a wavelength of 520 nm on a spectrophotometer. The proline content was calculated using a calibration curve, using crystal proline for its construction, with further conversion to dry and fresh mass of the sample (Bates et al., 1973).

Lipid peroxidation was determined by the content of malondialdehyde. To achieve this, a sample of plant material was homogenized in 3 ml of distilled water and 3 ml of trichloroacetic acid. The homogenate was centrifuged at 10000 × g for 10 minutes. Two ml of 0.5 % thiobarbituric acid was added to 2 ml of the supernatant and incubated in a water bath for 30 minutes at 95 °C. The tubes were centrifuged and optical density was measured at wavelengths of 532 nm and 600 nm. The concentration of MDA was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} (Costa et al., 2002).

To determine the content of chlorophyll in wheat plants, 100 mg of leaves was taken. The samples were cut with scissors, placed in test tubes and filled with 6 ml of 96 % ethanol. The test tubes were tightly closed with lids and placed in a dark place for 24 - 48 hours. After the time elapsed, the tubes were shaken and the optical density was measured at wavelengths of 665 nm and 649 nm. The content of chlorophylls "a", "b" was calculated using the formulas:

$Chl\ a = 13.7\ D665 - 5.76\ D649;$

$Chl\ b = 25.8\ D649 - 7.6\ D665$

where Chl a and Chl b are the concentration of chlorophyll "a" and "b", and D665, D649 are the values of extinction at the corresponding wavelength. Concentration of chlorophyll was recalculated per gram of fresh or dry mass of the sample (Lichtenthaler and Buschmann, 2001).

Data were expressed as means ± S.E., which were calculated in all treatments using MS Excel. Significant differences between means were analyzed by one-way analysis of variance ANOVA test to discriminate means. The data were processed using the Statistica version 10 software (Statsoft, Moscow, Russia).

3 RESULTS AND DISCUSSION

Simultaneous exposure to drought and the application of auxinic herbicide resulted in a decrease in the fresh mass of both roots and shoots of wheat plants (by 24 % and 32 %, respectively). However, plants treatment with *bacterial suspension* under these conditions contributed to an increase in the mass of the shoot (by 13 % compared to stressed plants without bacteria), but without an increment in the mass of roots.

It is known that under water and / or mineral deficiency, relative activation of root growth is a typical adaptive reaction in plants (Davies et al., 2005; Vysotskaya et al., 2009). In our experiments, the root / shoot mass ratio of plants grown under herbicide treatment combined with water scarcity, did not change compared to the control (Figure 1 B), since the fresh mass of both roots and shoots was reduced to the same extent (Figure 1 A), while the addition of growth-stimulating bacteria to the herbicide solution led to a decrease in this ratio due to the relative activation of shoot growth.

Accumulation of low-molecular-weight compounds is one of the early adaptive reactions of plants to the action of stressors of various nature, amino acid proline being one of such stress metabolites. An increase in the

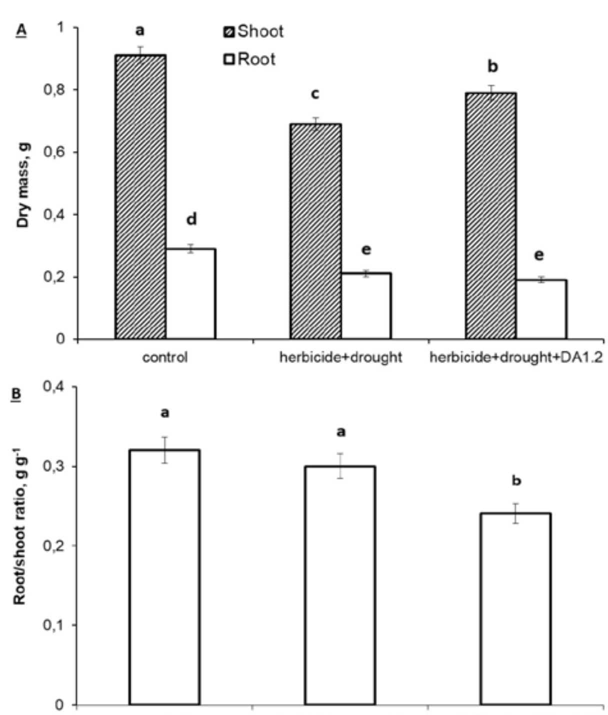

Figure 1: The dry mass of shoots and roots (A) and mass root/shoot ratio (B) of wheat plants 14 days after treatment. Significantly different means (n = 20) are labelled with different letters (ANOVA, LSD, $p \leq 0.05$)

concentration of proline in wheat leaves was observed in response to contamination with toxic metals, osmotic and temperature stress (Fatima et al., 2006; Yang et al., 2011; Mwadzingeni et al., 2016). Indeed, in our work, concentration of proline in plant shoots sharply increased (by more than 100 %) under combined action of water deficit and herbicide treatment, while introduction of the studied bacteria led to a decrease in proline content to the control level (Figure 2 A).

MDA is one of the metabolites of lipid peroxidation and its accumulation in plants shows that they are under severe oxidative stress (Reddy et al., 2004; Masoumi et al., 2011). A decrease in the MDA level indicates a decrease in the damaging effect of reactive oxygen species in leaves of wheat plants inoculated with *bacterial suspension* under conditions of combined stress, which contributed to maintaining their growth and indicated a favorable anti-stress effect of DA1.2 bacteria. Thus, the treatment of plants with herbicide during drought led to accumulation of MDA (15 % more than in the control), while introduction of bacterial strain *P. protegens* DA1.2 leveled the stress by reducing the growth of MDA concentration (Figure 2 B).

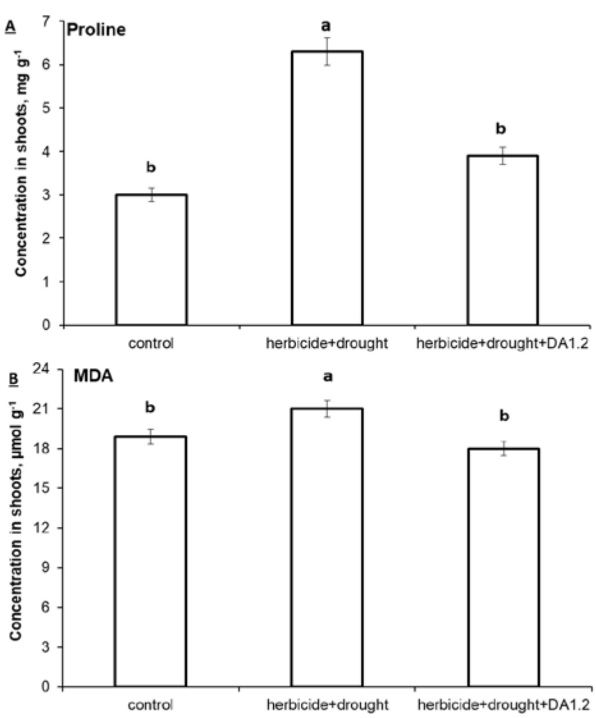

Figure 2: The content of proline (A) and MDA (B) in the shoots of wheat plants 3 days after treatment. Significantly different means (n = 9) are labelled with different letters (ANOVA, LSD, $p \leq 0.05$)

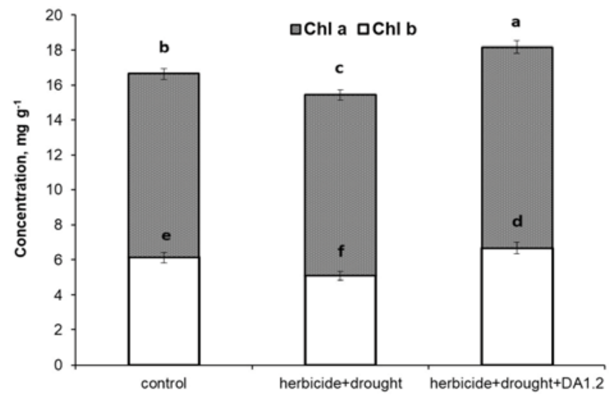

Figure 3: The content of chlorophylls a (Chl a) and b (Chl b) in the shoots of wheat plants 3 days after treatment. Significantly different means (n = 9) are labelled with different letters (ANOVA, LSD, $p \leq 0.05$)

Another important indicator of the plant status under stress conditions is the content of chlorophylls a, b (Nayyar, Gupta, 2006; Ashraf, Harris, 2013). The content of chlorophylls in wheat leaves was shown to decrease by 8 % (Figure 3) under combined "herbicide + drought" stress. The positive effect of bacteria was manifested in mitigating adverse action of the herbicide on photosynthetic apparatus. This was reflected in the quantitative content of pigments in plants: inoculation with bacteria led to an increase in the total content of chlorophylls under the combined stress by 17 %. High concentration of chlorophylls is a characteristic of healthy plants, as it is associated with greater efficiency of photosynthesis. Thus, the interaction of wheat with growth-stimulating bacteria of the strain DA1.2 improved plant status compared to plants untreated with bacteria under stress conditions.

Taking into account that under combined stress root mass of plant of both variants (treated and untreated with bacteria) remained equally low compared to the control, we evaluated possible involvement of hormonal regulation in the detected growth responses. The herbicide treatment (herbicide from the class of synthetic auxins) led to the accumulation of IAA in shoots by more than 2.5 times, while bacterial treatment significantly increased root IAA content (Figure 4 A). We suggested that this could be due to the ability of plants to absorb exogenous auxins. Otherwise, the herbicide could influence the content of endogenous IAA (Kudoyarova et al., 2017). This assumption does not contradict the existing reports (Sung, 1979). The observed imbalance in the auxin distribution between the shoot and root caused by the herbicide may be the reason of the decrease in resistance of plants to drought. And accumulation of aux-

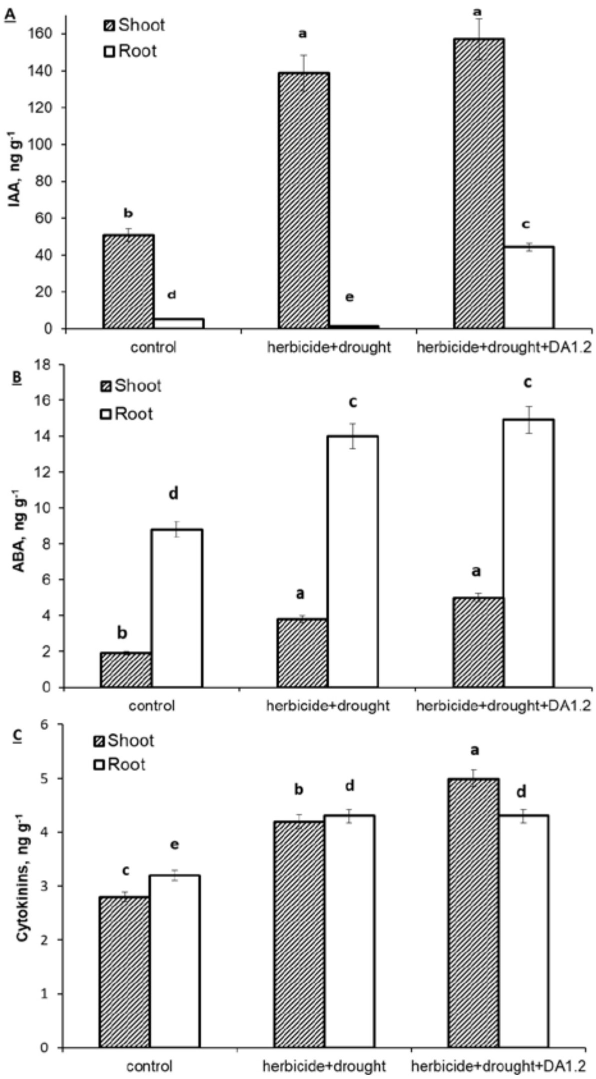

Figure 4: The content of IAA (A), ABA (B) and cytokinins (C) (calculated per g fresh mass) in shoots and roots wheat plants 3 days after treatment. Significantly different means (n = 9) are labelled with different letters (ANOVA, LSD, $p \leq 0.05$)

ins under the influence of bacteria in the roots serves as one of the mechanisms responsible for improving plant growth, plant status when treated with an herbicide under drought conditions.

Accumulation of ABA causes stomatal closure under conditions of water scarcity (Zhang et al., 2006; Leung et al., 2012). The decrease in cell turgor during dehydration dramatically activates synthesis of ABA, which accumulates mainly in the chloroplasts of leaf cells (Zhou et al., 2006; Venzhik et al., 2016). Accumulation of ABA in plants under stress was revealed. It was most strongly manifested in the roots, which could be the result of inhibition of their growth (Figure 4 B). As for cytokinins, stress led to an increase in their content (Figure 4 C), and with bacterial introduction, we observed the greatest accumulation of cytokinins in the shoot. Cytokinins are known to be essential for shoot growth (Martin et al., 2000), inhibit root growth (Werner et al., 2010), and are ABA antagonists (Dodd, 2005). Accumulation of cytokinins under the studied stress could compensate for the growth-inhibiting effects of the increased level of ABA, which were most pronounced in plants treated with bacteria.

4 CONCLUSIONS

It was shown that the herbicide-induced imbalance in the auxin distribution between shoot and root in plants may be the cause of a decrease in drought resistance. The bacterial suspension had a positive effect on the wheat plants treated with the auxinic herbicide under drought conditions, it was expressed in a decrease in the content of biochemical stress markers MDA and proline, preventing plant growth inhibition and normalizing the content of chlorophylls. These positive effects of growth-stimulating bacteria on wheat plants under combined stress caused by drought and herbicide treatment have been described for the first time. Thus, bacterial suspension of the strain *Pseudomonas protegens* DA1.2 can be considered as the basis of biological product for increasing plant tolerance.

5 ACKNOWLEDGMENTS

The work was performed within the framework of the state assignments of the Ministry of Education and Science of Russia project no. 075-00326-19-00 on topic № AAAAA19-119021390081-1 and project no. 075-03-2021-607 on topic № 122031000309-7 with equipment of the Agidel Center for Collective Use of the Ufa Federal Research Center of the Russian Academy of Sciences.

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doi:10.14720/aas.2023.119.1.2751 Original research article / izvirni znanstveni članek

Cultivation of irrigated tomatoes with ozonised water under a Mediterranean climate in Algeria

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Received June 29, 2022; accepted December 07, 2022.
Delo je prispelo 29. junija 2022, sprejeto 7. decembra 2022

Cultivation of irrigated tomatoes with ozonised water under a Mediterranean climate in Algeria

Abstract: This study presents the results of an experimental work carried out on tomato seedlings of the same varietal type, grown under a Mediterranean climate and irrigated with ozonised water at different ozonisation durations that vary from zero to 30 seconds. In order to assess the impact of aqueous ozone on the agronomic performances and the physicochemical characteristics of fruits. The tomatoes responded differently between themselves and the control batch. The results obtained showed precocity of germination, an increase in growth, development, and vigour in the experimental plants compared to the controls. The yield is proportional to the increase in the ozonisation time of irrigation water. During the reproductive phase of the plants, no negative effects were observed. The physicochemical characteristics of fruits could be evaluated; they are distinguished from higher sugar contents in the experimental tomatoes compared to controls, no great difference for the other criteria analysed, the latter were generally in accordance with the standardized values of the literature as well as with the applicable national regulations. Ozonisation of irrigation water is an effective and promising alternative method, which improves germination time and increases fruit yield without negatively affecting their marketability.

Key words: chemical properties; ozone; performances; sustainable land use; yield

Gojenje paradižnika zalivanega z ozonirano vodo v razmerah mediteranskega podnebja v Alžiriji

Izvleček: Raziskava predstavlja rezultate poskusa izvedenega na sadikah paradižnika iste sorte, gojenega v mediteranskem podnebju in namakanega z ozonirano vodo, tretirano z ozononom od 0 do 30 sekund. Namen raziskave je bil ugotoviti učinek ozonizirane vode na agronomske in fizikalnokemijske lastnosti plodov. Odziv rastlin paradižnika se je razlikoval med posameznimi obravnavanji in glede na kontrolo. Rezultati so pokazali zgodnejšo kalitev, hitrejšo rast in hitrejši razvoj ter večjo vitalnost obravnavanih rastlin v primerjavi s kontrolo. Povečanje pridelka je bilo proporcionalno povečanju časa ozonizacije vode za zalivanje. V reproduktivni fazi rastlin ni bilo opaženih nobenih negativnih učinkov ozonizacije. Ocenjene fizikalno-kemijske lastnosti plodov so pokazale večjo vsebnost sladkorjev v obravnavanih rastlinah v primerjavi s kontrolo. V ostalih analiziranih parametrih ni bilo večjih razlik v primerjavi s kontrolo in so bile nasplošno v skladu s standardnimi vrednostmi iz literature kot tudi z veljavnimi nacionalnimi standardi. Ozoniranje vode za zalivanje je učinkovita in obetavna alternativna metoda, ki izboljšuje čas kalitve, povečuje pridelek plodov paradižnika brez negativnih učinkov na njihovo tržno vrednost.

Ključne besede: kemijske lastnosti; ozon; uspevanje; trajnostna raba tal; pridelek

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1 INTRODUCTION

The tomato cultivation occupies an important place in the Algerian agricultural economy nearly 33.000 hectares are devoted annually to the cultivation of market garden and industrial tomatoes (MADR – Ministère de l'Agriculture et du Développement Rural, s. d.). its cultivation has adapted to different climates and the production of fresh tomatoes in Algeria has grown steadily over the past ten years, increasing from 64 million tonnes in 2009 to 164 million tonnes in 2020 (FAOSTAT, s. d.), the same for tomatoes intended for processing, they recorded exceptional performances during the year 2019-2020, with a global production of nearly 1.3 million tonne. In Algeria, the industrial tomato has experienced a considerable development in terms of area and production, going from 0.92 million tonne in 2013 to 1.65 million tonne in 2019. This notable performance made it possible to reduce imports and by therefore, to create added value to the national economy (MADR – Ministère de l'Agriculture et du Développement Rural, s. d.). Indeed, Algeria, the largest country in Africa, with an area of 2.4 million km^2 and it opens along 1200 km of coastline on the Mediterranean. The climatic areas are very diverse and the climate varies from the Mediterranean's type to the Saharan's type. On the Mediterranean coast, the summer is hot and dry; the winter is mild and rainy. Total annual rains falls increases along the coast from west to east, but decreases rapidly from the coast southward to the interior. Rains falls decreases after crossing the Atlas Mountains to the south (Algeria | Flag, Capital, Population, Map, & Language, s. d.).

Due to the nutritional qualities and antioxidants contained in tomato such as lycopene, β-carotenes and phenolic compounds, its consumption has been linked to the prevention of several types of cancer, such as breast cancer, coronary cancer, lung cancer, colon cancer and prostate cancer, in addition to protective effects against cardiovascular disease in the form of antiplatelet aggregation (Ajilogba & Babalola, 2016; Cámara et al., 2020). It helps to fight against the development of degenerative human diseases (Ilahy et al., 2018). In addition to these properties, the lycopene contained in tomatoes has been shown to modulate the immune system, stimulate hormones and other metabolic pathways, and regulate cell growth and the induction of detoxifying enzymes. A significant inverse correlation between lycopene levels and systolic and diastolic blood pressure has been observed in hypertensive patients. The tomato contributes to the reduction of hypertension (Medina-Remón et al., 2013) Foods rich in antioxidants reduce the risk of Alzheimer's disease by inhibiting oxidative stress, lycopene intake results in improved memory retention, attenuation of oxidative mitochondrial damage, and reduced neuro-inflammation (Min and Min, 2014; Prakash and Kumar, 2014).

In agriculture, ozone has been used for crop protection against pests that cause bacterial plant wilt and reduce yields, thus causing significant economic losses, it has also been used for foliar treatment, for the decomposition of pesticide residues in soils and to improve the microbiological quality of irrigation water (Díaz-López et al., 2021; Guo et al., 2019; Landa Fernández et al., 2019; Mitsugi et al., 2017). Indeed, ozone (O_3) is an unstable triatomic allotrope of oxygen, its half-life in water at room temperature is about 20 minutes, it is characterized by a high oxidation potential (2.07 volts) higher than that of chlorine (1.36 volts) (Holah et al., 2016). It decomposes rapidly into oxygen without leaving toxic residues or halogenated compounds (Isikber & Athanassiou, 2015; Segat et al., 2014). Due to these properties, ozone is used in various fields, such as drinking water disinfection, the treatment of industrial wastewater, the disinfection of medical equipment, in the food industry, in the agricultural sector, as a substitute for antibiotics and pharmaceuticals, in the textile and paper sector, for post-harvest treatment to increase the shelf life of fresh products stored in warehouses and cold rooms by reducing their enzymatic activities and destroying microorganisms (Remondino & Valdenassi, 2018; Rizzo et al., 2020; Wang et al., 2019; Wei et al., 2017), and in fish, meat and poultry processing plants (Al-Qadiri et al., 2019; Fundo et al., 2018; Habibi Najafi & Haddad Khodaparast, 2009; Zhang et al., 2016).

Ozone does not generate harmful by-products, it can be used in agriculture without any risk of soil and groundwater pollution (Remondino & Valdenassi, 2018), it is a non-thermal and green technique, environmentally friendly and promising for agriculture and the food industry.

The objective of this study is to evaluate the effect of irrigating tomato seedlings with ozonized water OZ1, OZ2 and OZ3 corresponding respectively to different durations of ozonisation of 10 seconds, 20 seconds and 30 seconds on agronomic characteristics (germination time, growth, development and yield) and physicochemical (titratable acidity, ash, soluble dry matter, carbohydrates, total and reducing sugars, etc.) characteristics by comparing them with irrigated plants only with non-ozonized tap water called controls (TW) of the same varietal type and grown under the same conditions.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL DESIGN

Experiments were carried out March-December period, during 2020 in the north-western region of Algeria, exactly in the region of Oran, on tomato seedlings of the same varietal type 'Saint-Pierre' and in undetermined port.

Seeds were sown in March in individual pots filled with potting soil, divided into four batches coded as follows: batch I (OZ1), batch II (OZ2), batch III (OZ3) and finally batch IV (TW), (see Figure 2). No phytosanitary treatment was used throughout the trial period.

Pot referenced as TW in Figure 1(a) was irrigated using tap water only, while pots OZ1, OZ2 and OZ3 were irrigated using tap water exposed to different gaseous ozone duration (OZ1: ozonized water during 10-s, OZ2: ozonized water during 20-s, OZ3: ozonized water during 30-s). Ozone generator FM-C900 (BEYOK ozone, China) was used for aqueous ozone generation for immediate use in the tests, as shown in Figure 1(b). Temperature was controlled during the preparation of the ozonized water. The frequency of irrigation was twice a week for the duration of the experiment.

The first germinations were observed after only five days of sowing for OZ1, OZ2 and OZ3 with the same appearance as the control, on the other hand, the germination of the TW pots was prolonged until the ninth day after sowing, i.e. approximately four days after the experimental plants. The plantlets were transplanted after five weeks of cultivation, in the month of April into large pots, the latter were transplanted deeply at the level of the cotyledons which will allow the development of a very important root system, and will ensure a good anchoring to the ground. All the plants were then staked and placed outdoors in a well-lit and sunny place (Figure 1(a)). Staking maintains the aerial part of the plant and offers better exposure of the leaves to light in addition to limiting losses by breakage of the branches under the weight of the fruits. Monthly average temperatures during the test period fluctuate between 20 °C to 30 °C, as shown in Figure 2, while the average monthly humidity changes between 76 % and 67 % (Nomades, s. d.).

During the crop cycle, the height of the tomato plants was measured, from ground level to the point of insertion of the bouquet, the averages as well as the height of the plants OZ1, OZ2 and OZ3 are compared to the control plants and the trends vegetative revealed, likewise, the following items are reported:

-The colour of the leaves, their length and smell;

-The duration of flowering after transplantation;

-The precocity of germination;

-Growth, vigour and development.

As the fruits ripen, the tomatoes were harvested by hand for each batch, the mass and the numbers of fruits were counted. The yield of the crops was evaluated at the end of the crop.

All ripe fruits were harvested between July and mid-December, corresponding respectively to the 3rd and 8th month after transplanting.

2.2 THE GENERATOR AND ITS OPERATION

To generate ozone by the corona discharge (CD) method, the generator refers to a high-energy electric discharge which, with the passage of a gas flow between

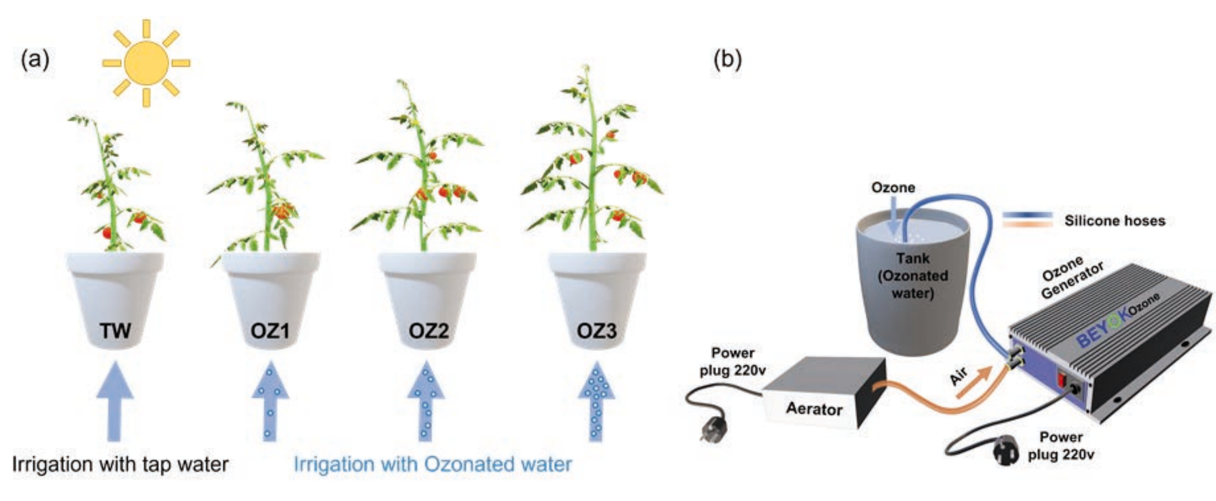

Figure 1: Experimental setup illustrating the trials

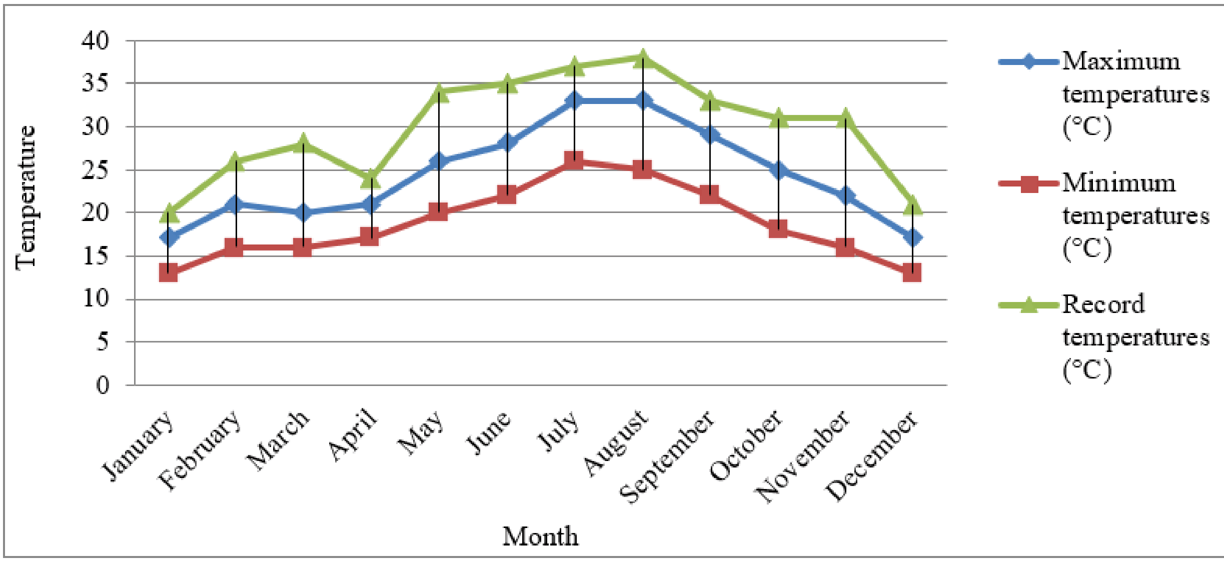

Figure 2: Temperature evolution during the experimental test period of the year 2020 (Nomades, s. d.)

two electrodes with asymmetric geometry and separated by a dielectric material between which a non-symmetrical field (crown) is established, thus causing the dissociation of the oxygen molecules (O_2) into free radicals of oxygen (O:). The latter are unstable, combined with other diatomic oxygen molecules, will form ozone (O_3), as shown in the below equations.

$$O_2 \rightleftharpoons 2[O^{\cdot}]$$

$$2[O^{\cdot}] + 2\,O_2 \rightleftharpoons 2O_3$$

Ozone production varies depending on oxygen concentration in feed gas, dielectric material property, discharge gap, current frequency and voltage. The electrodes are often cooled to remove excess heat and prevent the decomposition of ozone generated (Oner & Demirci, 2016; Pandiselvam et al., 2017).

2.3 SAMPLES PREPARATION AND PHYSICO-CHEMICAL ANALYSIS

The specificity of fresh fruits and vegetables (unprocessed), grown under the same conditions, is their evolutionary heterogeneity, their seasonality, their difference in shape, size, mass, density, growth and development. The analyses carried out in this study give objective indications on the quality of the experimental and control tomatoes and make it possible to compare the batches between them. They also make it possible to observe anomalies related to the quality of horticultural products and to ensure food safety for consumers. Tomato samples were randomly taken from equal quantities of fruit from each lot for chemical analysis. The tomatoes were washed and equilibrated under ambient temperature and pressure conditions, the fruits were cut into pieces and crushed before analysis. The analyses were carried out according to standardized methods in order to assess the quality of tomato fruits through the following parameters:

-The water content determined according to standard NA1133 / 1990;

-The pH measurements at 20 °C were carried out using a digital laboratory pH meter (inoLab pH 7310) according to standard NA 751/1990 corresponding to standard AOAC 981-12

- The titratable acidity according to the method (NA 691 Fiju N ° 3) in accordance with the ISO 750 recommendations;

-The ash contents determined according to standard NA 732/1990 in accordance with standard ISO 3595/1981;

-The soluble dry matter content (MSS) carried out at 20 °C using a refractometer according to ISO 2173-2003;

-Carbohydrate content; the total sugars and the reducing sugars determined by the method of G.Bertrand;

-The sodium chloride content determined according to the standard approved in Algeria, (Inter ministerial decree, Official Journal No. 49, 2013) in accordance with the ISO 3634-1979 standard.

2.4 UNCERTAINTIES AND SHORTCOMINGS

Our research work was carried out in pots and put in outdoor conditions, in a well-lighted sunny place. Throughout the cultivation cycle of the tomato seedlings, irrigation was carried out regularly and periodically in the evening, in order to guarantee a better result and ensure greater efficiency of the effect of ozone on the seedlings.

Gaseous ozone degrades spontaneously, it cannot be stored or transported from one place to another, and therefore it is preferred to be produced in site. The half-life time of ozone in water is related to certain parameters such as pH, temperature, light intensity and others. The pH and temperature control of ozonised water to limit ozone depletion was performed regularly during the preparation of ozonised water and before each use.

Ozone generators require very dry air; air humidity leads to reduced ozone production. The increase in water vapour in the feed gas leads to the formation of a large quantity of nitrogen oxide during electrical discharges. Nitrogen oxides can form nitrogen acids, which on the other hand lead to nitrates as fertilizers. On the other hand, the acids can cause corrosion over a long period. Therefore, an economic and financial evaluation would have been appropriate to assess the costs and benefits and take into account the natural depreciation of the value of the generator to determine the duration of the amortization of the investment.

3 RESULTS AND DISCUSSION

3.1 AGRONOMICS RESULTS

The results obtained following the irrigation of the tomato plants with ozonised water (OZ1, OZ2 and OZ3) and compared to the control plants (TW), are discussed according to the following criteria:

3.1.1 VIGOUR AND HEIGHT OF PLANTS

The height of the tomato plants irrigated with ozonised water was significantly higher than that of the control plants irrigated only with tap water. An increase in vigour in favour of experimental plants was observed. The heights and vigour of experimental tomatoes plants

Figure 3: Photo of tomatoes plants irrigated with: (a) ozonized water at 10-s (OZ1); (b) ozonized water at 20-s (OZ2); (c) ozonized water at 30-s (OZ3) and tap water (d) non-ozonized (TW)

Table 1: Average size of the four lots of experimental and control tomato plants

Designation	Size during transplantation (called Initial Size) (cm)	Size after two months (cm)	Growth from initial size
Control tomato plants (TW)	14	38.21	1.73
Tomato plants OZ1	15	43.08	1.87
Tomato plants OZ2	16.4	49.26	2.004
Tomato plants OZ3	18.8	57.5	2.06

increase with increasing irrigation water ozonisation durations (Figure 3).

The size of the plants for the four batches of tomatoes two months after transplanting reached heights varying between 38 cm and 57cm (see Table 1).

Plant's vigour showed a marked difference between batches (see Figure 3); while for size, growth appears to be favourable to OZ3 and OZ2 plants (Table 1). This heterogeneity in the height and vigour of the plants is due to the irrigation of the crops with ozonized water that favours the oxygenation of the roots. The conversion of ozone into oxygen provides plants and soil with a natural fertilizer since it contributes to the improvement of plant growth. Rozpądek et al. (2015) reported that aqueous ozone treatments on broccoli plants firstly showed accelerated growth and secondly, they reached marketable level faster.

The number of leaves and the leaf area of the experimental plants are higher compared to the control plants (Figure 3). This promotes photosynthesis thus accelerat-

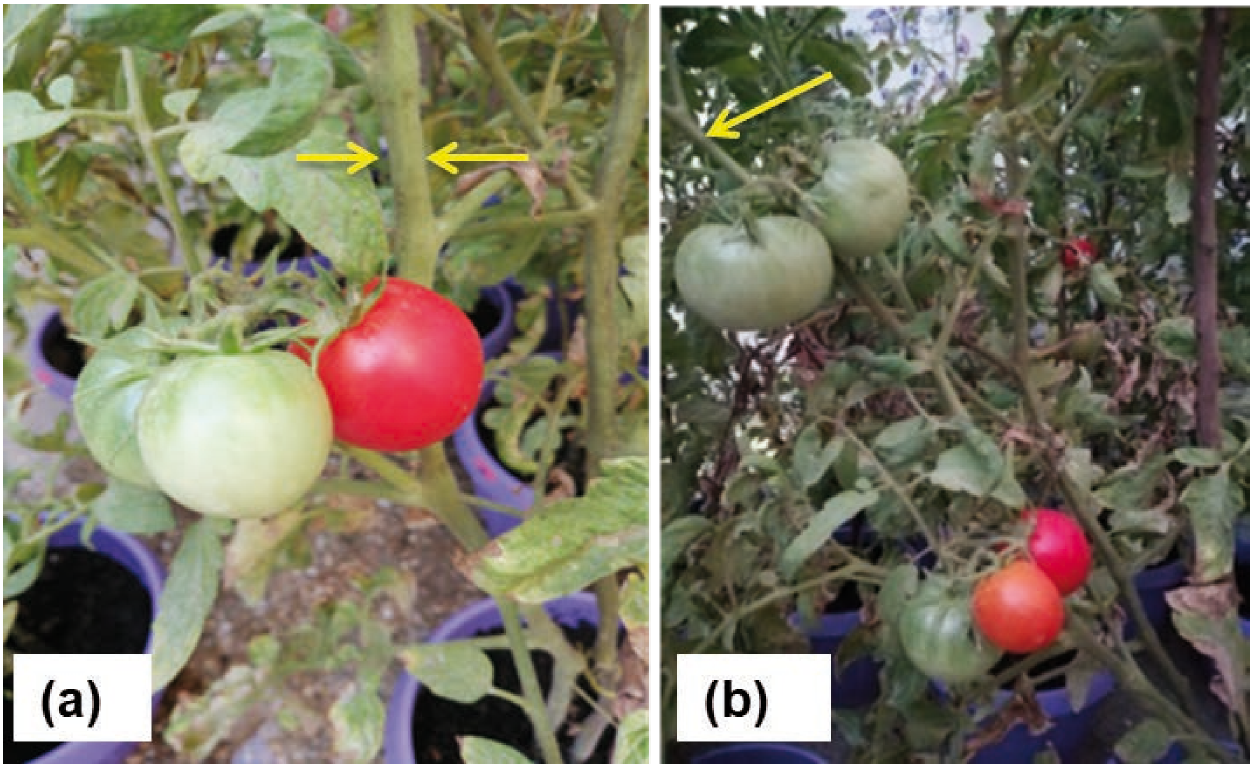

Figure 4: Represents (a): tomatoes plants irrigated with ozonized water; (b): tomatoes plants irrigated with non-ozonized water (control plants)

Figure 5: Photo of a ripe tomato irrigated with ozonized water; (a) The external view shows the persistent calyx with five sepals; (b, c) Longitudinal and transverse sections show that the berry originates from a bicarpellate ovary with axile placentation

ing growth, flowering and leading to an increase in yield. No negative impact was observed on the morphology and quality of fruits irrigated with ozonized water (Figure 4 and 5). These results corroborate with those obtained by Ohashi-Kaneko et al. (2009) , the latter demonstrated the non-toxic effect of ozone in the root system and described that treatment with ozonised water improves root respiration and increases the absorption of nutrients and the biomass production of tomatoes. The work of Martínez-Sánchez & Aguayo (2019) reported that the treatment of irrigation water with ozone improved the growth of pepper seedlings and increased the number of leaves and the development of secondary roots of plants, this facilitates adaptability and improves the yield of seedlings when they are transplanted into fields.

In addition, ozone oxidizes the organic matter present in the soil, inhibits the growth of pathogens in the root zone (Graham et al., 2011). Increases the availability of soil nutrients found in organic matter, improves soil structure and increases the rate of soil aeration. All of this collectively contributes to increase the growth of tomatoes irrigated with ozonised water (Najarian et al., 2018).

All these observations prove the influence of irrigation with ozonized water on tomatoes plants.

The tomato plants showed a difference in stems diameter ranging from 13.24 mm to 18.24 mm for the four batches (see yellow arrow in Figure 4) and no significant difference was observed concerning leaves colour and flowers colour (Figure 4).

3.1.2 PRECOCITY

Plants irrigated with ozonised water showed better precocity than the control plants. The difference in flowering and first harvesting times was significant. For the flowering time, the plants (OZ1, OZ2 and OZ3) flowered 85 days after sowing, on the other hand the control plants (TW) the duration was 100 days after sowing. Likewise for the first harvests, the tomatoes were obtained after 127 days for the OZ1, OZ2 and OZ3 and 142 days for the control tomatoes, i.e. around two-weeks (15 days) of difference compared to the experimental tomatoes. Flowering continued until November (i.e., 7 months after transplanting) for the four lots of tomatoes.

3.1.3 TOTAL YIELD

The total yield of harvested tomatoes fruits per unit area is summarized in Figure 6. The yield of the plants (OZ1, OZ2 and OZ3) is significantly higher compared to the control plants. Results are represented graphically in Figure 6 and indicate that yield increases with increasing the ozonisation time of the irrigation water. The highest

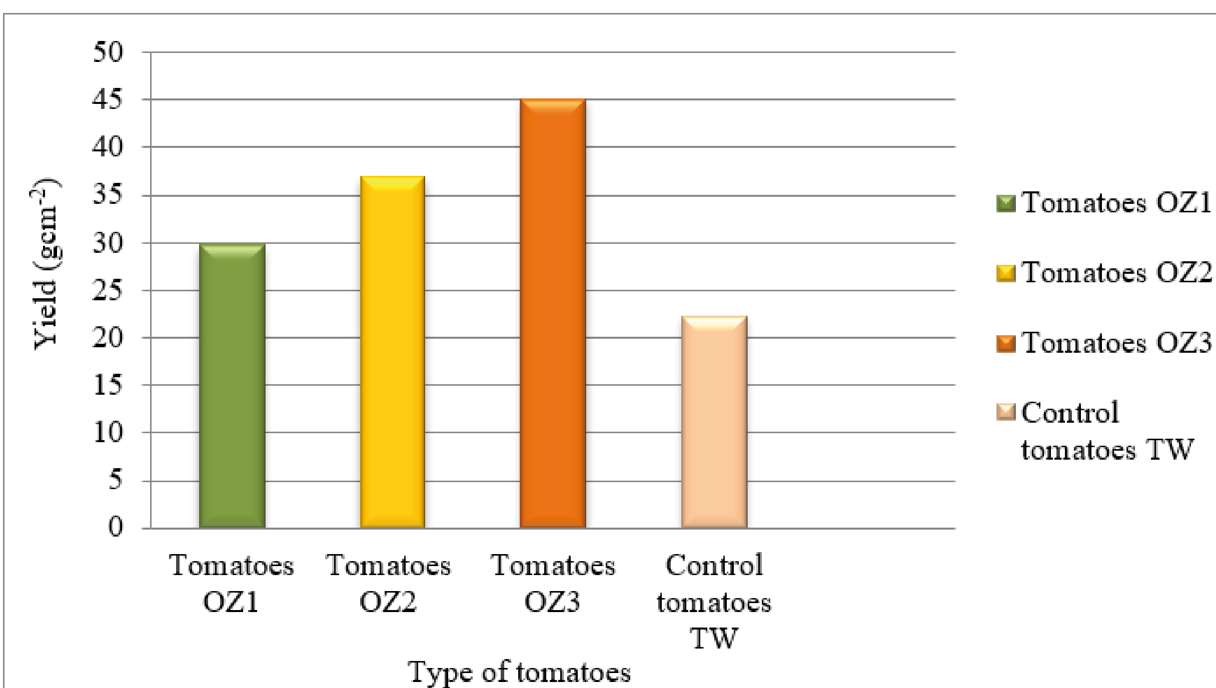

Figure 6: Yield of tomatoes irrigated with ozonized water (OZ1, OZ2 and OZ3) and non-ozonized (TW) water

yield was observed in OZ3 tomatoes with 33.62 % followed by OZ2 tomatoes with 27.57 % then OZ1 tomatoes with 22.19 % and finally 16.62 % for the TW control tomatoes.

The yield of OZ3 tomatoes is significantly higher compared to the other lots (OZ1, OZ2 and TW), it is twice the yield of control tomatoes, and it exceeds the yield of OZ2 tomatoes by 22 %, and the yield of OZ1 tomatoes by 50 %. On the other hand, the OZ2 tomatoes gave a yield of more than 23 % compared to the OZ1 tomatoes and less (-18 %) compared to the OZ3 tomatoes with a ratio of 2 compared to the control tomatoes (Figure 6).

The difference in tomato yield for the three batches (OZ1, OZ2, and OZ3) is proportional to the concentration of the ozonised water.

Plants irrigated with ozonised water were among the best performing plants in terms of germination time, germination rate and survival rate; the latter were higher compared to controls (Figure 3). The early germination, growth, development and flowering of tomato plants OZ1, OZ2 and OZ3 are linked to irrigating with ozonised water; according to the work of (Flores et al., 2019; Terao et al., 2019; Zhuang et al., 2017), ozonised water used at appropriate concentrations, improves tomato plant growth and yield, as well as fruit quality.

3.2 PHYSICOCHEMICAL CHARACTERISTICS OF TOMATOES

The difference in physical and chemical properties between the samples of tomatoes irrigated with ozonised water compared to the samples of control tomatoes is not significant; the obtained values of titratable acidity, pH, soluble solids, ash content and water content are of the same order of magnitude as the control tomatoes (Table 2). The pH values for the four lots of tomatoes studied are in accordance with the standards (pH < 4.90 acceptance standard for fresh tomatoes) and indicate a pH below 4.5 which gives the fruit protection against microorganisms sensitive to acidic pH, it is an advantage from the point of view of sanitary quality. Organic acids are naturally present in the fruits; the contents can vary according to the cultivars and the stage of maturation of the fruit. Total acidity is expressed in relation to citric acid and malic acid. The titratable acidity contents of the experimental tomatoes (OZ1, OZ2 and OZ3) do not show any significant difference compared to the control tomatoes (TW), (Table 2). The fruits analysed are of the same varietal type and were grown under the same conditions. The results obtained are in agreement with those reported by Moresi & Liverotti (2007) who indicated values between 0.3 and 0.5 %. Irrigation with ozonised water does not alter the contents of organic acids in tomato fruit. The citric and malic acid ratio of control tomatoes is slightly higher than that of OZ3 tomatoes followed by OZ1 tomatoes and finally OZ2 tomatoes. Fruit acidity decreases as ripening comes to an end.

By their nature, tomatoes are very rich in water, they contain 93 % to 95 % water (Grasselly et al., 2000), but this content can go up to 97.1 % according to Espiard (2002); the very high water content is a parameter which translates the fragility of the fruits with regard to shocks and limits their suitability for storage at ambient temperature. The moisture content of the tomatoes studied is in agreement with those reported by Grasselly et al. (2000).

The refractometry index makes it possible to measure the fraction of soluble dry matter which is correlated with the sugar content of fruits. The values obtained show a slight difference between the batches. The Brix degree of the OZ1 tomatoes slightly exceeds that of the control tomatoes, followed by that of the OZ2 tomatoes and finally the OZ3 tomatoes. The soluble dry matter content depends on the relative amounts of water and assimilates imported by the fruit during its growth (Grasselly et al., 2000). The values mentioned in table 2 of the four lots of tomatoes studied agree with those recorded by Espiard (2002) which is of the order of 2 to 4.5 °Brix.

The carbohydrate contents of OZ2 and OZ3 tomatoes are similar to each other and slightly higher than those of OZ1 and control tomatoes. The same is for the

Table 2: Physicochemical characteristics of experimental and control tomatoes

					Acidity					
Designation	TSS (°Brix)	Water content (%)	Ashes (%)	pH	Citric acid (%)	Malic acid (%)	Carbohydrate content (%)	Total sugars (%)	Reducing sugars (%)	Sodium chloride content (%)
Tomato OZ1	3.90	95.00	0.452	4.42	0.388	0.372	1.645	0.628	0.563	0.951
Tomato OZ2	3.67	94.39	0.467	4.41	0.416	0.39	2.114	2.88	0.189	1.040
Tomato OZ3	3.57	94.55	0.429	4.40	0.432	0.42	2.186	2.90	0.202	1.0397
Control tomato TW	3.86	94.91	0.519	4.43	0.418	0.40	1.720	0.795	0.74	1.085

total sugar and reducing sugar contents of OZ2 and OZ3 tomatoes, where sugars contents are similar or slightly higher compared to the contents of OZ1 and control tomatoes, this may be due to the increase in the ozonisation time of the irrigation water. The work of Onoue et al. (2018), concluded that the ozonized water treatment of *Brassica rapa* L. allowed the production of high quality vegetables with high contents of soluble sugars and L-ascorbic acid.

The ash content represents the total quantity of mineral salts present in the sample. Referring to Table 2, the results obtained from the four batches of tomatoes analysed are within the 0.3 to 0.5 % range reported by Espiard (2002). For the sodium chloride content, no significant difference was observed between the experimental tomatoes (OZ1, OZ2 and OZ3) and the control tomatoes. The mineral salts contained in the samples studied can contribute to the characterization of a geographical origin, the nature of the soil and the composition of the irrigation water.

In general, the physicochemical parameters of OZ2 and OZ3 tomatoes are slightly elevated compared to the controls while remaining within the standards, this is due to the increase in the ozonisation time of the irrigation water. Irrigation with ozonised water has no negative impact on the tomatoes studied. Overall, all the results obtained have been verified and are consistent with the work carried out on fresh tomatoes by other research.

4 CONCLUSION

The application of ozonised water in agriculture of tomato seedlings grown under a Mediterranean climate at Oran, north-western region of Algeria, was experienced by us for the first time. Knowing that Algeria is known for its diversification of climatic areas where the climate varies from the Mediterranean type to the Saharan type. This research is part of a series of studies which aims to know the impact of different concentrations of ozonised water on the agronomic and physicochemical performance of tomato plants and fruits, the aim of which will be to reduce the use of pesticides and chemical fertilizers while guaranteeing a good yield without altering the marketable quality of the fruits.

The results of our study concluded that the ozonisation of irrigation water applied to tomatoes seedlings of the same varietal type and grown under the same conditions while comparing them to controls led to earlier germination, better development and plant growth, increased vigour and yield. No phytosanitary treatment was used throughout the trial period and no detrimental effects were observed on fruit morphology and productivity. Despite the same growing conditions; the tomatoes plants gave different yields. yield increases with increasing irrigation water ozonisation time.

On the physicochemical level, the experimentation showed some differences between the batches, the sugar contents of the experimental tomatoes were higher compared to the controls and is proportional to the concentration of ozonized water. No significant difference was observed for the other criteria analysed. In general, the ozonisation of irrigation water is a promising technique for agriculture, environmental and economic challenges, thus allowing reducing pesticides, increasing qualitatively and quantitatively of crop yield and to provide consumers with healthy horticultural products. The economical profit gained by our method compensating the long-time damages to instruments.

The results obtained encourage the use of this technique by farmers in order to improve agronomic performance such as germination time, growth, vigour and yield of tomato plants and to ensure a good product that complies with health standards for consumers and exporters. Farmers can adopt this easy-to-use, low-cost technique, and thanks to renewable energy, ozone generators can be used even in remote areas of the country. In addition, ozone has proven its effectiveness and can be used as a substitute for pesticides, insecticides and fungicides. This could provide a basis for policy makers and legislators to curb the overuse of chemical fertilizers and pesticides that have deleterious effects on market garden produce.

Our research work required simple tools, pots in outdoor conditions. Greenhouse and open field conditions on a large number of samples and a large cultivated area are to be expected in our future research, this will make it possible to study on the one hand the evolution of plants, to evaluate the impact of the different concentrations of ozonised water on the plants and the fruits and on the other hand, to define the advantages and the disadvantages of each cultivation mode and compare the results obtained in order to select the best techniques from the point of view of earliness, yield and fruit quality. Cross tests of ozonised water and compost will also be recommended for the rest of the work. The compost formed from the remains of cultivation, kitchen scraps as well as fruit and vegetable peelings; is a rich source of trace elements and nutrients necessary for the development of tomato plants; likewise for poultry manure, it is generally used for acidic soils because of its high calcium content. Ozone oxidizes organic materials, decreases their toxicity and increases their biodegradability. It decomposes quickly into oxygen without generating toxic residues, hence its use in agriculture without any risk of soil and groundwater pollution.

The combination of ozone and compost can improve the composition of the soil and reduce the extensive use of chemical fertilizers and pesticides and thus open up prospects for a circular economy through the recycling of organic waste for sustainable agriculture and respectful of the environment.

Physicochemical, morphological, bacteriological, sensory and nutritional analyses will be recommended to assess the efficiency of the cultivation techniques used.

Studies must be continued in order to extend the use of ozone in the cultivation of cereals, vineyards, rice and coffee and even in the pharmaceutical industry. However, ozone concentrations must be determined for safe and effective use.

5 ACKNOWLEDGEMENTS

The authors would like to thank Professor Adil ALI OTHMAN, professor at the University of Sciences and Technology of Oran USTO-MB for the time granted, his patience, his help and his advice, many of his constructive comments have made it possible to improve this study.

6 FUNDING STATEMENT

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors that could be construed as a conflict of interest or that might have appeared to influence the work reported in this paper.

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doi:10.14720/aas.2023.119.1.2611 Original research article / izvirni znanstveni članek

Phenotypic diversity of date palm varieties (*Phoenix dactylifera* L.) from southwest Algeria estimated by fruit characteristics

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Received March 13, 2022; accepted December 13, 2022.
Delo je prispelo 13. marca 2022, sprejeto 13. decembra 2022

Phenotypic diversity of date palm varieties (*Phoenix dactylifera* L.) from southwest Algeria estimated by fruit characteristics

Abstract: This study aimed to identify the genetic diversity of date palm (*Phoenix dactylifera* L.) in the southwest of Algeria (wilaya of Adrar), as part of the Algerian date palm varieties conservation. The morphological characterization was adopted in terms of quality and quantity of 26 varieties. The main results showed a considerable genetic diversity of date palms in this agricultural territory. 7 cultivars counted in danger of extinction. The cluster analysis brought out significant differences between qualitative and quantitative traits. However, the cultivars of 'Deglet Talmine', 'Maatouk', and 'Timidouele' produced dates of high quantitative traits, however, of different qualitative traits. Also, some relationships were observed within clusters for other varieties. A variety named 'Khalt' was able to produce dates better than many cultivars. This work would help to know the focal traits of the plant genetic resources of date palm and preserve that are in extinction.

Key words: Adrar; cluster analysis; cultivar; dates; *Phoenix dactylifera* L.; qualitative characterization; quantitative characterization; variety

Fenotipska raznolikost sort dateljeve palme (*Phoenix dactylifera* L.) v jugozahodni Alžiriji, ocenjena na osnovi lastnosti plodov

Izvleček: Namen raziskave je bil določiti genetsko raznolikost dateljeve palme (*Phoenix dactylifera* L.) v jugozahodni Alžiriji (provinca Adrar), kot del programa ohranjanja alžirskih sort dateljeve palme. Morfološka opredelitev 26 sort je bila narejena na osnovi kakovostnih in količinskih parametrov. Glavni izsledki so pokazali, da je na tem kmetijskem območju precejšnja genetska raznolikost dateljeve palme. Sedem sort je bilo opredeljeno kot ogroženih pred izumrtjem. Klasterska analiza je pokazala značilne razlike med kakovostnimi in količinskimi lastnostmi sort. Sorte 'Deglet Talmine', 'Maatouk', in 'Timidouele' so bile najbolj rodovitne, a z različnimi kakovostnimi lastnostmi. Znotraj skupin sort so bile opažene še nekatere druge lastnosti. Tako je bila sorta 'Khalt' najrodovitnejša. Izsledki te raziskave bodo pomagali prepoznati ciljne lastnosti v naboru sort dateljeve palme za njihovo ohranjanje.

Ključne besede: Adrar; klasterska analiza; sorta; *Phoenix dactylifera* L.; kakovostna in količinska oznaka sort

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1 INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is a centuries-old crop plant farmed in many desert regions of the world, particularly in West Asia and North Africa (Al-Yahyai & Mumtaz Khan, 2015).

Its culture is perennial and classified under the genus *Phoenix*, a monocotyledonous family Palmae with 36 chromosomes ($2n = 36$) (Chao & Krueger, 2007). *Phoenix* species are dioecious, with offshoots being the most common propagation method; however, tissue culture and seed are other viable options. Propagation via seeds produces new genotypes or forms of date palm, which are the primary source of date palm variety (Elhoumaizi et al., 2002).

Around 3000 date palm cultivars have been identified worldwide (Khierallah et al., 2015) in which 940 cultivars were recorded in Algeria (Hannachi et al., 1998).

However, Algeria is known for its noble variety 'Deglet Noor', which is considered a crop of nutritional quality (Debabeche et al., 2021) with great socio-economic importance 54 % of the total production. It occupies the largest cultivated areas in Ziban, Oued Souf, and Oued Righ (MARDF, 2021).

Adrar is the fourth-largest producer of dates in the country, with an annual production of 93568.1 tons in 2020, where there are a number of 3798579 date palms (MARDF, 2021).

This rank has decreased mainly due to the extension of the adverse biotic threats. The vascular attack of *Fusarium oxysporum* f.sp. *albedinis* W.L. Gordon, (1965) named the bayoud, which is considered the most dangerous, is widespread in multiple palm groves of the municipalities of the wilaya also, the anthropogenic and abiotic threats are well noted in this region. Drought is one of the most important environmental stresses causing a significant drop in crop productivity (Guettouchi et al., 2017; Sarwat & Tuteja, 2017). So, adopting a strategy to preserve and restore the plant genetic resources of the date palm is necessary for the state and scientists.

A previous survey on identifying genetic diversity of Algerian date palm female cultivars employing morphological description was done in the southeast Algerian territory. Simozrag et al.(2016) and Bedjaoui & Benbouza (2020) distinguished 96 cultivars in the wilaya of Biskra based on the morphological traits of the vegetative and the fruit part of the date palm. Another morphological identification was made on 175 cultivars in Oued Righ and 56 in Oued Souf (Acourene et al., 2007).Guettouchi et al. (2017) described 19 cultivars in the wilaya of Boussada and Simozrag & Laiadi (2020) characterized 80 minor cultivars in the wilayas of Biskra and Oued Righ using molecular traits.

The characterization combining the morphological, biochemical, and molecular traits of trees provides definite genetic information to species (Kameswara Rao, 2004) and preserve the genetic heritage of plants (Al-Yahyai & Mumtaz Khan, 2015). One of the most prevalent approaches for identifying date palm diversity is to use morphological criteria wherein the traits related to the vegetative or the fruit organs have been highly praised for date palm characterization (Elhoumaizi et al., 2002b).

In fact, phenotypic diversity is a visible sign of date palm variety. It depicts the interaction impact between genetic variation and the environment (Jaradat, 2011). Considering that this study has been engaged through the investigation and participation in the genetic identification of the endogenous female accessions of Southwest Algeria.

Therefore this work aimed to characterize some of the fruit morphological traits which are accessible to the observer being part of the diversity identification and conservation of the Algerian date palm varieties existing in the wilaya of Adrar and to enrich date palm germplasm and develop a suitable breeding strategy.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

The research was conducted on the fruit of twenty six Algerian date palm varieties located in Algeria's southwest (wilaya of Adrar), between 27 ° 52 ′ 00 ″ N and 0 ° 17 ′ 00 ″ W, with an altitude estimated at 279 m. Adrar has a hot desert climate characterized by a mean annual temperature of 26.7 °C which means annual rainfall barely reached 16.26 mm in 2020.

Specific information on local names of date palm varieties, their type of reproduction, distribution, availability of their fruit in the market, and socio-economic value were carried out. A panel of expert provided it from Ben Abbes station of scientific and technical research center on arid regions and the farmers in the wilaya of Adrar (Table 1).

2.2 SAMPLING AND ANALYSIS

All the varieties are selected in palm groves of five municipalities of Adrar (Figure 1).The tree's sampling on each variety depended on their availability from one grove to another, and from 20 groves studied in each municipality at least 3 replicates were taken from each variety except for 'Khalt'. Twenty fruits from different bunches of each date palm tree were randomly sampled at the

Table 1: Identification data of the cultivars studied

Varieties	Code	Status of reproduction	Distribution	Harvest	Appreciation	Marketing
Abbad	ABB	Cultivated	Frequent	August	Excellent	Important
Abd Salam	ASA	Cultivated	Rare	October	Excellent	Important
Adam Bara	ABA	Cultivated	Rare	October	Excellent	Important
Adam Bola	ABO	Cultivated	Frequent	October	Excellent	Important
Adam Lebled	ALD	Cultivated	Frequent	August	Excellent	Important
Adam Mani	AMN	Cultivated	Frequent	October	Good	Medium
Adam Osalem	AOS	Cultivated	Infrequent	October	Medium	Medium
Cherka	CHE	Cultivated	Frequent	October	Good	Medium
DegletTalmine	DTN	Cultivated	Frequent	October	Medium	Important
El Mabrouka	ELM	Cultivated	Infrequent	October	Good	Medium
Feggous	FEG	Cultivated	Frequent	October	Excellent	Important
Hartane	HAR	Cultivated	Frequent	November	Excellent	Important
Hemira	HEM	Cultivated	Frequent	October	Good	Important
Hija	HIJ	Cultivated	Frequent	August	Excellent	Important
Khalt	KHL	Seedling	Unique	October	Low	Low
Maatouk	MAA	Cultivated	Frequent	October	Good	Important
Tademama	TDM	Cultivated	Frequent	October	Medium	Low
Takerbouchte	TKB	Cultivated	Frequent	December	Excellent	Important
Taorakhet	TAR	Cultivated	Rare	October	Low	Low
Tazerza	TZR	Cultivated	Abundant	October	Medium	Low
Timjohar	TMJ	Cultivated	Frequent	October	Good	Medium
Timebadda	TBD	Cultivated	Frequent	September-October	Good	Important
Timeliha	TML	Cultivated	Frequent	October	Medium	Medium
Timidouele	TMD	Cultivated	Frequent	October	Medium	Low
Tinaser	TNS	Cultivated	Frequent	October	Low	Important
Tinizioua	TNZ	Cultivated	Frequent	October	Medium	Low

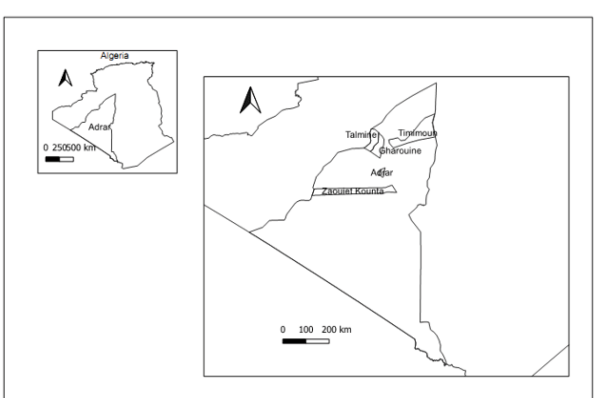

Figure 1: Location map showing the collected date palm varieties in the wilaya of Adrar

fully mature stage. The study was designed on different analyses on a phenotypic basis. The main traits were derived from the descriptor list for the date palm (IPGRI et al., 2005), concerning the fruit, including eight quantitative traits: fruit and seed sizes (cm), fruit and seed mass (g), and ratios of length and mass of the seed on their fruit also, the dates were identified by qualitative traits: the fruit shape, the fruit peel colour, the fruit consistency and the flesh texture.

2.3 STATISTICAL ANALYSIS

Correlations between qualitative and quantitative traits of the fruit were obtained with significance

$p < 0.05$. Cluster analysis was run for grouping cultivars that showed dissimilarity in several traits. Clustering was performed on eight quantitative fruit traits and four qualitative traits for everyone, using the Euclidean distance matrix and the un-weighted pair group method with arithmetic average (UPGMA). The qualitative traits were scored based on principal coordinates after doing Correspondence Analysis (CA). All statistical analyses were executed using PAST software (Hammer et al., 2001).

3 RESULTS

3.1 CORRELATION PLOT

The correlation plot confirmed that the most qualitative and quantitative traits studied are significantly correlated in the positive sense (Figure 2).

Fruit mass was positively and highly correlated with the fruit size (fruit length and diameter) and the fruit shape as fruit length had a positive correlation with seed length and fruit mass and peel colour of the fruit, and both were negatively correlated with the seed/fruit length and mass ratios. Fruit diameter was positively correlated with fruit's mass and shape and negatively correlated with the seed/fruit length and mass ratios. Otherwise fruit shape was negatively correlated with the seed/fruit mass ratio. Positive correlation was marked between fruit consistency and flesh texture and another positive correlation between seed size and seed mass.

3.2 QUALITATIVE PHENOTYPIC TRAITS

The visual observation traits are revealed in Table 2. Qualitative analysis of the dates showed remarkable phenotypic diversity in the shape, colour, consistency, and texture of dates. 65.38 % appeared of subcylindrical shape, 26.92 % were cylindrical, 11.54 % were triangular, 7.69 % were ovate elongated and subspherical, and 3.85 % was spherical. Most dates in maturity were dark in colour: 88.46 % pigmented red to black, and the rest were yellow to amber. Many dates were characterized by consistency from semi-dry to soft, 42.31 % for each one and 15.39 % were dry. However, 53.85 % of dates were of chewiness texture, followed by a cohesiveness texture with 30.77 % and hardiness with 15.38 %.

3.3 QUALITATIVE CLASSIFICATION OF DATE PALM FRUIT

Euclidean distance was used to estimate the phenotypic dissimilarity of the 26 varieties. Dissimilarity levels ranged from 0 to 2.50, determining the variety groups qualitatively related (Figure 3). The cluster analysis of qualitative traits resulted in two main clusters. The first

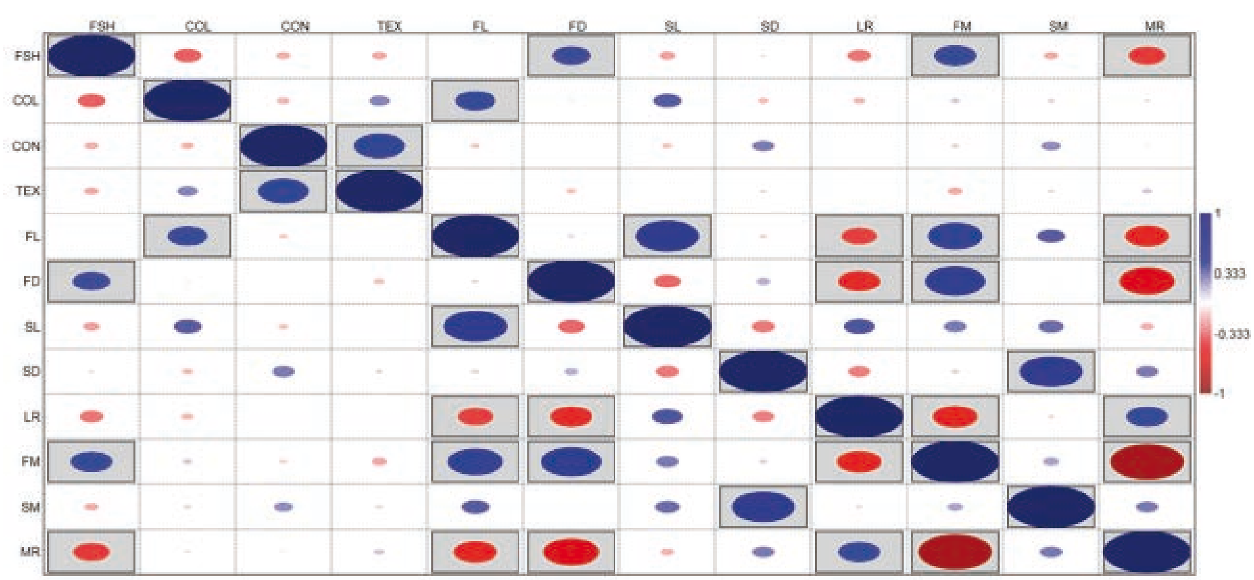

Figure 2: Correlation plot between qualitative and quantitative fruit traits. FSH - fruit shape, COL – peel colour, CON - fruit consistency, TEX - flesh texture, FL - fruit length, FD - fruit diameter, SL - seed length, SD - seed diameter, LR - seed/fruit length ratio, FM - fruit mass, SM- seed mass, MR - seed/fruit mass ratio

one is divided into three distinct sub-clusters: dates of 'Hartane', 'Maatouk', 'Timjohar', 'Tazerza', 'Feggous', 'Takerbouchte', 'Taorakhet', 'Tademama', 'El Mabrouka' and 'Adam Bola' grouped in a sub-cluster shared a dark colour of peel and soft consistency. They are characterized by an irregular shape and a chewiness and cohesiveness texture. The second sub-cluster contained 'Abbad', 'Tinizioua', 'Timidouele', 'Hija', 'Adam Lebled', 'Adam Bara', 'Timeliha', and 'Abd Salam' were very close, distinguished by the elongated shape of the fruit and colour shades from light to dark. They were similar in the semi-dry consistency and chewiness texture. The third sub-cluster recorded 'Timebadda', 'Cherka', 'Hemira' and 'Adam Mani', which had independence in fruit shape and peel colour between light and dark. Their dates were of semi-dry consistency and cohesiveness texture.

The second cluster was limited to only 'Adam Osalem', 'Khalt', 'Deglet Talmine', and 'Tinaser' entire of dry consistency and hardy texture compared to the other cluster. Their dates were not necessarily elongated, distinguished by the colour shades from light to dark.

3.4 QUANTITATIVE PHENOTYPIC TRAITS

The mean values and standard deviation of quantitative traits are reported in Tables 3 and 4.

Concerning the size, 61.54 % of dates seemed small

Table 2: Qualitative traits of fruit of date palm varieties

Varieties	Fruit shape	Colour of fruit peel	Fruit consistency	Flesh texture
Abbad	Subcylindrical and cylindrical	Amber-Brown	Semi-dry	Chewiness
Abd Salam	Subcylindrical	Yellowish orange-Dark orange-Garnet	Semi-dry	Chewiness
Adam Bara	Ovate elongated	Yellowish brown-Brown	Semi-dry	Chewiness
Adam Bola	Subcylindrical	Amber-Dark red	Soft	Cohesiveness
Adam Lebled	Subcylindrical	Brown	Semi-dry	Chewiness
Adam Mani	Subspherical	Brown	Semi-dry	Cohesiveness
Adam Osalem	Subcylindrical	Brown	Dry	Hardiness
Cherka	Triangular	Yellowish brown	Semi-dry	Cohesiveness
Deglet Talmine	Subcylindrical and triangular	Brown	Dry	Hardiness
El Mabrouka	Triangular	Reddish brown with mustard yellow	Soft	Chewiness
Feggous	Cylindrical	Garnet	Soft	Chewiness
Hartane	Cylindrical and subcylindrical	Red	Soft	Chewiness
Hemira	Subcylindrical	Dark red-Garnet	Semi-dry	Cohesiveness
Hija	Subcylindrical	Yellowish brown-Red	Semi-dry	Chewiness
Khalt	Subcylindrical	Blonde yellow-Brown	Dry	Hardiness
Maatouk	Ovate elongated and triangular	Garnet with dandelion yellow	Soft	Chewiness
Tademama	Cylindrical	Black	Soft	Cohesiveness
Takerbouchte	Spherical	Garnet	Soft	Cohesiveness
Taorakhet	Subcylindrical	Black	Soft	Cohesiveness
Tazerza	Subcylindrical	Garnet	Soft	Chewiness
Timjohar	Subcylindrical	Black	Soft	Chewiness
Timebadda	Subcylindrical and subspherical	Honey yellow	Semidry	Cohesiveness
Timeliha	Subcylindrical	Darkred–Darkbrown	Semi-dry	Chewiness
Timidouele	Cylindrical	Brown-Garnet	Semi-dry	Chewiness
Tinaser	Cylindrical and subcylindrical	Yellowish brown	Dry	Hardiness
Tinizioua	Cylindrical	Brown-Garnet	Semi-dry	Chewiness

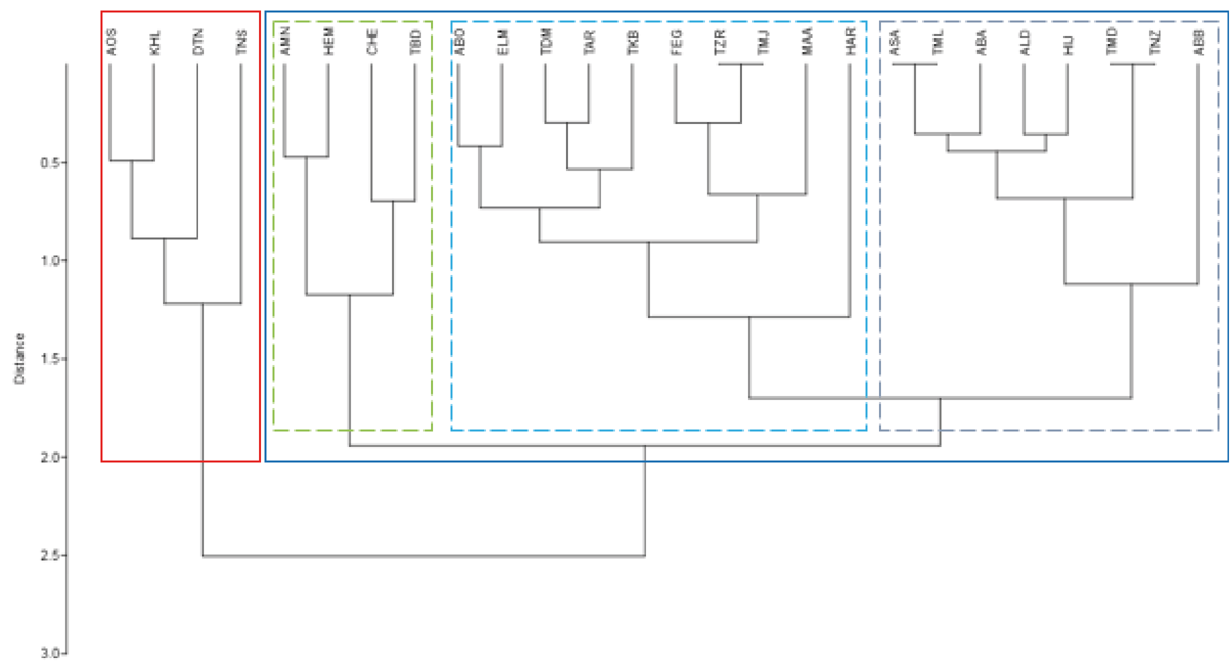

Figure 3: Hierarchical clustering of Algerian date palm fruit-based on four qualitative traits

(3 < *l* < 4 cm) as demonstrated by 'Abbad', 'Abd Salam', 'Adam Lebled', 'Adam Mani', 'Adam Osalem', 'Cherka', 'Feggous', 'Hija', 'Tademama', 'Takerbouchte', 'Timjohar', 'Timebadda', 'Timeliha', 'Tinaser', 'El Mabrouka' and 'Tazerza', followed by a rate of 34.61 % that were medium-sized (4.1 < *l* < 5 cm): 'Adam Bara', 'Adam Bola', 'Deglet Talmine', 'Hartane', 'Hemira', 'Khalt', 'Maatouk', 'Taorakhet', 'Tinizioua' and only 3.85 % represented by 'Timidouele' was large-sized (5.1 < *l* < 6cm). Traits in relation to fruit and seed length were shown that most dates represent a moderate size (0.5 < *r* < 0.67) except for 'Timidouele', which appears good (r < 0.5), and 'Timeliha' was bad (r > 0.67) (Table 3).

Table 4 revealed that 73.08 % of the dates had a good weight (*m* > 8 g) illustrated by 'Abd Salam', 'Adam Bara', 'Adam Bola', 'Adam Lebled', 'Adam Osalem', 'Cherka', 'Deglet Talmine', 'El Mabrouka', 'Feggous', 'Hartane', 'Hemira', 'Khalt', 'Maatouk', 'Takerbouchte', 'Taorakhet', 'Tazerza', 'Timebadda', 'Timidouele' and 'Tinizioua'. 23.08 % were moderate (6 < *m* < 8 g): 'Adam Mani', 'Hija', 'Tademama', 'Timjohar', 'Timeliha', 'Tinaser' and 3.85 % represented by 'Abbad' was of poor weight (*m* < 6 g). Traits concerning fruit and seed weight reported that 69.23% of the dates presented a ratio higher than 0.10, an indicator of a large seed volume relative to the fruit.

3.5 QUANTITATIVE CLASSIFICATION OF DATE PALM FRUIT

Euclidean distance was used to estimate the phenotypic dissimilarity of the 26 varieties. Dissimilarity levels ranged from 0.750 to 5.018, determining the accession groups quantitatively related (Figure 4). The cluster analysis of quantitative traits resulted in three main clusters. In the first cluster, close relationships were observed among 'Deglet Talmine', 'Maatouk' and 'Timidouele'. These dates record high traits of size and mass. However, 'Timeliha' in the second cluster was highly different from the two groups generated. Their dates presented very poor quantitative traits because of the high seed length. The third cluster was more grouped in distinct sub-clusters displaying the varieties with medium traits of fruit: 'Adam Bara', 'Adam Lebled', 'Hemira', 'Khalt', 'Hartane', 'Taorakhet', 'Adam Bola', which were very closely having the best quantitative traits in this sub-cluster. 'Tinizioua', 'Tazerza', 'Cherka', 'Timjohar', 'Tinaser' and 'Adam Osalem' were grouped in a sub-cluster, particular for dates of high seed mass, and 'Abbad', 'Tademama', 'Hija', 'Abd Salam', 'Takerbouchte', 'Timebadda' and 'Adam Mani' were grouped in another which seems poor. 'El Mabrouka' and 'Feggous' were clustered and appeared moderate due to their dates' high seed diameter and seed mass.

Table 3: Biometric traits of fruit of date palm varieties

Varieties	Fruit length (cm)	Fruit diameter (cm)	Seed length (cm)	Seed diameter (cm)	Seed / fruit length ratio
Abbad	3.59 ± 0.24	2.07 ± 0.11	2.09 ± 0.14	0.73 ± 0.02	0.58 ± 0.00
Abd Salam	3.78 ± 0.18	2.29 ± 0.06	1.95 ± 0.04	0.82 ± 0.01	0.52 ± 0.02
Adam Bara	4.27 ± 0.20	1.68 ± 0.67	2.71 ± 0.14	0.80 ± 0.03	0.64 ± 0.05
Adam Bola	4.47 ± 0.18	2.12 ± 0.10	2.62 ± 0.12	0.76 ± 0.03	0.59 ± 0.03
Adam Lebled	3.85 ± 0.04	2.38 ± 0.11	2.40 ± 0.03	0.83 ± 0.05	0.62 ± 0.01
Adam Mani	3.16 ± 0.14	2.16 ± 0.05	1.76 ± 0.07	0.92 ± 0.02	0.56 ± 0.04
Adam Osalem	3.94 ± 0.05	1.79 ± 0.03	2.33 ± 0.09	0.90 ± 0.08	0.59 ± 0.02
Cherka	3.74 ± 0.25	2.16 ± 0.10	2.04 ± 0.12	0.96 ± 0.05	0.55 ± 0.03
Deglet Talmine	4.52 ± 0.20	2.81 ± 0.17	2.30 ± 0.21	0.87 ± 0.04	0.51 ± 0.06
El Mabrouka	4.02 ± 0.27	2.17 ± 0.25	2.25 ± 0.13	1.18 ± 0.45	0.56 ± 0.01
Feggous	3.91 ± 0.28	2.27 ± 0.11	2.11 ± 0.09	1.08 ± 0.29	0.54 ± 0.02
Hartane	4.46 ± 0.13	2.09 ± 0.05	2.53 ± 0.03	0.84 ± 0.04	0.57 ± 0.01
Hemira	4.36 ± 0.19	2.13 ± 0.10	2.43 ± 0.17	0.89 ± 0.12	0.56 ± 0.06
Hija	3.49 ± 0.07	2.02 ± 0.15	2.06 ± 0.10	0.70 ± 0.04	0.59 ± 0.02
Khalt	4.45 ± 0.51	2.06 ± 0.23	2.61 ± 0.24	0.82 ± 0.08	0.59 ± 0.05
Maatouk	4.19 ± 0.06	2.33 ± 0.02	2.19 ± 0.10	0.88 ± 0.05	0.52 ± 0.02
Tademama	3.49 ± 0.08	2.08 ± 0.45	2.14 ± 0.02	0.82 ± 0.02	0.61 ± 0.02
Takerbouchte	3.10 ± 0.09	2.51 ± 0.17	1.90 ± 0.06	0.88 ± 0.02	0.61 ± 0.03
Taorakhet	4.37 ± 0.15	2.10 ± 0.10	2.54 ± 0.05	0.78 ± 0.04	0.58 ± 0.01
Tazerza	4.03 ± 0.03	2.02 ± 0.07	2.11 ± 0.09	0.89 ± 0.05	0.52 ± 0.03
Timjohar	3.85 ± 0.19	1.57 ± 0.57	2.23 ±0.13	0.90 ± 0.07	0.58 ± 0.03
Timebadda	3.32 ± 0.01	2.18 ± 0.16	1.88 ± 0.08	0.89 ± 0.04	0.57 ± 0.02
Timeliha	3.70 ± 0.43	1.85 ± 0.16	2.64 ± 0.06	0.82 ± 0.11	0.72 ± 0.08
Timidouele	5.10 ± 0.26	2.30 ± 0.10	2.42 ± 0.03	0.81 ± 0.01	0.47 ± 0.03
Tinaser	3.91 ± 0.19	1.86 ± 0.03	2.28 ± 0.05	0.80 ± 0.03	0.58 ± 0.02
Tinizioua	4.36 ± 0.16	2.14 ± 0.01	2.35 ± 0.09	0.88 ± 0.01	0.54 ± 0.02

4 DISCUSSION

The morphological traits of dates present the identity of date palms at the harvest moment. This research was conducted to discover more phenotypic diversity between qualitative and quantitative traits among the investigated varieties. Positive significant correlations were showed between date traits where distinction between varieties has been carried out. Previous similar results were found by Bedjaoui & Benbouza (2020) regarding the positive correlation between fruit mass and seed and fruit length.

Discrimination between varieties highly appeared in qualitative terms for the fruit consistency and texture. This diversity is due to plant material's ability to benefit from a significant genotypic heterogeneity especially as the study region is spread over a large area.

But it could also be due to environmental factors (Jaradat, 2011), which include differences in hydro-edaphic factors and crop management, affecting the physico-chemical contents and also the mechanical and rheological traits of the fruit (Ismail et al., 2006) as these factors have an influence on quantitative traits. It should be noted that since the mode of cultivation in the wilaya of Adrar is multi-variety, no special cultivation practice for any variety of date palm can be recorded.

Our findings appeared similar to those of Simozrag & Laiadi (2020) where they studied the genetic diversity based on nuclear microsatellite markers. Significant diversity was recorded between 'Cherka' and 'Taker-

Table 4: Mass traits of fruit of date palm varieties

Varieties	Fruit mass (g)	Seed mass (g)	Seed/fruit mass ratio
Abbad	5.99 ± 1.06	0.73 ± 0.13	0.12 ± 0.01
Abd Salam	8.90 ± 1.23	0.86 ± 0.11	0.10 ± 0.02
Adam Bara	10.38 ± 1.06	1.03 ± 0.22	0.10 ± 0.02
Adam Bola	11.85 ± 0.65	0.88 ± 0.12	0.07 ± 0.01
Adam Lebled	10.49 ± 0.28	0.92 ± 0.22	0.09 ± 0.02
Adam Mani	7.36 ± 0.59	0.93 ± 0.07	0.13 ± 0.00
Adam Osalem	8.31 ± 0.24	0.88 ± 0.10	0.11 ± 0.01
Cherka	8.47 ± 0.81	1.10 ± 0.19	0.13 ± 0.02
Deglet Talmine	22.08 ± 1.49	0.94 ± 0.18	0.04 ± 0.01
El Mabrouka	10.53 ± 2.21	1.23 ± 0.26	0.12 ± 0.01
Feggous	10.66 ± 2.45	1.17 ± 0.29	0.11 ± 0.00
Hartane	10.72 ± 0.92	1.15 ± 0.14	0.11 ± 0.00
Hemira	11.52 ± 0.80	1.02 ± 0.01	0.09 ± 0.01
Hija	7.96 ± 2.13	0.59 ± 0.12	0.08 ± 0.02
Khalt	9.90 ± 3.35	1.09 ± 0.26	0.11 ± 0.02
Maatouk	14.30 ± 0.88	0.93 ± 0.14	0.06 ± 0.01
Tademama	6.53 ± 0.43	0.81 ± 0.06	0.12 ± 0.00
Takerbouchte	9.74 ± 1.12	0.94 ± 0.05	0.10 ± 0.01
Taorakhet	11.74 ± 1.45	0.95 ± 0.11	0.08 ± 0.01
Tazerza	9.52 ± 0.32	1.04 ± 0.06	0.11 ± 0.00
Timjohar	6.15 ± 0.33	0.94 ± 0.06	0.15 ± 0.00
Timebadda	8.57 ± 1.37	0.87 ± 0.10	0.10 ± 0.00
Timeliha	6.60 ± 0.73	1.00 ± 0.23	0.15 ± 0.02
Timidouele	14.63 ± 1.61	0.87 ± 0.10	0.06 ± 0.00
Tinaser	6.66 ± 0.75	0.88 ± 0.09	0.13 ± 0.01
Tinizioua	9.02 ± 0.44	1.07 ± 0.13	0.12 ± 0.02

bouchte' varieties and a great diversity between 'Timeliha', 'Hartane', 'Tinaser', 'Deglet Talmine', 'Timidouele' and 'Hemira' varieties.

It is possible that this diversity may be owing to commercial consideration which is mainly due to the appreciating varieties at the expense of other varieties (Table 1). For some cases, soft consistency negatively affects the demand for the fruit and its cultivation. It is only because of the risk of damage to the fruit. It concerned mainly the dates of 'Tazerza', 'Tademama' and 'Taorakhet' varieties in addition, chewiness texture even in dates of semi-dry consistency has the same effect on the dates of 'Timidouele' and 'Tinizioua' varieties (Table 1),which are proved by Acourene et al.(2007) who indicated that 'Tazerza' variety threatened with erosion in Oued Righ.

However, the dry consistency and hardiness texture of the dates are less appreciated than other types but 'Deglet Talmine' and 'Tinaser' varieties are the most distinguished ones (Table 1) due to the demand from neighboring states.

Low production of young shoots throughout the date palm cycle for cultivars of 'Abd Salam' and 'Adam Bara' is considered a major factor in the genetic decline of date palm.

5 CONCLUSIONS

This study revealed an immense wealth of the date palm genetic resources in southwest Algeria. Biodiversity analyses using fruit qualitative and quantitative traits showed a high degree of diversity among date palm varieties.

The cultivars of 'Deglet Talmine', 'Maatouk' and 'Timidouele' produced dates of high quantitative traits despite different qualitative traits. Some relationships were also observed within clusters for the multitude of varieties. 'Khalt' variety produced dates of quantitative traits better than some cultivars.

Based on these phenotypic results, the breeder could have a primitive idea about the date palm's choice of plant genetic resources.

In this study, 7 cultivars counted in danger of extinction: 'Abd Salam', 'Adam Bara', 'Tazerza', 'Tademama', 'Taorakhet', 'Timidouele' and 'Tinizioua'.

Such work is an overview of the genetic identification of date palms. Further characterization of the date palm vegetative part is suggested, and biochemical and molecular analysis should be completed to allow precise identification.

In situ collections do not suffice with the extension of the adverse threats. There is an urgent need to establish field gene banks to preserve date palm genetic resources less frequented and less in demand, and to provide suitable germplasm for propagation.

6 ACKNOWLEDGMENT

This research work was supported by the Scientific and Technical Research Center on Arid Regions, Biskra, in Algeria.

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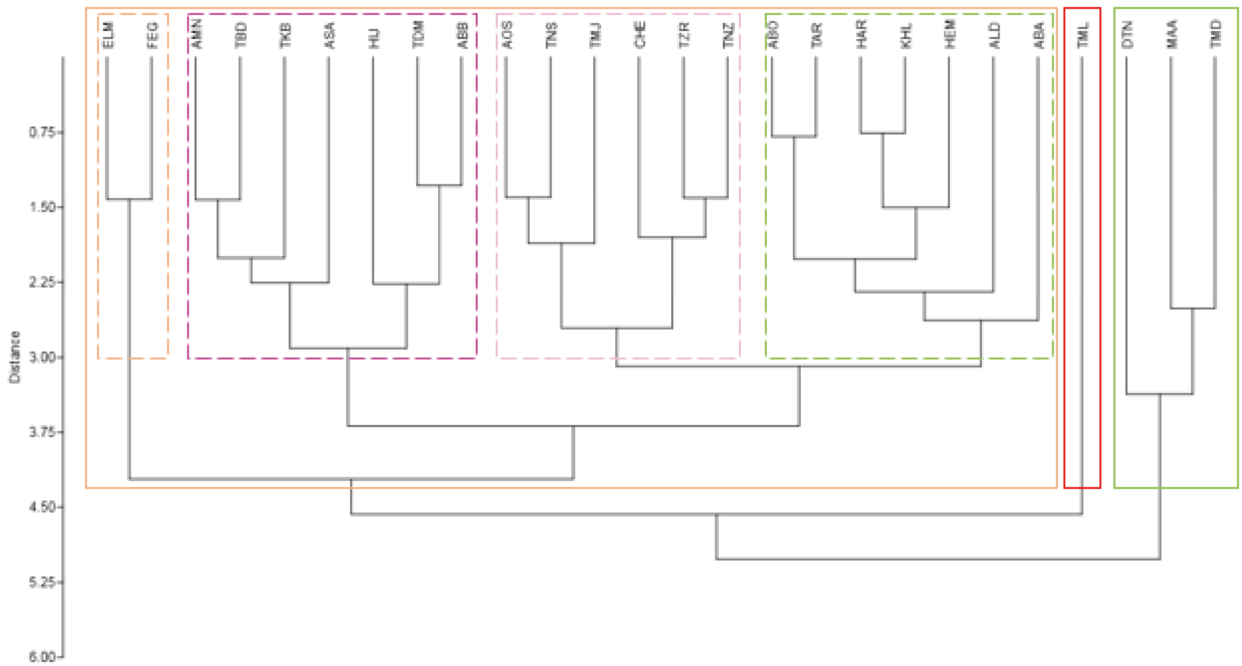

Figure 4: Hierarchical clustering of Algerian date palm fruit-based on eight quantitative traits

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doi:10.14720/aas.2023.119.1.2671 Original research article / izvirni znanstveni članek

Seed pre-sowing treatments and essential trace elements application effects on wheat performance

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Received April 28, 2022; accepted December 17, 2022.
Delo je prispelo 28. aprila 2022, sprejeto 17. decembra 2022

Seed pre-sowing treatments and essential trace elements application effects on wheat performance

Abstract: Current study was conducted to evaluate the effects of different seed priming and foliar spray of micronutrients on bread wheat performance in semi-arid region in Northwest of Iran. Pre-sowing treatments were S1: no pre-sowing treatment (intact seeds), S2: hydro-priming, S3: bio-priming (seed inoculation with plant promoting rhizobacteria consortium: *Azotobacter chroococcum* + *Azospirillum lipoferum*), S4: micronutrient seed priming and foliar feeding include, check (0): distilled water spray, Fe: foliar spray of iron, Zn: foliar spray of zinc. All seed priming treatments significantly increased plant height, tiller number, canopy width, total biomass, spike mass, seed number per spike and seed yield compared to intact seeds. A brief comparison of the effect of seed priming and fertilizer treatments showed that the effects of priming treatments on improving growth and seed yield was more obvious than fertilizer treatments. The greatest increase in seed yield and yield components was recorded for plants grown from bio-fortified seeds by essential trace elements. However, comparison of fertilizer treatments showed that growth parameters were significantly affected by Zn application. From the present study, it may be concluded that combined seed priming through pre-sowing hydration, soaking in micronutrients and microbial inoculation is useful to enhance wheat production and agricultural sustainability for smallholder farmers in semi-arid region.

Key words: bio-priming; hydro-priming; nutrient priming; seed yield

Učinki obravnavanja semen pred setvijo in dodajanje elementov v sledeh na uspevanje pšenice

Izvleček: Raziskava je bila izvedena za ovrednotenje učinkov predtretiranja semen in foliarnega nanosa mikroelementov na uspevanje krušne pšenice v polsušnih območjih severozahodnega Irana. Predsetvena obravnavanja semena so bila: S1- brez obravnavanj (intaktna semena), S2 - predtretiranja z vodo (hydro-priming), S3 - bio-priming (semena inokulirana z mešanico bakterij, ki promovirajo rast rastlin - *Azotobacter chroococcum* + *Azospirillum lipoferum*), S4 - semena predtretirana z mikrohranili in kasnejšim foliarnim dodajanjem mikrohranil, kontrola (0): pršenje listov z distilirano vodo, Fe: foliarno pršenje z železom, Zn: foliarno pršenje s cinkom. Vsa predtretiranja so značilno povečala višino rastlin, število stranskih poganjkov, širino krošnje (nadzemnega dela rastlin), celokupne biomase, maso klasov, število semen na klas in pridelek semena v primerjavi z intaktnimi semeni. Primerjava učinkov predtretiranja semen in obravnavanj z gnojili je pokazala, da so bili učinki predtretiranj semen na povečanje rasti in pridelka bolj očitni kot učinki gnojenja. Največje povečanje pridelka semen in njegovih komponent je bilo zabeleženo pri rastlinah, ki so zrasle iz semen predtretiranih z mešanico bakterij in esencielnih hranil v sledeh. Primerjava obravnavanj z gnojili je pokazala, da je bilo povečanje rastnih parametrov značilno boljše pri uporabi Zn. Iz te raziskave bi lahko zaključili, da bi bila kombinacija obravnavanja semen s predsetvenim tretiranjem z vodo, namakanjem semen v raztopini mikrohranil in inokulacijo z mikrobi koristna za povečanje pridelave pšenice in trajnosti pridelave pri manjših kmetih v polsušnih območjih.

Ključne besede: bio-priming (predtretiranje z mikrobi); vodno predtretiranje; predtretiranje s hranili; pridelek semena

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1 INTRODUCTION

The semi-arid regions of West Asia and North Africa (WANA) are dominated by erratic and unpredictable precipitation, low yield and cereal rain-fed farming system (Rayan, 2011). Poor crop stand establishment is one of the major abiotic restrictions encountered by resource-poor farmers in marginal semi-arid region (Sime & Aune, 2020). The farming system in these regions are characterized by slow seed emergence, low vigor of seedlings, patchy plant stands, and frequent crop failure or yield reduction (Camara et al., 2013). This problem is especially evident in the cereal fields of semi-arid WANA regions, as drought-prone environments, where cereal germination tends to be irregular and can extend over long periods. Late seed germination and low seedling growth rate may result in poor crop stands with high gaps in canopy. This condition is more important especially in semi-arid areas and will lead to loss of moisture through evaporation or an increase in the population of weeds and an increase in the competition for receiving light and nutrients with crop plants (Kaur et al., 2018). Thus accelerating and homogenizing the germination process is a prerequisite for a good crop establishment and helps to increase yield eventually (Samad et al., 2014). However, several physiological approaches and agronomic managements may be employed to increase the crop establishment. Among these approaches, pre-sowing techniques or seed priming are a low cost and safe solution to improve crop stand establishment (Wajid et al., 2018). Between pre-sowing seed treatments priming is a simple method that seeds can adsorb some amount of water in a controlled environment, so that germination processes begin without the appearance of roots. There are various pre-sowing procedures that have been used to improve vegetative growth and seed yield. Depending on the plant species, seed shape and internal components of the seed, various priming treatments can be applied for stimulating the germinative *metabolic* activities (Paparella et al., 2015). The most commonly used methods are hydro-priming, soaking in a distilled water (Damalas et al., 2019). Hydro-priming is the most cost effective and practical method that needs only water to prime seeds. It easily involves soaking seed in water (usually overnight), surface-drying, and then sowing the same day. Priming promotes germination rate and uniformity due to some kind of metabolic repair of seeds during imbibition, build-up of germination-enhancing metabolites, osmotic modification, and a simple decrease in imbibition lag time (Harris et al., 1999; Ashraf and Foolad, 2005; Roslan et al., 2020). On-farm seed priming is a form of hydro-priming, which consists of soaking seeds in water for a number of hours, usually overnight, surface drying them (to allow limited storage) and sowing soon after. On-farm seed priming has been reported to improve emergence, crop establishment, and yield besides improving economic benefits in dryland agriculture (Sime & Aune, 2020).

Microorganisms are the crucial contributors in numerous ecological processes, viz., nutrient cycling, improving the supply of essential elements, plant growth and development (Sarkar et al., 2021). Seed bio-priming is a pre-sowing routine where seeds are treated with some beneficial microbial cells (Singh et al., 2020). Bio-priming with plant promoting rhizobacteria (PGPR) can increase plant growth compared to intact plants by improving the nutrients availability, improving plant resistance and tolerance to disease and some abiotic stresses (Roslan et al., 2020). Managing agrochemicals for crop production always remains a classic challenge for us to maintain the doctrine of sustainability and it seems that bio-priming may provide a maintainable and reasonable solution. It has been reported that seed inoculation with beneficial microorganism can increase accumulation of some certain organic molecules (sugar, proline, polyamines) and secondary metabolites (polyphenols, flavonoids) as a defense mechanism under biotic or abiotic stress (Sarkar & Rakshit, 2020). Seed inoculation with beneficial microbial cells is a practical skill that can make integrated nutrient management plans more efficient (Devika et al., 2021). Further, it is also suggested that seed priming in nutritional solution improves plant growth and the nutrient status (micro and macronutrients) under unfavorable conditions, which are crucial for optimizing yield and quality status of grains (Singhal et al., 2021).

On the other hand, semi-arid fields of WANA are subjected to severe risk of desertification due to poor and shallow soil and low organic matter content, high pH, low water holding capacity and plant nutrients. Unavailability of crop nutrients in appropriate amount and form to crops is one of the major crop productivity constraints in the developing countries (Lal, 2013). Soil organic matter and nutrients have been severely depleted owing to continuous cultivation and non-application of sufficient and proper fertilizers, ignoring of fallowing in crop rotations and prevalence of severe erosion and the productivity of these soils has consequently declined. Zinc and iron deficiencies are common throughout the developed and developing world and lack of these essential trace elements can limit the growth and productivity of a wide range of crops, including wheat. It seems that in order to ensure food security and increase crop yield, in addition to pre-sowing seed treatments, some fertilizer managements should be considered in these areas. We hypothesized that on-farm seed priming (soaking seeds in water for a predetermined duration before sowing), seed inoculation with PGPR (*Azotobacter and Azospirillum)*, foliar spray of micro-nutrient fertilizers or their combination increases the agro-economic benefits in wheat production. Each of the technologies can separately increase wheat productivity. However, their combination can further improve economic yields of crop plants and financial returns, thus it can be considered as a potentially viable option for resource-poor farmers. The objective of this study was, therefore, to estimate the individual and combined effects of on-farm priming, essential trace elements on wheat agronomic performances, in the semi-arid northwest of Iran.

2 MATERIALS AND METHODS

2.1 SITE DESCRIPTION

Experiment was implemented at the Research Farm of University of Maragheh, Northwest Iran (latitude 37°23' N, longitude 46°16' E and altitude 1485 m. The site has a semi-arid, moderate cool, Mediterranean climate with mean annual rainfall around 380 mm/year. Summer (May to September) has a mean maximum temperature of 31 °C and mean minimum temperature of 18 °C. Winter (December to the end of March) has mean minimum temperature of -2 °C and a maximum of 11 °C. Between the driest and the most humid months, the difference in precipitation is close to 64 mm. The variation in mean annual temperature is around 25.2 °C. Rainfall from June to October is relatively light, and the highest rate of evapotranspiration then occurs. Application of supplemental irrigation is necessary during the dry spell. Generally, wheat-barley-fallow cropping system is adapted by the majority of the farmers in studied areas. Soil sample taken from a maximum depth of 30 cm (in composite) was analyzed for its physio-chemical characteristics. The soil of the field was a clay loam containing 0.43 % of organic matter (OM) with pH 7.65 and electrical conductivity (EC) of 0.84 $ds \cdot m^{-1}$ (Table 1). The soil of experimental sit had low content of Zn (0.73 ppm) and Fe (1.62 ppm).

2.2 SEED PRIMING AND ESSENTIAL TRACE ELEMENTS APPLICATION

The experiments were arranged as split-plot (4 × 3), based on the randomized complete block design (RCBD) with three replications. Four seed priming treatments as main plots and three micronutrients foliar spray assigned to sub-plots. Seeds of wheat genotype Sardari were used in this experiments which were collected from Dryland Agricultural Research Institute (DARI), Maragheh, Iran. The best soaking duration and optimal concentration of essential trace elements for priming wheat seed were determined in a series of laboratory experiments at Plant Production Department, University of Mragheh. Seeds and priming solutions ratio was kept as mass/volume 1 : 5 (w/v). Seed priming technique was practiced by soaking wheat seeds in respective solutions of distilled water as hydro-priming (8 h), micronutrient as nutrient-priming (16 h, Ψs = -0.80 MPa), bio-priming with plant promoting rhizobacteria consortium (10^7 CFU ml^{-1}: *Azotobacter chroococcum* Beijerinck 1901 + *Azospirillum lipoferum* Reinhold (Beijerinck 1925) Tarrand et al. 1979 (Approved Lists 1980) for 8 h). Untreated wheat seeds were considered as non-primed control. For nutrient seed priming Iron, Zn and Mn were. Iron was used as Fe–EDTA (8.5 mM Fe; Fermolife, Iran), Zn and Mn used as 4 mM Zn (ZnSO4·H2O; Henan Lihao Chem Plant, China) + 2.5 mM Mn ($MnSO_4 \cdot 7\ H_2O$; Henan Lihao Chem Plant, China) solution. Then seeds were rinsed with plenty of distilled water and were left to air-dry until their mass reach about the initial ones. Also micronutrient fertilizers (iron nano chelate, zinc nano chelate and distilled water as control) applied three times during initiation of tillering stage, booting and milky stage as foliar spray with a concentration of 1000 ppm.

2.3 SOWING, IRRIGATION, WEED CONTROL AND HARVESTING

The experimental field was ploughed once in early autumn and harrowed twice to bring the soil to fine tilth one week before planting in the third decade of February. The recommended dose of fertilizer (150 kg N and 80 kg P_2O_5 ha^{-1}) was applied in the form of urea and triple superphosphate at the time of seed bed preparation. Sowing was done on March 8, 2020. Each experimental plot was 8 m^2 consisting of twelve rows, 4 m long and 15 cm apart. Seeds were sown 2 cm apart at 5 cm depth. Following seed sowing, light irrigation was done. Weeds were controlled by systemic selective chlorophenoxy herbicides including 2, 4-D and MCPA (U 46 Combi Fluid-Nufarm, Portugal). Plants were grown under irrigated condition that received both natural rainfall and four time irrigation irrigations (surface or flood irrigation) were applied during planting, jointing, milking stage and grain filling stage. The amount of irrigation water was calculated to restore water content in the root zone to field capacity. Depth of net irrigation water fraction was ~124 mm. Rest of the agronomic operations like soil preparation and weed management etc. were kept alike for all experimental units.

Table1: Physico-chemical properties of field soil (depth of 0-30 cm), Maragheh, in Northwest of Iran

Soil texture	Sand (%)	Silt (%)	Clay (%)	Zn (ppm)	Fe (ppm)	Organic matter (%)	EC ($ds.m^{-1}$)	pH	CEC (Cmolc kg^{-1})	K ($mg\ kg^{-1}$)	P ($mg\ kg^{-1}$)	N (%)
Clay loam	25	31	44	0.73	1.62	0.43	0.84	7.65	18.2	287.3	14.8	0.17

2.4 DATA COLLECTION AND ANALYSIS

Plant height, canopy width, number of tillers per plant, spike length, 1000- grain mass and mass, number of grains per plant, straw yield, total biomass, yield per plant, and biological yield were taken at maturity stage. Chlorophyll content (SPAD values) of flag leaves were recorded using a SPAD-502 meter (Konica-Minolta, Japan) at heading stage. Twelve independent SPAD measurements were made per treatment, using several different plants (Ling et al., 2011). Harvest index (HI) was calculated according to the following formula: *Harvest index* (%) = *Grain yield / Biological yield* × 100. All data were subjected to variance analysis (ANOVA) for each character to determine crop parameter response to seed priming and foliar application of essential trace elements. Statistical analysis of the data was performed using GLM procedure of SAS 9.1 version software package (*SAS* Institute Inc., Cary, NC, USA). The least significant difference (LSD) at 5 % was used to compare between the means. Pair-wise Pearson's correlation coefficient was calculated among twenty agronomic traits. Cluster analysis was performed for the traits and treatment combinations. The principal components analysis (PCA) based on Everitt & Dunn (1992) was used.

3 RESULTS

Analysis of variance (ANOVA) showed that plant height was strongly affected by seed priming treatments ($p < 0.01$) and micronutrient fertilizers also affected this trait ($p < 0.05$). Mean comparison of plant height sowed that the highest plant height was recorded in plants grown from bio-primed seed (controlled seed hydration and inoculation with PGPR consortium containing *Azotobacter* and *Azospirillum*) and hydro-primed seed. The mentioned pre-sowing treatments increased the plant

Table 2: Effect of seed priming and foliar spray of micronutrients on morphological and growth characteristics of Common wheat (*Triticum aestivum* L.) in Northwest of Iran

Treatments		PH	TL	SL	CW	BT	CHF
Seed priming							
	S_1	68.55^{c}	2.93^{b}	9.42^{a}	17.08^{b}	7.12^{c}	34.72^{c}
	S_2	74.98^{ab}	3.84^{ab}	9.27^{a}	17.80^{a}	9.92^{b}	39.86^{ab}
	S_3	78.07^{a}	4.75^{a}	9.20^{a}	18.02^{a}	11.28^{a}	41.90^{a}
	S_4	72.97^{b}	4.70^{a}	8.66^{b}	18.11^{a}	11.41^{a}	37.30^{b}
Fertilizer	0	71.18^{b}	3.66^{b}	9.47^{a}	17.54^{b}	9.76^{b}	32.18^{b}
	Fe	73.45^{ab}	3.91^{ab}	8.97^{b}	17.57^{b}	11.51^{a}	38.26^{a}
	Zn	76.30^{a}	4.59^{a}	9.43^{b}	18.15^{a}	11.10^{a}	41.40^{a}
				Statistical significance			
S		**	**	**	*	**	**
F		*	ns	*	*	*	**
S*F		ns	ns	**	ns	ns	*
CV		5.32	13.85	4.90	3.77	10.29	6.21

S: seed priming treatment, S1: no pre-spwing treatment (intact seeds), S2: hyropriming, S3: Biopriming (hydropriming + seed inoculation with biofertilizer containing *Azotobacter* and *Azospirillum*), S4: micronutrient seed priming, F: fertilizer treatment, Check (0): without fertilizer application, Fe: foliar spray of iron, Zn: foliar spray of zinc. CV: coefficient of variation (%), PH: plant height, TL: tiller number, SL: spike length (cm), CW: canopy width (cm), BT: biomass (g), CHF: flag leaf chlorophyll content (SPAD unit). Values in a column with the same letter (s) or without any letter (s) do not differ significantly, whereas values with dissimilar letters are statistically different. Ns = not significant, * = significant at 5 % level of probability, ** = significant at 1 % level of probability

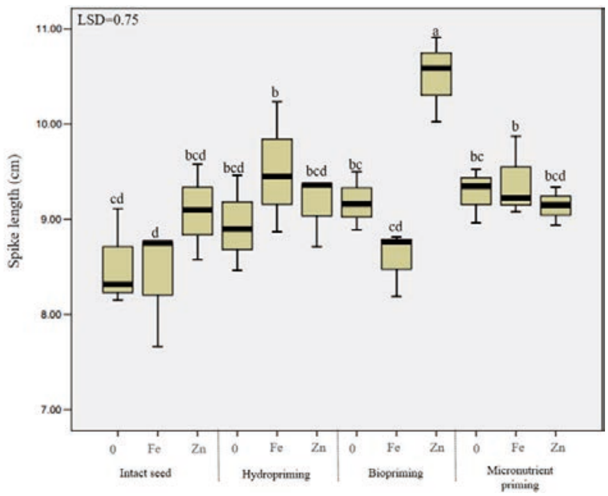

Figure 1: The effect of different seed priming treatments and foliar application micronutrients on spike length of spring wheat. Vertical bars in each column are standard error. Between the columns with different names there are statistically significant differences

height by 13 % and 6 %, respectively, compared to the control. Among fertilizer treatments, the highest plant height was observed under zinc foliar application condition, which was about 7 % higher than the control. Evaluation of tiller number per plants showed that bio-priming and micronutrients priming relatively increased the number of tillers per plant by up to 60 % compared to the plants grown from intact seeds (control). However, hydro-priming treatment could also have a significant effect on this trait and increase the number of tillers by 31 % compared to the control. Assessment of exogenous application of micronutrient fertilizers indicated that the greatest effect on tiller number was related to zinc application (Table 2).

A similar trend also was recorded for response of canopy width to priming treatments and foliar application of essential trace elements. So that the plants grown from primed seeds had an average of about 6 % larger canopy width compared to the control. Alternatively, the utilization of zinc significantly increased the canopy width compared to the control (4 %). However, the effect of iron foliar application on canopy width was not significant and there was no significant difference between iron application and control (Table 2).

Evaluation of spike length showed that although seed treatments caused a significant increase in length of this organ, this increase was more noticeable in bio-primed seed and under foliar application of zinc fertilizer (37 %) when compared with control conditions (Figure 1). Zinc foliar application in non-priming conditions, iron foliar application in hydro-priming and nutrient priming conditions were more effective than other foliar spraying treatments.

Assessment of plant biological mass showed that seed priming significantly affected this parameter, so that plants grown from primed seed had 50 % more biological mass than plants grown from intact seeds. Likewise, foliar application of Fe and Zn increased the biological mass by 18 % and 13 % (Table 2). Evaluation of chlorophyll content showed that seed priming increased the chlorophyll content of flag leaf. However, the highest chlorophyll content was recorded in plants grown from bio-primed seeds and sprayed with zinc, followed by plants grown from hydro-primed seeds and sprayed with iron (Figure 2).

Evaluation the effects of seed priming and micronutrient fertilizers on seed yield components are presented in Table 3. The effect of priming treatments on yield components was significant, however, the effect of foliar application of micronutrient was not very significant. Plants grown from primed seeds (hydro-priming, bio-priming and nutrient priming) showed the higher spike mass, seed mass per spike, fertile spikelet number and spike number in unit area when compared with control.

Assessment of 1000-seed mass showed that although all seed priming treatments increased the 1000-seed mass, the highest 1000-seed mass were recorded in plants grown from bio-primed and hydro-primed seeds under zinc and iron foliar application conditions. It is noteworthy that the application of zinc under bio and hydro-priming conditions had the greatest effect on grain mass and significantly increased this seed yield component (Figure 3).

Examination of the number of seeds per spike

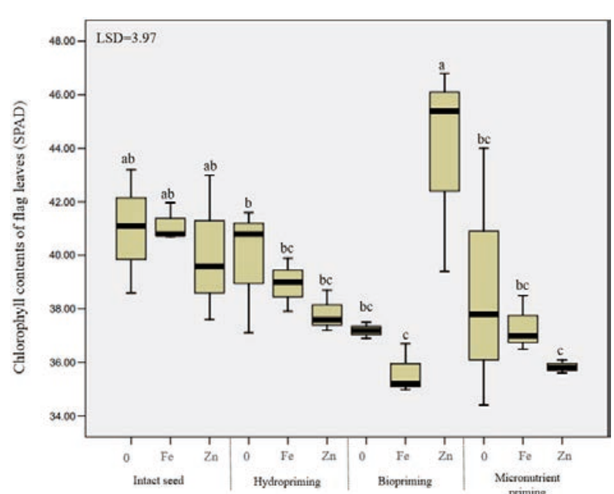

Figure 2: Influence of pre-sowing seed treatment and micronutrient fertilizer on chlorophyll content of flag leaf in spring wheat in semi-arid region of Iran

Table 3: Impact of seed pre-sowing treatment and micronutrients on seed and yield component of common wheat in semi-arid region of Maragheh

Treatments		SPM	SNP	SMS	FSN	SNM	TSM	SY	HI
Seed priming									
	S_1	1.60[b]	21.80[d]	1.22[b]	38.65[b]	368.51[b]	53.44[bc]	2536.4[c]	49.76[ab]
	S_2	1.82[a]	24.02[b]	1.40[a]	44.80[a]	506.32[a]	54.59[ab]	2665.0[bc]	49.19[ab]
	S_3	1.82[a]	26.41[a]	1.41[a]	45.04[a]	526.23[a]	55.64[a]	2906.3[ab]	50.23a
	S_4	1.81[a]	23.73[bc]	1.40[a]	46.12[a]	568.83[a]	52.53[c]	3073.2[a]	48.26[b]
Fertilizer	0	1.72[b]	22.26[c]	1.32[b]	43.05[ab]	455.56[a]	53.08[b]	2611.4[a]	49.84[a]
	Fe	1.73[b]	23.95[b]	1.39[ab]	42.63[ab]	483.34[a]	54.60[a]	2874.2[a]	48.36[a]
	Zn	1.85[a]	25.03[a]	1.43[a]	45.28[a]	538.57[a]	54.48[a]	2900.1[a]	49.74[a]
					Statistical significance				
S		*	*	*	**	*	**	*	*
F		ns	*	ns	ns	ns	*	ns	ns
S*F		*	*	**	ns	ns	**	*	ns
CV		10.29	9.37	9.12	8.35	9.26	5.43	18.66	5.24

S: Seed priming treatment, S1: no pre-sowing treatment (intact seeds), S2: hyropriming, S3: hydropriming + seed inoculation with biofertilizer (*Azotobacter* and *Azospirillum), S4:* micronutrient seed priming, F: fertilizer treatment, Check (0): without fertilizer application, Fe: foliar spray of iron, Zn: foliar spray of zinc. CV: coefficient of variation (%), SPM; spike mass, SNP: seed number per spike, SMS: seed mass per spike, FSN: fertile spikelet number, SNM: spike number in unit area, TSM: thousand seed mass (g), SY: seed yield (kg ha^{-1}), HI: harvest index (%).Values in a column with the same letter (s) or without any letter (s) do not differ significantly, whereas values with dissimilar letters are statistically different. Ns = not significant, * = significant at 5 % level of probability, ** = significant at 1 % level of probability

showed that seed bio-priming and application of zinc produced the highest amount of this trait (Figure 4). Bio-priming, hydro-priming and nutrient priming had the greatest effect on the number of seed per spike, respectively. However, the efficiency of foliar spraying treatments at different levels of priming was different and the highest foliar application efficiency was observed in

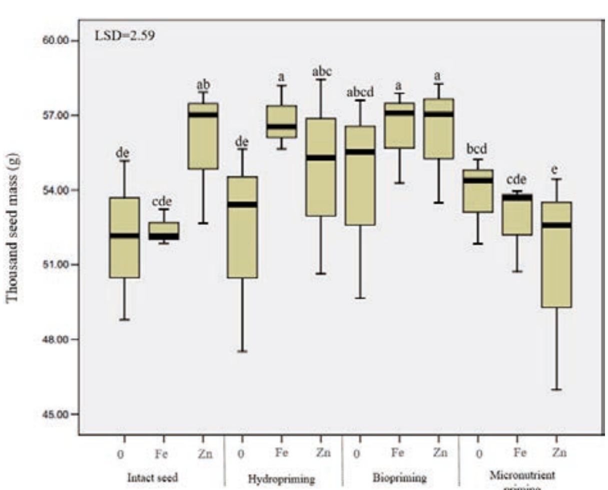

Figure 3: The effects of seed invigoration techniques and iron and zinc fertilizers on seed mass of spring wheat

hydro-priming and bio-priming conditions. Seed yield evaluation showed that the highest amount was recorded in bio-primed plants by zinc foliar application. Although all priming treatments increased the seed yield, the greatest effect was related to bio-priming and hydro-priming treatments. Foliar spraying had the greatest effect on bio-primed plants (Figure 4).

Although the pre-sowing seed treatments significantly improved seed yield when compared with control (intact seed), the best performance was related to plants grown by trace fertilizers. Evaluation of seed yield showed that the highest amount was recorded under bio-priming condition along with application of essential trace elements (Zn and Fe) and followed by Zn received plant under hydro-primed condition (Figure 5).

Principal component analysis (PCA) was employed to provide an overview of the capacity to distinguish combined treatments. First principal component clearly separated the non-primed seeds from other priming techniques (Figure 6). PCA could separate the foliar treatments by second component. PCA evidently separated the micronutrients application from control. Also second principal component segregated the Zn application along with bio-priming was very close to seed yield component. Also PCA provided the correlation coefficient between any two traits by the cosine of the angle

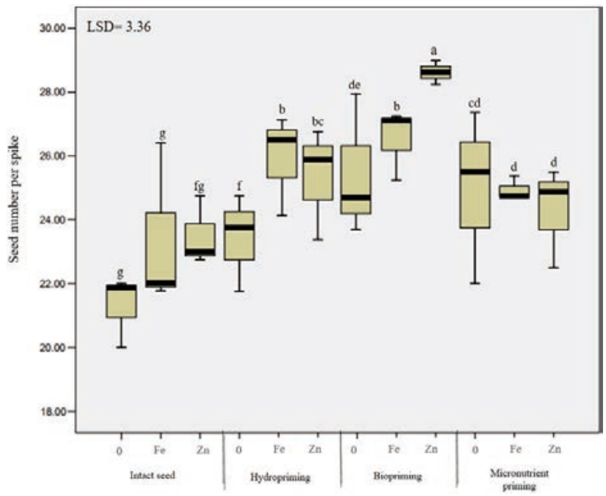

Figure 4: The effect of different seed priming treatments and foliar application micronutrients seed number in spike of spring wheat in semi-arid region in northwest Iran

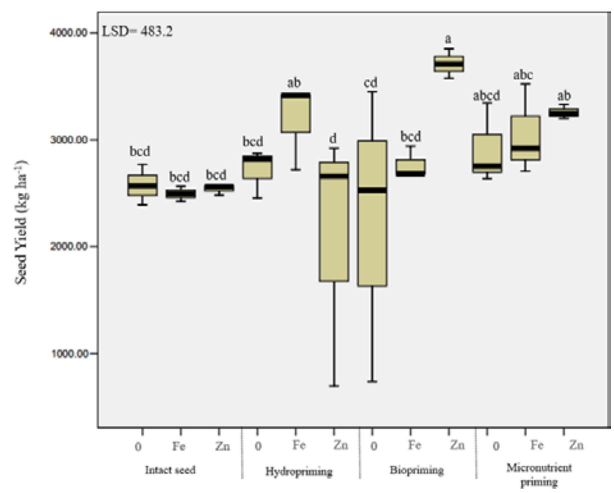

Figure 5: Influence of pre-sowing seed treatment and micronutrient fertilizer on seed yield of spring wheat in semi-arid region of Iran

between their vectors of related traits. A strong positive association between seed yield, chlorophyll content of flag leaf, seed mass, ground cover, fertile spikelet number and seed mass indicated by the small obtuse angles between their vectors (r = cos 0 = +1).

4 DISCUSSION

Seed priming is used to achieve rapid and uniform seed germination and seedling emergence to enhanced crop production performance. In the present study, we found that seed bio-priming and hydro-priming increase height and tiller number as compared to control treatment. The results were similar to those of earlier studies, which reported that seed priming enhanced seedlings fresh mass, hypocotyl, when compared with unprimed seedlings (Sarkar and Rakshit, 2020; Anwar et al., 2020). These findings are suggested that seed priming incredibly enhanced seedlings growth. Considering the key role of plant hormones auxin and gibberellin in regulating plant height and tiller production, it seems that these treatments have increased height by accelerating the plant initial growth as well as changes in phyto-hormone levels as well as hormone ratios (Zhuang et al., 2019).

Results revealed that canopy width improved by both priming treatments and Zn foliar application. Canopy width as one of the most important vegetative traits can affect yield. Increasing crop canopy structure can improve canopy photosynthetic productivity and thereby crop yield potential (Feng et al., 2016). The findings of the current study are consistent with those of Wajid et al. (2018) who showed that different seed priming approach increased the leaf area in wheat. The increase in canopy width is probably due to the acceleration of germination and rapid establishment of the seedling and the optimal use of ecological conditions. This can be due to a wide range of factors such as hormones and faster exploitation of growth factors such as water, nutrients and light. Increasing the canopy width can affect the source-sink relationship. Traits such as plant height, canopy width, number of tillers, chlorophyll content can directly play a role in increasing the rate of photosynthesis and also improve the ability of the source to supply photo-assimilates for growing sinks. Besides, traits such as spike length, number of florets per spikelet and number of seeds, as well as grain size or mass are traits that are directly related to the size of the sink and indirectly reflect the activity of the sink. It seems that seed priming affected both sink and source activity and size in current study. Sink- source relations can regulate biomass production and assimilate allocation in plants (Burnett, 2019). In plants grown from non-primed seed the reduction in the photosynthetic area resulted in reducing the assimilation formation and translocation towards the sink, which led to reduce the seed yield and related attributes. Furthermore, the balance between carbon- and nitrogen-containing metabolites is an important indicator of source-sink status. The rapid expansion of root systems in primed seeds allows the plant to absorb nitrogen, which can lead to more photosynthesis and more activity of the source as well as the sink. Further absorption of nitrogen can cause further supply of free amino acid to sink. The ratio of free amino acids to sucrose expresses the relative availability of nitrogen and carbon, with a high ratio indicating an excess of available nitrogen and a low ratio indicating

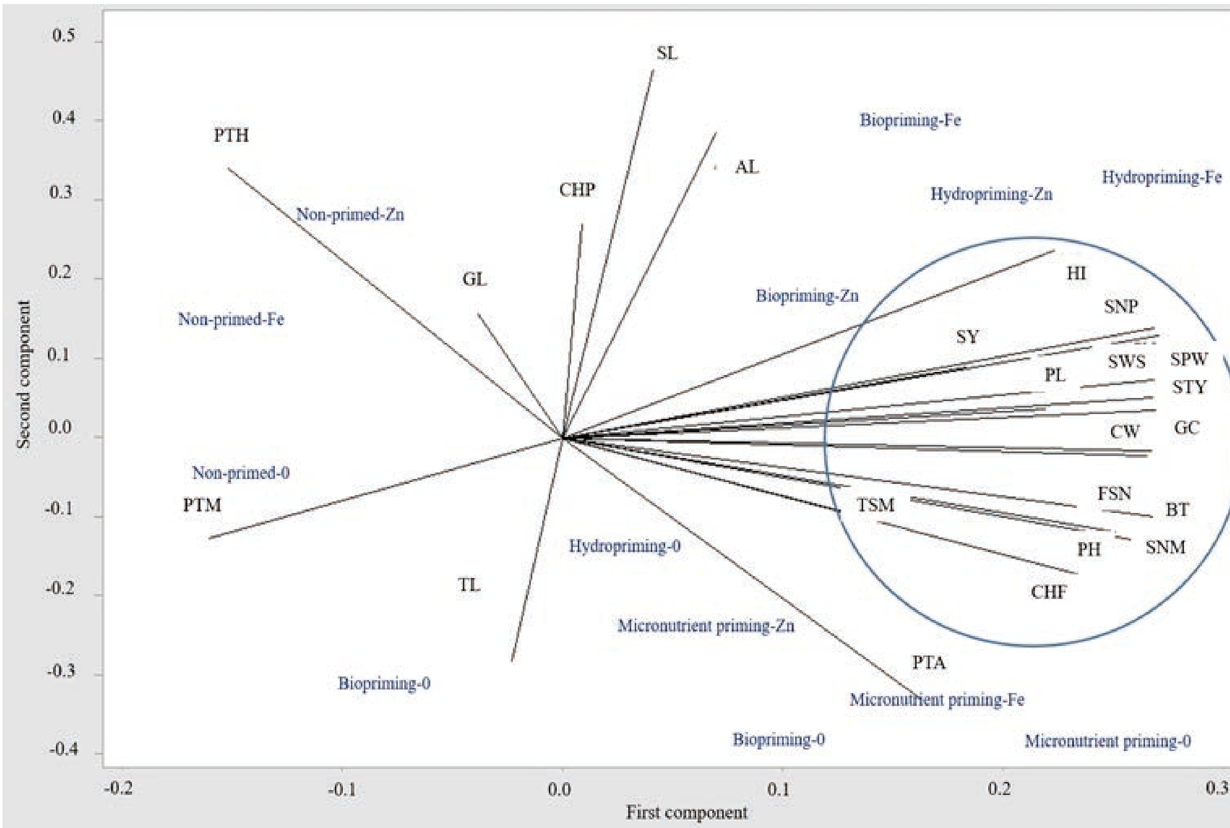

Figure 6: Plot of the first two PCAs showing relation among various agronomical traits of wheat. PL: peduncle length, SL: spike length, AL: length of the awn, SPM: Spike mass, BT: biomass, FSN: fertile spikelet number, SNP: seed number per spike, SES: seed mass per spike, STY: straw yield, GL: grain length, TL: tiller number, CW: canopy width, SY: seed yield, GC: ground cover, CHF: chlorophyll content of flag leaf, CHP: chlorophyll content of penultimate leaf, TSM: thousand seed mass, PTH: day from planting to heading, PTA: day from planting to anthesis, PTM: day from planting to maturity

an excess of available carbon. This balance is attuned to enable plants to improve their growth and development. Also there was found Zn application affected both source and sink size. This finding supports previous research into this brain area which links Zn and Sink- source relations (Wang et al., 2021).

The results of this study showed a significant superiority of bio-priming in improving the evaluated traits. Bio-priming is an important method to reinforce the mechanisms of seed-microbe-soil plant interactions. However, many mechanisms behind these associations and mode of action are still not well understood and need further investigation. This superiority is supported by Mirshekari et al. (2012) who writes that seed bio-priming with PGPR consortium (*Azotobacter* + *Azospirillum*) significantly increased yield component of barley including dry matter accumulation, seed mass, harvest index, biological yield, and seed yield. It seems that PGPR capable of colonizing the rhizosphere or plant roots facilitate nutrient availability for plant uptake (Jacoby et al., 2017). Seed bio priming can improve plant performance through modification of soil enzymes such as cellulose, protease, catalase, dehydrogenase, acid phosphatase, alkaline phosphatase, phytase and amylase enzymes (Mengual et al., 2014). Seed bio-priming also can induce plant growth by alternation of the phytohormone contents (indole-3-acetic acid, gibberellic acid, and salicylic acid) or decreasing the content of abscisic acid (Sarkar et al., 2021). Furthermore, seed bio-priming through inoculation with beneficial root-associated bacteria increases speed and uniformity of germination and modified the seedling nutritional condition by increasing ascorbic acid, protein, flavonoid, and total phenolic contents; and also via reorganization of defense process such as improving antioxidant potential by increasing hydroxyl radical scavenging activity, free radical scavenging activity, redox capacity, and iron chelating capacity affected seed yield (Jain et al., 2014). The latter case is of great importance in semi-arid regions, so that the wheat plant in these regions at the end of its development period are faced with drought and heat stress.

The positive response of many traits to Zn application under non-priming condition without may to some extent indicate a severe deficiency of this element in the soil of the site study. Due to the central role of applied rhizobacteria for bio-priming (*Azotobacter* and *Azospirillum*) in nitrogen assimilation, it seems that Zn can interact with mentioned PGPR. Zn is an essential micronutrient in plant growth and development. The major role of Zn in plants is to act as the cofactor for enzymes involved in N metabolism, such as alcohol dehydrogenase. This enzyme play a critical role in plant growth, development, adaptation with anaerobic soil conditions. Therefore, Zn deficiency reduces anaerobic root metabolism and seedlings' capacity under restriction of oxygen in the soil (Tuiwong et al., 2022). However, our finding revealed that utilization of Zn fertilizers alone is not be sufficient for increasing wheat performance in semi-arid region, so that the application of suitable seed priming and micronutrient nano-fertilizer application had the best effect on both vegetative growth and seed yield. This may be the result of synergistic relationships between the fertilizer management and priming treatments. Overall, seed priming improves wheat performance under semi-arid conditions through improved germination metabolism and accelerating crop stand establishment, resulting in accelerated growth and development even under unfavorable condition of semi-arid regions.

5 CONCLUSIONS

Our results showed that seed priming significantly improved wheat plants growth characteristics and yield component. On-farm pre-sowing seed treatments, whereby seeds were soaked in water for a predetermined duration followed by surface-drying (to facilitate handling) and inoculated with bio-fertilizers (PGPR) before sowing, resulted in the highest seed yield and best performance. On the other hand micronutrient deficiencies especially Zn and Fe are common in studied site as a representative of semi-arid Mediterranean region. The present study found that foliar application zinc significantly improved vegetative growth and yield components when compared to control. However, seed treatment in solutions containing essential trace elements can be successfully used to improve wheat quality attributes like tiller number, spike number and seed yield. Keeping in view all results, we concluded that among the seed priming treatments bio-priming (inoculation with PGPR consortium containing *Azotobacter* and *Azospirillum)* and hydro-priming along with foliar application of zinc was the most effective and recommendable for wheat field in studied region. Despite the efforts so far reported to further improve seed priming, novel ideas and cutting-edge investigations need to be brought into this technological sector of agri-seed industry. Further research work is needed to optimize the effectiveness of pre-sowing seed inoculation with different bio-fertilizer and with using of more varieties of wheat in combination of other agents/factors.

6 ACKNOWLEDGEMENTS

This research was supported by a grant-in-aid for scientific research from the University of Maragheh and funded by the Ministry of Science, Research and Technology, Iran.

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doi:10.14720/aas.2023.119.1.2890 Original research article / izvirni znanstveni članek

Dormancy-breaking treatments for enhancing seed germination in plant *Kitaibela vitifolia* Willd.

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Received October 20, 2022; accepted December 23, 2022.
Delo je prispelo 20. oktobra 2022, sprejeto 23. decembra 2022

Dormancy-breaking treatments for enhancing seed germination in plant *Kitaibela vitifolia* Willd.

Abstract: Vine-leaved kitaibelia (*Kitaibela vitifolia* Willd.), also known as balkanmalva or chalice flower, is a critically endangered plant species with a high risk of extinction in the wild. A reason given for this is, among others, a low germination rate primarily caused by dormancy. The present study evaluated the seed germination and seedling growth parameters of vine-leaved kitaibelia in response to eight different pre-sowing treatments. The final germination percentage ranged from 0 % to 55 %, depending on the pre-sowing treatment. The most effective method for breaking dormancy and increasing vine-leaved kitaibelia seed germination was the treatment with seeds soaked in H_2SO_4 for 5 min. The mechanical scarification of vine-leaved kitaibelia seeds also improved germination as compared to control treatment, while treatments with nitric acid and gibberellic acid were not effective in enhancing seed germination. All evaluated seedling growth parameters were not affected by pre-sowing treatments. Considering that successful germination and seedling establishment are crucial for the regeneration of vine-leaved kitaibelia further studies are required in order to identify other pre-sowing treatments that could further enhance seed germination and, consequently, seedling development.

Key words: climate chamber; greenhouse; habitat restoration; seedling growth; plant protection

Postopki prekinitve dormance za pospeševanje kalitve kitajbelovke (*Kitaibela vitifolia* Willd.)

Izvleček: Kitajbelovka (*Kitaibela vitifolia* Willd.), poznana tudi kot balkanski slezenovec, je kritično ogrožena rastlinska vrsta z veliko nevarnostjo izumrtja v naravi. Med drugim je razlog za to majhna sposobnost kalitve, ki jo povzroča dormanca. V raziskavi so bili ovrednoteni kalitev semen in parametri rasti kitajbelovke kot odziv na osem različnih predsetvenih obravnavanj. Odstotek kalitve je bil med 0 % in 55 %, odvisno od predsetvenega obravnavanja. Najučinkovitejša metoda prekinitve dormance kalitve pri kitajbelovki je bilo namakanje semen pred kalitvijo v H_2SO_4 za 5 min. Mehanska skarifikacija semen je tudi izboljšala kalitev v primerjavi s kontrolo med tem, ko obravnavanji semen z dušikovo kislino in giberilini nista bili učinkoviti pri pospeševanju kalitve. Na vse ovrednotene rastne parametre sejank predkalitveno obravnavanje ni vplivalo. Upoštevaje, da sta uspešna kalitev in uspešen razvoj sejank odločilna za obnavljanje kitajbelovke, je iskanje primernih predsetvenih obravnavanj ključno za pospeševanje kalitve in razvoj sejank te rastline.

Ključne besede: klimatska komora; rastlinjak; obnova habitata; rast sejank; zaščita rastlin

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1 INTRODUCTION

Bosnia and Herzegovina (BIH) is a country in South-eastern Europe, situated in the western part of Balkan Peninsula (Gekić et al., 2022). As a result of unique orography, special geological past and pedo-climatic conditions, BIH is one of the richest European countries in terms of plant species diversity (Redžić, 2007).

Unfortunately, plant diversity in the BIH has been declining at an alarming rate in recent years, mainly because of habitat loss, climate change and all forms of pollution resulting from human activity. Accordingly, there is a pressing need to preserve plant diversity, especially endemic plants that are usually more vulnerable to environmental changes. One of such plant species is vine-leaved kitaibelia (*Kitaibela vitifolia* Willd.) also known as balkanmalva or chalice flower.

The given plant belongs to family Malvaceae and genus *Kitaibela* that was named in honour of Paul Kitaibel, a famous Hungarian Botanist. The natural distribution of vine-leaved kitaibelia is Balkan Peninsula and in its natural surroundings can grow up to 3 m. The stems are robust, simple or sparingly branched, with unusual vine-like leaves and large showy cup-shaped white flowers. The 5-petaled flowers are about 5 cm across. The petals are slightly longer than wide, with cuneate to oblate form and rounded or slightly notched apex. The fruit is a schizocarp, consisting of numerous one-seeded mericarps usually separating at maturity (Šilić, 1984). Vine-leaved kitaibelia is endemic to the western Balkan peninsula and the area of distribution of this plant species is constantly decreasing, especially in Bosnia and Herzegovina. According to the 'Red list of endangered wild species and subspecies of plants, animals and fungi for Federation of BIH', vine-leaved kitaibelia is a 'critically endangered' plant species with a high risk of extinction in the wild (OG F BIH, 2014).

One of the potential limiting factors for the survival of wild plants in its natural environments is seed dormancy, which can be defined as the temporary blockade of the germination of a viable seed (Carrera-Castaño et al., 2020). Vine-leaved kitaibelia also produces seeds that are dormant upon maturity. Seed dormancy allows seeds to overcome periods that are unfavourable for seedling growth and is therefore important survival strategy of plants in their natural habitats (Li et al., 2022). On the other hand, insufficient level of seed germination or delayed germination may influence plant regeneration in its natural habitat (Chen, 2022).

There are two types of seed dormancy; exogenous and endogenous. Exogenous dormancy is caused by unfavourable conditions outside of the seed's embryo and is often broken down into three subgroups: (1) physical - caused by seed impermeability to oxygen or water, (2) chemical - caused by biologically inactive growth hormones that are present in the coverings around the embryo, and (3) mechanical - caused by hard seed that restrict growth. Endogenous dormancy occurs due to physiological or morphological changes within the seed's embryo. It includes: (1) physiological dormancy - caused by hormonal imbalance in the embryo, (2) morphological dormancy - caused by an undeveloped embryo, and (3) morphophysiological dormancy (combination of both) (Jursík et al., 2003).

Seed dormancy can be overcome by different pre-sowing dormancy-breaking treatments such us dry heat treatment, hot water treatment, mechanical scarification, chemical scarification, stratification and treatment with growth regulators (Phuyal et al., 2022). Physical, mechanical and chemical treatments reduce exogenous seed dormancy by removing or permeabilizing the seed coat, while stratification and treatment with growth regulators reduce endogenous seed dormancy by neutralizing seed germination inhibitors (Lavallee et al., 2021). However, inappropriate dormancy-breaking treatment can result in failure to break dormancy and at worst kill the seeds (Kildisheva et al., 2020). A better understanding of the mechanism and regulation of vine-leaved kitaibelia seed dormancy as well as dormancy breaking treatments ensures a greater chance for successful propagation of this plant, and thus for its preservation both in natural habitats and in ex-situ collections.

Therefore, the main goal of this study was to find out the appropriate dormancy-breaking treatments that maximize total germination and produce more vigour seedlings of vine-leaved kitaibelia. It was hypothesized that dormancy-breaking treatments applied in this study can facilitate the germination of the seeds in both the laboratory and greenhouse experiments.

2 MATERIALS AND METHODS

2.1 SEED COLLECTION

In this study, vine-leaved kitaibelia seeds were collected in September 2021 from the adult plant growing in the botanical garden which is part of the National Museum of Bosnia and Herzegovina (Latitude: 43.8563° N, Longitude: 18.4131° E). All seeds that were damaged or had irregular shapes, were removed. After collection, seeds were kept in paper bags in dark place at room temperature until further analysis.

2.2 DORMANCY-BREAKING TREATMENTS

Stratification (seeds exposed to low temperature), scarification (physically damage the seed coat to make it permeable to water and gases) and chemical methods (soaking the seeds in different chemicals) are the methods used for breaking seed dormancy (Kaur et al., 2020). In this study, the vine-leaved kitaibelia seeds were submitted to the following dormancy-breaking treatments: (T1) stratification at 4 ˚C for 7 days; (T2) immersion in 250 mg l^{-1} gibberellic acid solution (GA_3) for 24 h; (T3) immersion in 500 mg l^{-1} GA_3 solution for 24 h; (T4) immersion in 0.1 % HNO_3 for 24 h; (T5) immersion in 0.2 % HNO_3 for 24 h; (T6) mechanical scarification with sandpaper; (T7) immersion in sulfuric acid for 5 min; (T8) control treatment (without any seed manipulation). The volume of solution used in applied dormancy-breaking treatments was sufficient to completely submerge the seeds. Before any dormancy-breaking treatments, the seeds were soaked in distilled water for 24 hours.

2.3 GERMINATION EXPERIMENTS

Two experiments, i.e. lab experiment and greenhouse experiment, were conducted to evaluate the impact of different dormancy-breaking treatments on germination and seedling growth of vine-leaved kitaibelia. Germination test was emphasised in the lab (petri dish) experiment, while seedling emergence and vigour were emphasised in the greenhouse (pot) experiment. Both experiments were carried out as a randomized complete block design. In each experiment, a total of 8 treatments were tested, and 100 seeds per treatment were evaluated. Each treatment was replicated five times.

Germination tests were conducted by placing 20 seeds in a sterile plastic Petri dishes (9 cm diameter) lined within two sheets of filter paper. The filter papers in Petri dishes were moistened daily with distilled water during the experiment. To minimize the effect of environmental factors, germination test was carried out under controlled conditions in a growth chamber at 25 ˚C ± 3 ˚C and 80 % relative humidity under a 12 h photoperiod. Germination was considered complete once the radicle had protruded 2 mm in length (Tobe et al., 2005).

The following germination parameters were evaluated: seed germination time, germination pattern, percent germination, germination energy and shoot and seminal root length. Germination was recorded daily, from the day of sowing through to the end of the experiment. Shoot and seminal root length was measured manually with a graduated ruler at the end of the experiment (10th days of the experiment).

Seed germination time (SGT) represents the number of days from first observed germination to where there was no more germination. Germination pattern was determined by counting the number of seeds that germinated at the different days after sowing (Viswanath et al., 2002).

The percent germination (GP) illustrates the number of germinated seeds on the final day of a germination test and it was calculated according to Eq. 1:

$$GP = \frac{germinated\ seeds\ at\ the\ end\ of\ experiment}{total\ seeds} \times 100 \qquad (1)$$

Germination energy (GE) is a measure of the speed of germination and hence, a measure of the vigour of seedlings. It represents the number of seeds that germinated within a definite period under optimum or stated condition. GE was determined on Day 5 after setting up the germination test (Elezz and Ahmed, 2021) and it was calculated according to Eq. 2:

$$GE = \frac{germinated\ seeds\ after\ 5\ days}{total\ seeds} \times 100 \qquad (2)$$

In order to see if the positive response to dormancy-breaking treatments would occur outside the germination chamber, pot experiment with treated seeds was also performed. The pot experiment was carried out under controlled conditions, in the greenhouse located in Kakanj (Latitude: 44.1280° N, Longitude: 18.1178° E) from the mid-June 2022 to mid-September 2022. Seeds obtained through breaking-dormancy treatments were sown with 3 cm depth in each pot (6 cm diameter x 5 cm high) containing a compost and sand mixture (one seed per pot). All pots (30 pots per treatment) were placed in a greenhouse under the same conditions (temperature ranging from 15 to 28 °C with 75 % of relative humidity). The pots were irrigated as needed to keep the soil moist during the experiment. Number of emerged seedlings was counted at 30 days after planting, while the seedling height and number of leaves and flowers per seedlings were evaluated at the end of the experiment (90 days after the start of the experiment). Plant height was measured manually with a graduated ruler.

2.4 STATISTICAL ANALYSIS

All data collected was subjected to Analysis of Variance using Microsoft Excel software program. When Fisher's F values were significant, the analysis was continued by comparing the means using the least significance difference (LSD) test at the threshold of $p < 0.05$.

3 RESULTS

3.1 LAB EXPERIMENT

Generally, germination started early; in most pre-sowing treatments where germination occurred, the time period from sowing to the first emergence of seedlings ranged between four and six days. The only exception was in the sulphuric acid treatment where germination started on the third day after sowing. In all treatments where germination occurred, seed germination was completed within eight days from the beginning of the experiment, indicating that the vine-leaved kitaibelia seed germination time lasted from 3 to 4 days, depending on the pre-sowing treatment. Most pre-sowing treatments reached their peak germination 4th or 5th day after sowing. In this study, pre-sowing treatment with sulphuric acid for five min (T7) resulted in a higher germination compared with other seed-dormancy-breaking treatments. The mechanical scarification of vine-leaved kitaibelia seeds (T6) also improved germination as compared to control treatment. Unfortunately, in this study pre-sowing treatments with GA_3 and HNO_3 (T2 - T5) did not significantly improve the seed germination. Moreover, germination was completely inhibited when the seeds treated with 250 mg l^{-1} GA_3 (T2) and 0.2 % HNO_3 (T4) (Table 1).

The highest percentage germination of vine-leaved kitaibelia seeds (55 %) was observed in treatment with seeds soaked in H_2SO_4 for 5 min (T7). The next best results were found in mechanical scarification treatment (T6) and cold stratification treatment (T1). Germination was 13.3 % and 6.7 %, respectively for these treatments, not differing among them. The lowest germination percentages were obtained by treatments T2, T4, T8, T3 and T5, which presented germination varying from 0 to 3.3 %, respectively, with no statistical difference.

Germination energy was determined on Day 5 after setting up the germination test and it varied from 0 % to 43.3 % among the pre-sowing treatments. The highest germination energy (43.3 %) was calculated for T7, followed by T6 (13.3 %) and T1 (5 %) (Table 1).

Table 1: Germination percentage and germination energy in response to different pre-sowing treatments

Pre-sowing treatment	Percent Germination (%)	Germination Energy (%)
T1 (stratification at 4 ˚C for 7 days)	$6.7 \pm 15.0^{bc*}$	5 ± 2.0^{c}
T2 (GA_3 250 mg l^{-1} for 24 h)	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}
T3 (GA_3 500 mg l^{-1} for 24 h)	3.3 ± 5.0^{c}	0.0 ± 0.0^{c}
T4 (0.1 % HNO_3 for 24 h)	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}
T5 (0.2 % HNO_3 for 24 h)	3.3 ± 5.0^{c}	3.3 ± 2.0^{c}
T6 (mechanical scarification)	13.3 ± 5.0^{b}	8.3 ± 3.5^{b}
T7 (sulfuric acid treatment for 5 min)	55.0 ± 15.0^{a}	43.3 ± 5.0^{a}
T8 (control treatment)	1.7 ± 5.0^{c}	1.7 ± 2.5^{c}
$LSD_{0.05}$	7.9	1.8

Averages denoted by the same letter indicate no significant difference ($p \leq 0.05$)

Table 2: Effects of different pre-sowing treatments on shoot and seminal root length

Pre-sowing treatment	Shoot length (mm)	Seminal root length (mm)
T1 (stratification at 4 ˚C for 7 days)	9.0 ± 6.0^{a}	15.0 ± 6.0^{ab}
T2 (GA_3 250 mg l^{-1} for 24 h)	0.0 ± 0.0^{b}	0.0 ± 0.0^{c}
T3 (GA_3 500 mg l^{-1} for 24 h)	9.1 ± 7.4^{a}	15.3 ± 9.6^{ab}
T4 (0.1 % HNO_3 for 24 h)	0.0 ± 0.0^{b}	0.0 ± 0.0^{c}
T5 (0.2 % HNO_3 for 24 h)	8.4 ± 6.1^{a}	14.3 ± 6.1^{b}
T6 (mechanical scarification)	10.2 ± 5.3^{a}	20.0 ± 8.9^{a}
T7 (sulfuric acid treatment for 5 min)	10.6 ± 6.0^{a}	17.1 ± 7.3^{ab}
T8 (control treatment)	10.3 ± 5.8^{a}	18.3 ± 6.6^{ab}
$LSD_{0.05}$	4.7	5.3

Averages denoted by the same letter indicate no significant difference ($p \leq 0.05$)

At the end of the experiment (10 days after the start of the germination test), plumule and radicle length were determined for sprouted seeds of each petri dish (Table 2).

The longest mean shoot was noted in T7 (treatment with H_2SO_4 for 5 min), followed by T8 (control treatment) and T6 (mechanical scarification); however, no significant difference was observed between the treatment where germination occurred in regards to shoot length. The longest mean seminal root was also noted in T6, T7 and T8 treatments, not differing among them. Statistically significant difference in seminal root length between all pre-sowing treatments where germination occurred was only observed between T7 (seeds soak in H_2SO_4 for 5 min) and T5 treatment (seeds soak in 0.2 % HNO_3 for 24 h).

3.2 GREENHOUSE EXPERIMENT

Effect of pre-sowing treatments on vine-leaved kitaibelia seed germination, evaluated in the greenhouse, is presented in Figure 1. Among the eight applied pre-sowing treatments, only three of them (T1, T6 and T7) stimulated seed germination.

The highest percentage germination (65 %) was observed in T7 (sulfuric acid treatment for 5 min), followed by T6 (mechanical scarification) and T1 (stratification at 4 ˚C for 7 days). Interestingly, the mechanical scarification and seed treatment with sulfuric acid for 5 min resulted in greater germination in potted soils than in Petri dishes, while the seed stratification at 4 ˚C showed an opposite trend; i.e., germination was higher in Petri dishes.

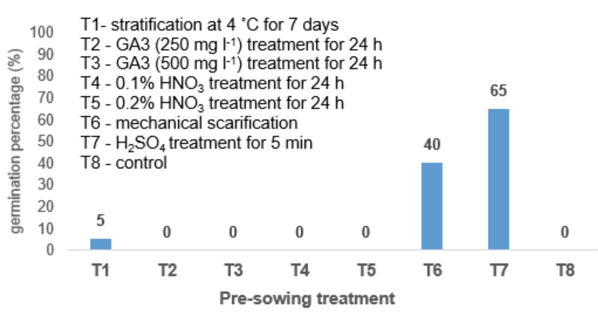

Figure 1: Vine-leaved kitaibelia seed germination in response to different pre-sowing treatments

Effect of pre-sowing treatments on growth parameters of vine-leaved kitaibelia seedlings in the pot experiment is presented in Table 3. Seedling height as well as the number of leaves and flowers per seedlings were evaluated at the end of the experiment (90 days after the start of the greenhouse experiment).

The highest mean seedling height was noted in T6 (mechanical scarification), followed by T1 (stratification at 4 ˚C for 7 days) and T7 (sulfuric acid treatment for 5 min); however, no significant difference was observed between them. Furthermore, these pre-sowing treatments (T6, T1, T7) had no significant influence on the number of leaves and flowers of vine-leaved kitaibelia seedlings.

4 DISCUSSION

Many wild plants, including vine-leaved kitaibelia, produce seeds that require a period of dormancy before they will germinate (Wang et al., 2021). Since the seed dormancy can reduce the vine-leaved kitaibelia restoration success, it is very important to identify the most efficient method for overcoming seed dormancy. Among the eight pre-sowing treatments applied in this study, only few of them significantly stimulated vine-leaved kitaibelia seed germination, and that is, T7 (sulfuric acid treatment for 5 min), T6 (mechanical scarification) and T1 treatment (stratification at 4 ˚C for 7 days). The most efficient treatment to break vine-leaved kitaibelia seed dormancy was treatment with sulfuric acid in both laboratory and greenhouse experiment. It seems that immersion seeds in concentrated sulfuric acid remove or permeabilize the seed coat, leading to a significant increase in seed germination as compared to control and other dormancy-breaking treatments. It could lead to a conclusion that dormancy in vine-leaved kitaibelia seeds is imposed by seed coat impermeability which prevents oxygen and/or water permeating into the seed or by hard seed coat which mechanically restricts embryo expansion, thus preventing shoot and seminal root emergence. Seed dormancy caused by an impermeable or hard seed

Table 3: Seedling growth parameters in response to different pre-sowing treatments

Pre-sowing treatment	Seedling height (cm)	Number of leaves per seedlings	Number of flowers per seedlings
T1 (stratification at 4 ˚C for 7 days)	81.9 ± 13.1	43.3 ± 20.0	1.1 ± 4.0
T6 (mechanical scarification)	82.4 ± 18.0	40.6 ± 19.0	1.4 ± 5.0
T7 (sulfuric acid treatment for 5 min)	80.9 ± 14.4	38.1 ± 18.0	1.8 ± 4.0

coat is known as physical dormancy, and that is, the major type of exogenous dormancy (Baskin et al., 2006).

Interestingly in this study, the seed germination tests with 0.1 % and 0.25 % nitric acid did not enhance vine-leaved kitaibelia seed germination as compared with control treatment. The assumption is that the low concentration of nitric acid caused inadequate removal of seed coat, resulting with poor seed germination. The results of the study also showed that the mechanical scarification with sandpaper had a significant effect on germination as compared to control treatment. These findings support the hypothesis that mechanical scarification can cause cracks in the seed coat, thus allowing for water movement to the embryo to trigger germination (Salazar & Ramírez, 2018). Similar findings were reported by Orsenigo et al. (2019) and Gao et al. (2021).

In this study, there were no significant differences in germination percentage and germination energy among treatments with GA_3 (T2 and T3) and control treatments (without any seed manipulation) in both laboratory and greenhouse experiment. The data suggested that the GA_3 in concentration of 250 mg l^{-1} and 500 mg l^{-1} failed to overcome dormancy of vine-leaved kitaibelia seeds. Tang et al. (2019) also found that treatments with gibberellic acid did not improve the seed germination of *Sorbus alnifolia* (Siebold & Zucc.) K. Koch. Similar results were obtained by Stejskalová et al. (2015) in sycamore seeds. Contrastingly, there are many studies that have demonstrated improved seed germination by treatment with GA_3 (Gashi et al., 2019; Cornea-Cipcigan et al., 2020; Uçarli, 2021; Guariz et al., 2022).

Many researchers agree that the hormonal signals, especially those of abscisic acid (ABA) and gibberellic acid (GA), play a dominant role in the regulation of seed dormancy and germination. ABA and GA are widely recognized as essential endogenous factors that regulate seed germination; ABA represses seed germination, while GA release seed dormancy and promote germination (Tuan et al., 2018; Ali et al., 2022). It is also well known that changes in the balance of a seed's ABA and GA levels can exert a significant influence in a seed germination (Finkelstein et al., 2008). However, in the present study, increased GA content did not improve the germination of vine-leaved kitaibelia seeds. On the basis of the above, it can be assumed that the dormancy in vine-leaved kitaibelia seeds is primarily controlled by factors outside the embryo. However, to our knowledge, there is no study that has examined the influence of GA treatments or any other pre-sowing treatments on vine-leaved kitaibelia seed germination. Further investigation is therefore needed to investigate the impact of the present findings.

In this study, cold stratification i.e., seeds stored at 4 °C for 7 days, enhanced the vine-leaved kitaibelia seed germination as compared to control in both laboratory and greenhouse experiment; however, the germination percentage and germination energy of cold stratified seeds was significantly lower than that of seeds treated by sulfuric acid or mechanically scarified seeds.

The results obtained in this study also showed that there were no significant differences among pre-sowing treatments in regards to shoot length in vine-leaved kitaibelia seeds. A similar pattern was found for seminal root length. Statistically significant difference in seminal root length between all pre-sowing treatments where germination occurred was only observed between T7 (sulfuric acid treatment) and T5 (0.2 % nitric acid treatment).

In this study, no significant differences were observed for all evaluated vine-leaved kitaibelia seedling growth parameters (seedling height and number of leaves and flowers) among pre-sowing treatments. This implies that the pre-sowing treatments may improve seed germination; however, this does not necessarily lead to improved seedling growth and development. The results of the present study are consistent with this hypothesis.

5 CONCLUSIONS

In general, the results revealed that the seed treatment with sulfuric acid was the most effective pre-sowing treatment from the view of seed germination both in the laboratory and greenhouse experiment. The mechanical scarification of kitaibelia seeds also improved germination as compared to control treatment, while treatments with nitric acid and GA_3 were not effective in enhancing seed germination. All evaluated vine-leaved kitaibelia seedling growth parameters were not affected by pre-sowing treatments. Future experiments should focus on identifying other pre-sowing treatments that could further enhance vine-leaved kitaibelia seed germination. The mechanism of seed breaking dormancy and germination of vine-leaved kitaibelia also needs further study.

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doi:10.14720/aas.2023.119.1.2227 Original research article / izvirni znanstveni članek

Graphical analysis of forage yield stability under high and low potential circumstances in 16 grass pea (*Lathyrus sativus* L.) genotype

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Received June 02, 2021; accepted January 15, 2023.
Delo je prispelo 2. junija 2021, sprejeto 15. januarja 2023

Graphical analysis of forage yield stability under high and low potential circumstances in 16 grass pea (*Lathyrus sativus* L.) genotype

Abstract: Introducing grass pea genotypes with wide adaptability across diverse environments is important. Dry forage yield of 16 grass pea genotypes, tested in a RCBD design with three replicates across 4 locations over 3 seasons in Iran. The GGE biplot method based on SREG model facilitated a visual evaluation of the best genotypes. The first two principal components accounted for 77 % of the GE interaction and revealed six winning genotypes and four mega-environments. The average location coordinate (ALC) was used to examine both yield performance and stability and indicated IFLA-1913, IFLA-1961, IFLA-1812, and IFLA-2025 were the best genotypes. Based on the ideal-genotype approach, genotype G5 was better than all other genotypes and showed both high forage yield and stability across locations. According to G + GE sources of variations, the genotypes (IFLA-1913, IFLA-1961, IFLA-1812, and IFLA-2025) were the most suitable varieties for the grass pea-producing regions in semi-arid and rain-fed conditions. An ideal location should be both discriminating of the genotypes and representative of the average location, but we could not find such location in this research. Results confirmed that G5 (IFLA-1961) has high stability and high yield performance (4.92 t ha^{-1}), and could introduce as favorable genotype for commercial variety release.

Key words: average location coordinate; biplot; GGE (Genotype+ Genotype Environment interaction)

Analiza stabilnosti pridelka krme 16 genotipov navadnega grahorja (*Lathyrus sativus* L.) v ugodnih in slabih okoljskih razmerah

Izvleček: Vzgoja genotipov navadnega grahorja z veliko prilagodljivostjo v različnih okoljih je zelo pomembna za pridelavo krme. Pridelek suhe krme 16 genotipov navadnega grahorja je bil preiskušen v popolnem naključnem bločnem poskusi s tremi ponovitvami na štirih lokacijah v treh rastnih sezonah v Iranu. Grafična analiza odnosov med genotipi in različnimi okolji je na osnovi SREG (Site Regression model) modela omogočila ovrednotenje najboljših genotipov. Prvi dve glavni komponenti sta razložili 77 % interakcij med genotipi in okoljem (GE) in odkrili šest zmagovalnih gentipov v štirih mega okoljih. Za preverjanje najboljših genotipov glede pridelka in njegove stabilnosti je bila uporabljena poprečna koordinata lokacije (ALC), ki je označila genotype kot so IFLA-1913, IFLA-1961, IFLA-1812, in IFLA-2025 kot najboljše. Na osnovi koncepta idealnega genotipa je bil genotip G5 boljši kot vsi ostali, saj je imel velik in stabilen pridelek krme na vseh preučevanih lokacijah. Glede na vire razlik v interakcijah med genotipi in genotipi in okoljem (G + GE) so bili genotipi IFLA-1913, IFLA-1961, IFLA-1812, in IFLA-2025 najprimernejše sorte navadnega grahorja za pridelavo krme v razmerah polsušnih in z dežjem namakanih območih. Idealna lokacija bi morala biti prepoznana po genotipu in reprezentativni poprečni lokaciji, a takšne v tej raziskavi niso našli. Rezultati so potrdili, da bi lahko bil genotip G5 (IFLA-1961) z veliko stabilnostjo in velikostjo pridelka (4,92 t ha^{-1}) lahko uveden kot priporočena komercialna sorta.

Ključne besede: povprečna koordinata lokacije; biplot; GGE (genotip + interakcije genotip-okolje)

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1 INTRODUCTION

Pulses from the Leguminosae family contains about 13,000 species which their seeds can be fractionated to obtain starch and protein concentrates as well as a by-product of the process, dietary fiber. The genus *Lathyrus* comprises approximately 160 species, primarily native to temperate regions of the world (Gcdt, 2007). Grass pea (*Lathyrus sativus* L.) is an important major pulse crop in some Asian countries where it is produced for feed and food. It was cultivated around 8000 BC due to archaeological investigations in the Middle East, and its seeds were observed among founded archaeological items (Lambein and Kuo-Genth, 1997). Grass pea (2n = 2x = 14) as an ancient crop, is found in Eurasia, North America, temperate South America and East Africa and perhaps one of the first crops to be domesticated, but its origin is not known; however, its presumed center of origin is Southwest and Central Asia (Smartt et al., 1994). It has been used as a pulse and its production as a forage crop has resulted in little evolutionary progress which result in its extensive dispersion worldwide while its excessive consumption may provoke the neurological form of lathyrism, so some efforts have been performed for selection and development of cultivars of low toxicity.

Also, the cultivation of grass pea has an important role as a useful rotation crop in the soils adapted annual cereals and pulses crops and is presently considered as a model crop for sustainable agriculture with a great future because it is unique in that it can thrive under adverse environmental conditions such as drought and flooding. In the past decade's little effort has been performed towards genetic improvement of this crop as food, due to its successful utilization as a forage crop while it has potential as an alternative pulse in many cropping systems around the world as it is very tolerant of drought stress and is not affected by extreme rainfall (Croft et al., 1999). Despite its obvious advantages, relatively little effort has been done in the breeding of this hardy pulse crop while exception of its neurotoxin problem, is produced in significant quantities in many parts of the world and recently its breeding is now being performed in many countries through germplasm collection and evaluation, as well as breeding programs. Iran has grass pea breeding program in recent years with the forage yield as the most important objective due to the large demand for forage, supported by the national institute known as Dryland Agricultural Research Institute (DARI). Development of new grass pea cultivars is done to meet the requirements of consumers' demands (especially high forage yield), and breeders need consistent access to newly genetic improved plant materials.

In multi-environmental testing trials genetically, improved lines are evaluated in different locations and years before the final recommendation of cultivars. For forage yield, the relative performance of genotypes varies from an environment to another environment, i.e., there is a genotype by environment (GE) interaction which is the result of changes in the relative ranking of the genotypes or changes in the amounts of differences among genotypes from one environment to another, making it difficult to detect which genotypes should be chosen (Kang, 2002). The effectiveness of selection is also decreased by the large magnitudes of GE interaction, and various efforts have been performed to overcome the problems created by GE interaction. The estimates of GE interaction provide useful information on the existence and magnitude of GE interaction, but give no information of the response of individual genotypes with the test environment, and therefore no measurement of the stability of individual genotypes. Now, interest has been focused on the GGE biplot analysis, and approach proposed by Yan et al. (2000) which developed a graphical analysis of multi-environment trial data considering both the G (genotype effect) and GE interaction effect (G+GE), simultaneously via biplot presentation. The GGE biplot approach is a type of linear bilinear model suitable for grouping environments and genotypes which is known as the site regression (SREG) model and related biplots are drawn from graphing the first two principal components (PC1 and PC2). There is little information on the stability and yielding ability of grass pea under rainfed conditions and this study was designed to; (i) evaluate the forage production of genotypes; (ii) determine the GE interaction; and (iii) study the adaptation of genotypes using GGE biplot method for commercial recommendation as well as identification of mega-environments in target grass pea producing locations.

2 MATERIALS AND METHODS

2.1 EXPERIMENTS

Sixteen grass pea genotypes from diverse origins were used to examine GE interactions and forage yield stability analysis (Table 1). Seeds of these genotypes were supplied by the International Center for Agricultural Research in Dry Areas (ICARDA) and most of them were developed with the support of ICARDA. The genotypes were planted at four locations during three growing years (2017-2019) under rain-fed conditions. In each environment (year-location), genotypes were grown according to the randomized complete block design with three replicates. The plots were 4.5 m long and 1 m apart (four rows with spacing of 25 cm between rows) and the seeding

Table 1: The origin of 16 grass pea (*Lathyrus sativus* L.) genotypes evaluated in four locations across three years in Iran

Code	Name	Origin
G1	IFLA-1707	Morocco
G2	IFLA-1864	Bangladesh
G3	IFLA-1813	Pakistan
G4	IFLA-1913	Nepal
G5	IFLA-1961	Nepal
G6	IFLA-1553	Morocco
G7	IFLA-1857	Bangladesh
G8	IFLA-1812	Pakistan
G9	IFLA-1547	Morocco
G10	IFLA-2341	Bangladesh
G11	IFLA-2025	Bangladesh
G12	IFLA-2750	Bangladesh
G13	Naghadeh	Iran
G14	Sel.290	Iran
G15	Sel.449	Iran
G16	Sel.587	Iran

rate was 150 seeds per m^2 in all the environments. The fields were not supplied with irrigation and fertilizer and the weed control was carried out by hand during crop growth. In all environments, for excluding border effects, only the central two rows were harvested (0.5 ×4.0 m plots equal to 2.0 m^2) for forage yield recording at 50 % flowering stage and then obtained data was converted to tons per hectare scale for statistical analysis.

2.2 DATA ANALYSIS

The GGE biplot approach was performed considering the simplified model for the first two principal components as:

$$Y_{ij} - y_{.j} = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \varepsilon_{ij}$$

where: Y_{ij} is the mean dry forage yield of genotype i in environment j; $y_{.j}$ is the mean of environment j; $\lambda_1 \xi_{i1} \eta_{j1}$ is the first principal component (PC1); $\lambda_2 \xi_{i2} \eta_{j2}$ is the second principal component (PC2); λ_1 and λ_2 are the eigenvalues related to the PC1 and PC2, respectively; ξ_{i1} and ξ_{i2} are scores of the PC1 and PC2 axes for G effects; η_{j1} and η_{j2} are the scores of the PC1 and PC2 for E effects; and ε_{ij} is the error term or residual of the model. The GGE biplot was constructed by first subjecting the GGE matrix (the environment-centered data) to singular-value decomposition (SVD). The used symmetric scaling method has the advantage that PC1 and PC2 have the same unit (square root of original unit t ha^{-1} in terms of dry forage yield). Burgueno et al (2003) developed a SAS program for obtaining the SREG analysis while to facilitate the use of GGE biplot method Yan (2001) developed a Windows application called GGE biplot and both packages were used for performing all statistical analyses in the present investigation.

3 RESULTS AND DISCUSSION

3.1 ANALYSIS OF VARIANCE

Mean forage yield for grass pea genotypes across each environment (year-location) were comprised via LSD test (Table 2). The summary of the yearly combined analysis of variance (Table 3) showed that all sources of variations were significant by the F test and these results demonstrate the existence of locational heterogeneity and also indicate significant differences among the genotypes since their responses were not coincident in the test locations. Variance components for L, G, and GL interaction based on the yearly data showed their relative magnitudes as the L was always the most important source of variation (relative to G and GL interaction) accounting for 94.6, 86.9 and 84.6 % of the total variance (G + L + GL). When the SREG model was fitted, the first two PCs explained about 77 % (PC1 = 44.3 % and PC2 = 32.5 %) of G + GE variation for grass pea multi-environmental trails (Table 4). In this research, F-test Gollob (1968) was applied to test the significance of PCs for the SREG model which indicated two significant PCs. The amount of GE interaction for the dry forage yield of 16 grass pea genotypes tested across four locations was larger than that of G effect, but smaller than that of E effect (Table 3). The genotypes indicated both crossover and additive types of GE interaction which led to differential rankings of genotypes across locations, thereby making selection difficult under the rain-fed circumstances. The relative contributions of G and GE interaction effects to the total variation for dry forage yield observed in this research are similar to those found in other crop GE interaction investigations in rain-fed climates (Berteroa et al., 2004; Sabaghnia et al., 2013). This founding proposes that it would be difficult to gain an indirect response to selection over all of the grass pea target plant materials of locations from selection in a few locations as well as environments, ignoring the GE interaction. In other words, GE interaction makes it difficult to select the most favorable (high yield and most stable) and so it must be considered in breeding programs because it reduces the selection promotion (Yau, 1995). Finally, results of yearly

Table 2: Mean forage yield for grass pea multi-environmental trials, 2017 to 2019

	2017				2018				2019				
	Gac.	Kho.	Meh.	Shi.	Gac.	Kho.	Meh.	Shi.	Gac.	Kho.	Meh.	Shi.	Mean
G1	1.79	3.66	1.00	10.10	2.11	5.22	1.94	8.80	7.21	3.69	6.80	3.73	4.67
G2	1.19	4.12	0.94	8.54	2.32	6.25	1.56	8.52	7.62	3.33	8.27	3.62	4.69
G3	0.94	3.42	1.22	8.06	1.74	5.78	1.96	6.24	8.86	3.39	12.00	4.49	4.84
G4	1.39	1.59	1.05	7.74	2.05	4.26	2.01	7.60	4.51	2.49	14.00	5.11	4.48
G5	1.61	2.27	1.14	9.48	2.55	3.13	2.19	8.20	7.46	2.88	13.87	4.22	4.92
G6	1.45	3.10	0.98	8.16	1.93	5.10	2.00	8.06	5.17	3.42	11.47	5.03	4.66
G7	1.86	2.83	1.11	8.56	2.46	4.09	2.00	6.67	7.71	4.08	12.40	4.94	4.89
G8	1.10	2.94	1.10	9.63	1.41	4.70	2.04	8.11	5.94	2.21	12.27	4.71	4.68
G9	1.01	3.10	0.90	7.82	2.53	5.32	2.01	7.16	6.41	4.02	13.07	4.54	4.82
G10	1.25	2.82	0.97	8.30	1.82	5.96	1.64	6.12	7.78	3.12	12.13	3.59	4.62
G11	1.67	2.46	1.58	8.54	2.27	5.77	2.84	6.74	9.71	3.59	13.73	5.27	5.35
G12	1.41	2.89	0.74	7.87	1.52	4.90	1.26	5.43	4.83	2.65	10.27	4.32	4.01
G13	1.70	2.46	1.10	6.24	1.84	8.04	2.06	6.90	6.76	2.90	13.60	3.82	4.78
G14	1.79	2.70	0.98	6.94	1.52	5.76	2.08	5.73	6.29	3.00	8.00	2.15	3.91
G15	2.27	3.09	1.18	5.91	2.10	5.48	2.09	5.01	7.24	2.74	11.20	1.77	4.17
G16	1.50	2.96	1.09	6.73	1.45	6.17	1.93	5.12	5.40	3.09	13.87	2.49	4.32
LSD†	0.70	0.80	0.24	2.35	1.24	1.96	0.16	2.21	2.46	1.69	4.08	2.16	0.69

Locations are: Gac., Gachsaran; Kho., Khoramabad; Meh., Mehran and Shi., Shirvan
† LSD (0.05) = 0.69 t ha^{-1} for comparison of mean dry forage yield within an environment (location–year)

analysis of variance indicated the large variation due to location which is irrelevant to genotype assessment as well as mega-environment identification (Gauch et al., 2008), legitimatized the use of SREG (Yan et al., 2000) as a proper method for analyzing the multi-environmental trials data of the current study.

3.2 MEGA-ENVIRONMENTS

The establishment of six mega-environments, that is, environments defined by winner genotypes which are the farthest from the biplot origin, is indicated in Fig. 1. The winner genotypes (G1, G5, G11, G14, G15 and G16)

Table 3: Genotype (G), location (L), and genotype × location (GL) variance terms for grass pea multi-environmental trials, 2017 to 2019

		2017		2018		2019	
Sources of Variation	DF†	SS‡	% of §	SS	% of §	SS	% of §
Location (L)	3	1479.6**	94.6	886.2**	86.9	2130.1**	84.6
Replication within L	8	18.7		27.8		205.6	
Genotype (G)	15	19.9*	1.3	27.8*	2.7	126.2**	5.0
GL	45	63.9**	4.1	106.0**	10.4	261.9**	10.4
Error	120	72.4		111.0		325.8	

** and * Significant at the 0.01 and 0.05 level, respectively.
† DF is degrees of freedom.
‡ SS is sum of squares.
§ % of L+G+GL

Table 4: Site regression (SREG) analysis of variance for dry yield of 16 grass pea genotypes

Sources of Variation	DF†	SS‡	MS§	% of GE
Environment (E)	3	320.89	106.96**	
Genotype (G)	15	32.11	2.14**	
GE	45	105.36	2.34**	
SREG Model				
PC1	17	60.97	3.59**	44.3
PC2	15	44.72	2.98**	32.5
Residual SREG	24	31.79	1.32ns	23.1

**, * and ns significant at the 0.01 and 0.05 level, and nonsignificant, respectively.
† DF is degrees of freedom.
‡ SS is sum of squares
§ MS is mean squares.

are located on the borders of the polygons, and the mega-environments are separated by lines perpendicular to the polygon but only the genotypes G1, G5, G11 and G14 determined the mega-environments I (Shirvan), II (Gachsaran), III (Mehran) and IV (Khoramabad), respectively. In other words, these genotypes are recommended for locations included within each mega-environment but genotypes G15 and G16 did not give the highest yield in any of the locations. The mega-environment III, Mehran location, was distinct from the mega-environments due to high PC2 values which is demonstrating that Mehran location contributed most to the GE interaction, and was therefore recommended for future investigation of adaptability. In a more detailed trial analysis, locations with the same GE response pattern (located in the same mega-environment) can be discarded but in this study, our locations felled in different mega-environments and none of them can be discarded. In other words, this result demonstrates the benefits of the GGE biplot method since locations with different patterns of genotype response and unlike patterns of GE interaction are maintained. Therefore, the polygon view of GGE biplot method suggests that there exist four possible grass pea mega-environments in Iran but this pattern requires validation through other multi-environment trials. As discussed by Sabaghnia et al. (2012), the above inferences

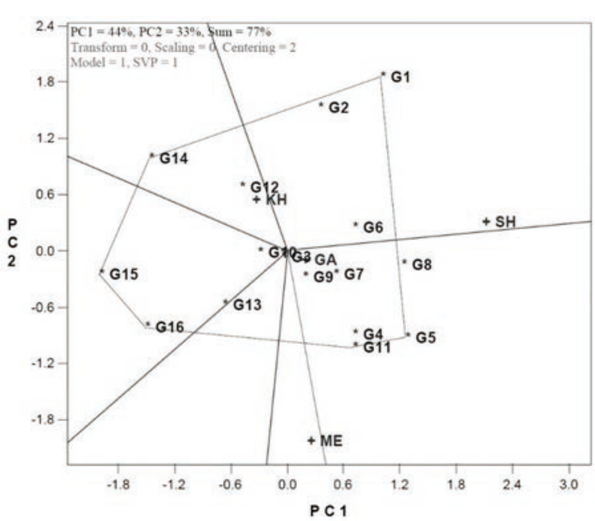

Figure 1: Site regression (SREG) biplot identification of winning genotypes and their mega-environments. Sixteen grass pea genotypes grown in four locations: GA, Gachsaran; KH, Khoramabad; ME, Mehran; and SH, Shirvan. Commonly PC1 indicates the additive effects and the PC2 shows the interaction effects

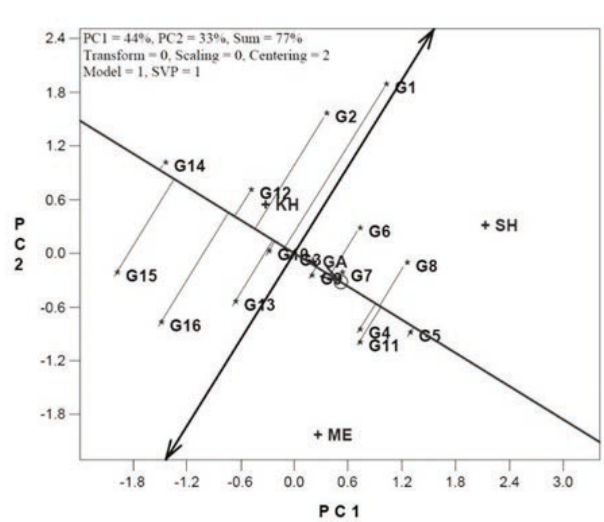

Figure 2: Site regression (SREG) biplot of mean and stability of sixteen grass pea genotypes for dry yield and specific genotype × environment interactions. Four locations are: GA, Gachsaran; KH, Khoramabad; ME, Mehran; and SH, Shirvan. Commonly PC1 indicates the additive effects and the PC2 shows the interaction effects

about observed patterns are mostly verified from the original data because GGE model is fitted to the original data incompletely, and the model consequence is valuable for recommendation purposes since, as reported by Yan (2002) and applied to GE modelling by Gauch et al. (2008).

The mean forage yield performance and stability property of the genotypes were examined by defining an average location coordinate (ALC) and an average location is indicated virtually by a circle and indicates the positive end of the ALC x axis (Fig. 2). The average yield performance of the genotypes is approximated by the projections of their markers on the ALC axis. According to Yan et al. (2000), in the GGE biplot method, the PC1 shows the genotype adaptability due to the high association of adaptability and high yielding while the PC2 shows the stability (genotypes low PC2 would be stable). In this research, the length of the average location vector was adequate to detect genotypes based on forage yield mean performances and some superior genotypes with above-average means (such as: G4, G5, G8 and G11) were selected, whereas the others were discarded while genotype G5 was the most stable genotype as well as high yielding (Fig. 2). Conversely, G1 was the least stable genotype (variable performance) but yielded with average means while genotype G14 was the least yielding. Our results confirmed that genotype G5 (IFLA-1961) has high stability and high yield performance (4.92 t ha^{-1}), therefore is introduced as the most favorable genotype. The need for the application of SREG model based GGE biplot for the determination of the premier genotypes is to facilitate the identification of such genotypes (Yan et al., 2007). This research has clearly that the SREG model can analyze GE interaction patterns plus G main effect and reveal the associations of genotypes and locations successfully as well as prepare a worthwhile prediction. Although, according to Sabaghnia (2010), the multivariate methods such as SREG model are too sophisticated to prepare a simple measure of yield stability but using graphical facilities of biplot presentation and their integration via GGE biplot method can eliminate such sophistication and provide a simple method for interpretation of GE interaction.

3.3 IDEAL GENOTYPE

An ideal genotype is one that has both high yield performance as well as high stability and the center of concentric circles (Fig. 3) indicates its position which is defined by a projection onto the mean-environment axis that equals the longest vector of the genotypes that had above-average mean yields and by a zero projection onto the perpendicular line as an index of minimum variation across all locations. The closer a genotype to this position is the more valuable it is and such an ideal genotype may not exist in reality, it only can be applied as an index for comparison (Yan and Tinker, 2006). In the biplot of ideal genotype, the ranking of genotypes is performed based on the genotype-focused scaling which assumes that both stability and yield are equally important thus,

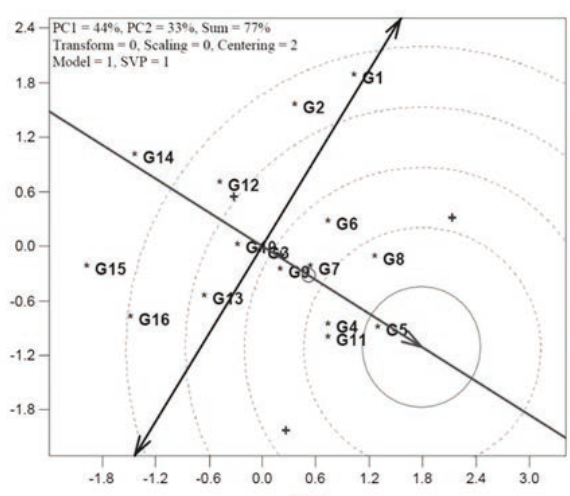

Figure 3: Site regression (SREG) biplot of ideal genotype and comparison of the sixteen grass pea genotypes with the ideal genotype. Commonly PC1 indicates the additive effects and the PC2 shows the interaction effects

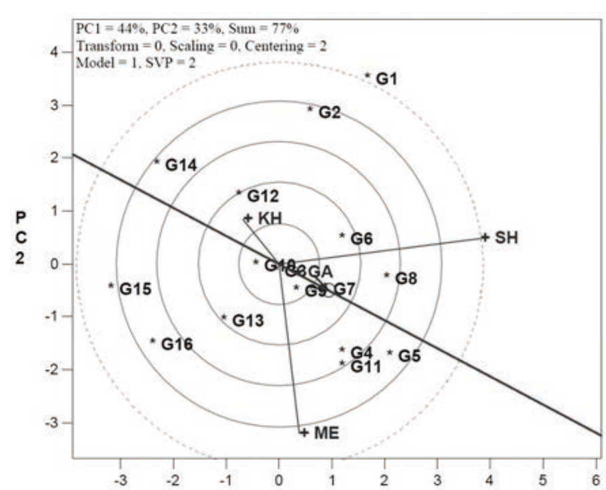

Figure 4: Site regression (SREG) biplot of discrimitiveness versus representativeness of testers. Four locations are: GA, Gachsaran; KH, Khoramabad; ME, Mehran; and SH, Shirvan. Commonly PC1 indicates the additive effects and the PC2 shows the interaction effects

G5 which is close to the center of concentric circles, was an ideal genotype in terms of yield potential and stability compared with the other grass pea genotypes. Following G5, genotypes G4, G8 and G11 located in the next concentric circle were also considered as superior genotypes regarding both yield and stability (Fig. 3). The PC1 and PC2 scores of SREG model indicates the yield and stability, respectively as they are comparable to the G main effect or yield performance and adaptability index (line slope coefficient) of the joint linear regression model in the method of Eberhart and Russel (1966). The relative contributions of stability and yield performance to the detection of the most favorable genotypes found in this research by ideal genotype view of GGE biplot method are similar to those found in other crop stability investigations in bean (Kang et al., 2006), lentil (Karimizadeh et al., 2013), and field pea (Yihunie and Gesesse, 2018).

3.4 DISCRIMITIVENESS AND REPRESENTATIVENESS

The discriminative and representativeness properties of the test locations for grass pea dataset were explored by GGE biplot method and results are shown in Fig. 4 and similar to Fig. 2, the average location coordinate (ALC) passes through the average location and the biplot origin relative to genotype mean yield performance and the small circle is the average location, and the arrow pointing to it is used show the direction of the ALC. The locations that have shorter vectors are less informative in contrast to those with longer vectors, thus the locations Shirvan and Mehran were the most discriminating locations based on their vector length which are mostly in the warm zone which is characterized by low rainfall (Table 2). However, these discriminating locations can distinguish among tested genotypes and results of genotypes' comparison is more reliable. Also, the angle between a location vector and the ALC represents the representativeness of the location; the large angle causes less representativeness of location, thus locations Gachsaran and Khoramabad were most representative whereas Shirvan and Mehran were the least representative (Fig. 4). The most representative locations (Gachsaran and Khoramabad) are the dry zone and are characterized by high seasonal precipitation and a high risk of drought (Table 5). However, these representative locations can be regarded as the best agent among tested locations and can show characteristics of warm and dry areas. An ideal location should be both discriminating of the genotypes and representative of the average location, but we could not find such location in this research. To make breeding progress testing locations should be a combination of high and low yielding locations and in most cases testing and selection of genotypes are performed under high potential circumstances and selected genotypes usually exhibit poorly under low potential circumstances in contrast to genotypes that are selected under both circumstances (Setimela et al., 2007). To make a breeding gain, selection should be done under both low and high potential locations which permits the plant breeder to identify genotypes that will improve yield performance for both circumstances.

4 CONCLUSIONS

We identified the mega-environments I (Shirvan), II (Gachsaran), III (Mehran) and IV (Khoramabad) for grass pea production. The G5 (IFLA-1961) was an ideal genotype in terms of yield potential and stability compared with the other grass pea genotypes. According to the discriminativeness and representativeness properties of the test locations for grass pea, Shirvan and Mehran were the most discriminating locations. Finally, we found G5 (IFLA-1961) as high stable and high yield (4.92 t ha^{-1}) genotype for commercial variety release.

5 ACKNOWLEDGMENT

We wish to thank Dr. W. Yan for making available a version of GGEbiplot as "testBiplotxlsx". Contributions of the cooperators of the Iran grass pea performance trials are also gratefully acknowledged.

Table 5: Agro-climatic characteristics of test locations in multi-environmental trails of grass pea

Location	Longitude	Latitude	Altitude (m)	Rainfall (mm)	AYT†	Max	Min
Gachsaran	30°18′N	50°59′E	668	443	15.7	23.6	7.9
Khoramabad	33°39′N	48°28′E	1125	520	17.9	26.0	9.9
Mehran	33°07′N	46°09′E	136	275	18.0	24.6	11.3
Shirvan	37°27´N	57°55´E	1091	227	14.8	21.6	8.0

†AYT, average yearly temperature

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doi:10.14720/aas.2023.119.1.2639 Original research article / izvirni znanstveni članek

Effect of removing the spring flush and irrigation on the reflowering and late ripening of cactus pear *Opuntia ficus-indica* (L.) Mill.

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Received March 31, 2022; accepted December 29, 2022.
Delo je prispelo 31. marca 2022, sprejeto 29. decembra 2022

Effect of removing the spring flush and irrigation on the reflowering and late ripening of cactus pear *Opuntia ficus-indica* (L.) Mill.

Abstract: The effect of removing the spring flush and three irrigation doses on cactus pear's reflowering and late ripening were studied. Removing the spring flush (scozzolatura practice) and irrigation have a significant effect ($p \leq 0,05$) on the reflowering and late ripening of cactus pear. In not irrigated and not scozzolaturated plants (NINSP), the flowering extended from March 10 to May 25, 2017, and the ripening extended from May 20 to August 15, 2017. Whereas, in irrigated and scozzolaturated plants (ISP) the reflowering extended from June 15 to August 24, 2017, and the ripening extended from July 21 to November 20, 2017. The number of growths was higher in ISP (92-103 per plant) than in NINSP (81 per plant). Fruit yield was higher in NINSP (56.2 t ha^{-1}) than in ISP (26.2-36.5 t ha^{-1}), but fruit mass (123-131 g) and fruit length (7.83-7.92 cm) were higher in ISP than in NINSP (fruit mass: 103 g and fruit length: 7.72 cm). The rate of juice in fruits was also higher in ISP (49-55 %) than in NINSP (43 %), and the content of sugars was higher in NINSP (15.20 °Brix) than in ISP (13.83-14.56 °Brix).

Key words: *Opuntia ficus-indica*; cactus pear, scozzolatura; flowering; ripening; fruit yield, fruit quality

Učinek odstranjevanja spomladanskih cvetnih nastavkov in namakanja na ponovno cvetenje in pozno zorenje plodov opuncije *Opuntia ficus-indica* (L.) Mill.

Izvleček: V raziskavi je bil preučevan učinek odstranjevanja nastavka prvih pomladanskih cvetnih nastavkov in treh namakanj na ponovno in pozno zorenje plodov opuncije. Odstranjevanje nastavka pomladanskih cvetov (postopek «skocolatura» -scozzolatura practice) in namakanja sta imela značilen učinek ($p \leq 0,05$) na ponovno cvetenje in pozno dozorevanje plodov opuncije. Pri nenamakanih in neodstranjenih prvih cvetnih nastavkih (NINSP) je cvetenje potekalo od desetega marca do petindvajsetega maja 2017, zorenje plodov pa od dvajsetega maja do petnajstega avgusta, 2017. Pri namakanih in skocolatiranih rastlinah (ISP) je ponovno cvetenje potekalo od petnajstega junija do štiriindvajsetega avgusta, zorenje plodov pa se je podaljšalo na obdobje od enaindvajsetega julija do dvajsetega novembra, 2017. Število plodov je bilo večje pri ISP rastlinah (92-103 na rastlino) kot pri NINSP rastlinah (81 na rastlino). Pridelek plodov je bil večji pri NINSP (56,2 t ha^{-1}) kot pri ISP rastlinah (26,2-36,5 t ha^{-1}), tudi masa plodov (123-131 g) in dolžina plodov (7,83-7,92 cm) sta bili večji pri pri ISP kot pri NINSP rastlinah (masa plodov 103 g in dolžina plodov 7,72 cm). Delež soka v plodovih je bil pravtako večji pri ISP (49-55 %) kot pri NINSP rastlinah (43 %), a vsebnost sladkorjev je bila večja pri NINSP (15,20 °Brix) kot pri ISP rastlinah (13,83-14,56 °Brix).

Ključne besede: *Opuntia ficus-indica*; plodovi; skocolatura (scozzolatura); cvetenje; zorenje; pridelek plodov; kakovost plodov

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1 INTRODUCTION

In the northern hemisphere, the main flush of flowers of cactus pear is set in spring. But the plant can set another or several flushes of flowers when the climatic conditions are favorable or under advanced management practices like irrigation, fertilization or the removal of the spring flush of flowers and cladodes. The removing of the spring flush is called 'scozzolatura' practice in Italy where it's used to induce a second flush of flowers and cladodes a few weeks later, and a late ripening in September-November (Barbera et al., 1991 and 1992; Barbera & Inglese, 1993; Nerd & Mizrahi, 1994; Mulas, 1997; Anonymus, 2015). The scozzolatura practice was discovered for the first time in Sicily by the beginning of the 19th century. The second flush of flowers depends on the cultivar's aptitude for reflowering, the growing medium's environmental factors, and the time and degree of removing the spring flush (Barbera et al., 1991). Several authors reported that the rate of reflowering is related to the number of removed cladodes, even if their removal is less than that of floral buds (Inglese et al., 1994). The number of floral buds resulting from the scozzolatura practice decreases with increased cladodes left on the plant after this practice (Anonymous, 2015). The full flowering stage (50 % flowers in bloom) may be the favorable period for the scozzolatura practice, leading to the sufficient right of the second flush of flowers and cladodes, and this period could be prolonged till the end flowering (100 % flowers in bloom) (Barbera et al., 1991; Ochoa et al., 2009; Anonymus, 2015). Whereas early removing did not reduce the number of flowers of the second flush, but led to early ripening of the late fruiting (15 to 40 days precocity in comparison with removing applied between the stage of full flowering and end flowering) (Anonymous, 2015). Removing used during the stage of fruit enlargement (after full flowering) negatively affects the number of flowers and the set fruits of the second flush (Barbera et al., 1991; Brutsch & Scott, 1991; Anonymous, 2015; Boujghagh & Bouharroud, 2015). In south Morocco, Boujghagh and Bouharroud (2015 have removed the spring flush during four flowering stages of cactus pear *O. ficus-indica:* (S1) the early stage of flowering (1 % flowers in bloom), (S2) the full flowering stage (50 % flowers in bloom), (S3) the end flowering stage at 50 % fall corollas, and (S4) end flowering stage after 50 % fall corollas. Their results have shown that removing the spring flush of flowers and cladodes in any flowering stage led to a second flush of flowering. Removing applied between S2 and S4 has given a higher rate of reflowering. Other authors (Anonymous, 2015) also reported that removing between the S2 and S4 stages leads to a high number of fruits. Removing the spring flush at S1, S2, S3, and S4 made it possible to delay the reflowering for 25 days with S1, 62 days with S2, 94 days with S3, and 115 days with S4 in comparison to the seasonal flowering of spring. Removing the spring flush at these stages also make it possible to delay the fruit ripening period for 25 days with S1, 43 days with S2, 64 days with S3, and 81 day with S4 compared to the seasonal ripening period (Boujghagh & Bouharroud, 2015). In Argentina, Ochoa et al. (2009) have studied the effects of the scozzolatura practice and irrigation on the reflowering and late fruiting of cactus pear *O. ficus-indica*. Their results have shown that not irrigated plants are flowering before the irrigated plants, and removing the spring flush delays the reflowering for 38 days in not irrigated plants and 85 days in irrigated plants compared to irrigated and not scozzolatured plants.

Fruits from the scozzolatura practice are larger and more delicious than seasonal fruits; they also contain more pulp and fewer seeds (Barbera & Inglese, 1993; Mulas, 1997; Ochoa et al., 2009). Regarding the emission of growths after the scozzolatura, the number of emitted growths is 10 to 40 % less than the spring flush of growths (Inglese, 1995).

The seasonal ripening period of cactus pear in Morocco is in summer between mid-July and mid-August. This seasonal crop exceeds the request of consumers for the fruit, which led to overproduction. The valorization possibilities of this seasonal crop are still limited, and the management practices that can delay the ripening period, mainly removing of the spring flush, prove to be interesting. Even though the scozzolatura practice is known in several countries, the reflowering consequences and the management aspects of the practice are still subject of research studies to be developed. Little information is available on the effect of removing the spring flush and irrigation on the reflowering and late ripening of cactus pear, and on fruit yield and quality (Barbera et al., 1991; Brutsh & Scott, 1991; Ochoa et al., 2009). The goal of this work was to study the effects of the scozzolatura practice and irrigation on the late ripening of cactus pear which is to sell fruits with an interesting price on the local market.

2 MATERIALS AND METHODS

Experiments were carried out in the semi-arid area of Souss Massa in South Morocco, at the experimental station of Hassan II Institute of Agronomy and Veterinary Medicine in Agadir, latitude 30°22' North, longitude 9°39' West and 32 m altitude. Annual rainfall in this area is low (207 mm), the average temperature is for 7 °C in January and 40 °C in August. The soil of the trials site is as follows: 4.3 % coarse sand, 30.1 % fine sand, 26 % coarse silt, 22.8 % fine silt, and 17.6 % clay. The parcel of trials is equipped with a drip irrigation system and each line of plantation is equipped with a ramp where drippers are spaced 40 cm and their flow rate is 4 liters per hour, and each plant is irrigated with two drippers (flow rate of 8 liters per hour). Trials were carried out on an 18-year-old plantation of cactus pear *O. ficus-indica* 'Aissa'. Plants had an average width of 1.6 m and the average height of 2 m. They were spaced 3 m between lines and 1.5 m between plants (2230 plants ha^{-1}). Irrigation was applied in a single watering and irrigation dose (ID) was determined according to CEMAGREF (1992):

$$ID = f \times (HCC\text{-}HPFP) \times z \times PSH/100$$

ID: Irrigation dose in mm. f: Cultural coefficient (depends on the culture): f = 0.5 for a mean culture with mean rooting. HCC: Humidity at a ground capacity of the field (mm per m depth in soil) (30 %). HPFP: Humidity at permanent withering point (mm per m depth in soil) (15 %). z: The ground depth occupied by most of the roots (60 cm for cactus pear). PSH: Percentage of humidified ground (100 % for full saturation of the ground and 50 % for half saturation)

For full saturation of the ground with water (100 %): $ID = 0.5 \times (30 - 15) \times 60 \times 100/100 = 45$ mm, and for half saturation of the ground (50 %): $ID = 0.5 \times (30\text{-}15) \times 50/100 = 22.5$ mm

Watering duration (wd) is determined as follow:

$$wd = ID/hourly\ rainfall$$

wd: watering duration (in an hour). ID: irrigation dose in one application (in mm). Hourly rainfall (mm/hour) = drippers flow (4 liters per hour) x number of drippers (2) per area really occupied by roots (1 m^2) = 8 mm per hour

For full saturation of the ground with water: wd = 45/8 = 6 hours watering in one application and for half saturation of the ground: wd = 22.5/8 = 3 hours watering in one application

Experimental design was a split-plot with two parameters: (i) irrigation with three treatments: I0 (without irrigation), I1 (irrigation dose of 45 mm in one application for full saturation of the ground), and I2 (irrigation dose of 22.5 mm in one application for half saturation of the ground); (ii) the scozzolatura practice with two treatments: without application of the scozzolatura practice (NS) and with application of the scozzolatura practice (SP). The scozzolatura practice was applied during the full flowering stage (50 % flowers in bloom) on April 27th, 2017. Irrigation treatments were the great plots of parcels and the scozzolatura practice treatments were the small parcels or experimental units with two plants per small parcel.

Statistical analysis of data was carried out with MINITAB software. It focused on the analysis of variance with two parameters, and data analysis was also completed with a comparison of means.

Observations started on February 2017 once a week from the emission of floral buds till the end of the ripening stage of scozzolaturated plants (SP). They focused on the enumeration of flowers and cladodes before and after the scozzolatura practice and irrigation. Observations also were related to the duration of the flowering and ripening stages from the beginning of each stage (5 % flowers in bloom for flowering and 5 % ripened fruits for ripening) till the end of each stage (100 % flowers in bloom for flowering and 100 % ripened fruits for ripening). Fruit yield was determined on two plants per treatment of irrigation or scozzolatura practice and per bloc. Fruit mass and size were determined on a sample of 20 fruits per plant per treatment of irrigation or scozzolatura practice and per bloc; the sample of fruits was randomly harvested on the four orientations of the plants (east, west, north and south). Fruit mass was determined with a balance of 0.01 g of precision and fruit size (length and diameter) and peel thickness were determined with a caliper. The ripening stage is reached when fruit color change from the green to yellowish green, and the percentage of fruits at ripening stage was determined as follow (Oelofse et al., 2006):

$$\%\ fruits\ at\ ripening\ stage = number\ of\ fruits\ at\ ripening\ stage\ /\ total\ number\ of\ fruits\ on\ the\ plant \times 100$$

The organoleptic parameters of the fruit focused on the juice content and sugars (°Brix) in the fruits, the titratable acidity and the pH of the juice. The same sample of fruits used in the determination of fruit mass and size was used for the chemical analysis of the fruits. The number of seeds by fruit was counted. The content of sugars in the fruits was determined with an electronic refractometer HI 96801, and the fruit juice rate was determined by crushing fruits with a mixer and filtering juice in 1 mm sieve to separate seeds from the juice. The percentage of juice in the fruit (J) was determined as follow:

$$\%\ juice\ in\ the\ fruit\ (J) = Juice\ mass/pulp\ mass \times 100$$

The pH of the juice was determined with a pH meter and the titratable acidity was carried out by the titration of the juice using NaOH 0.1 N and phenolphthalein as an indicator of color change. Titratable acidity (QCA) was determined as follows (IFU, 2017):

$$QAC = 0.64\ x\ V\ \text{NaOH}$$

QAC: Quantity of citric acid (g citric acid per liter of juice). V NaOH: volume NaOH 0.1 N used in the titration.

3 RESULTS AND DISCUSSION

3.1 EFFECTS OF REMOVING THE SPRING FLUSH AND IRRIGATION ON THE REFLOWERING AND LATE RIPENING OF CACTUS PEAR

Removing spring flush had a significant effect ($p \leq 0.05$) on the reflowering and late ripening of cactus pear. In not irrigated plants (NI), the period of flowering of not scozzolaturated plants (NSP) (the spring flush of flowering) extended from March 10th till May 25th 2017, and the period of reflowering of the scozzolaturated plants (SP) (the second flush of flowers) extended from June 10th till July 25th 2017. The average number of flowers per plant was 159 in NSP and 111 in SP. The ratio number of flowers resulting from the scozzolatura practice /number of flowers of the spring flush is 0.69. This is following the results of several authors (Barbera et al., 1991; Anonymus, 2015) who confirmed that the rate of reflowering in cactus pear after the scozzolatura practice is strongly related to the rate of the spring flush. The ratio number of flowers after the scozzolatura practice / number of flowers of the spring flush varies between 0.7 and 1 if the removing of the spring flush is practiced between full flowering and end flowering. Our rate of flowering (0.69) exceeds the rate which is reported by Barbera et al. (1991) (0.50) who indicated that this rate might be sufficient for the second flush of flowers after the scozzolatura practice.

Irrigation had a significant effect ($p \leq 0.05$) on the reflowering of cactus pear. In irrigated and scozzolaturated plants (ISP), the reflowering period extended from June 15 to July 30, 2017 for I2 treatment of irrigation (50 % saturation of the ground) and from June 20 to August 24, 2017 for I1 treatment of irrigation (100 % saturation of the ground). Whereas in not irrigated and scozzolaturated plants (NISP), the reflowering period extended from June 10 to July 25, 2017 (Figure 1). ISP have emitted an average number of 61 flowers per plant for I1 treatment and 85 per plant for I2 treatment. Whereas NISP has emitted an average number of 111 flowers per plant. INSP have emitted an average number of 183 flowers per plant for I1 treatment and 179 per plant for I2

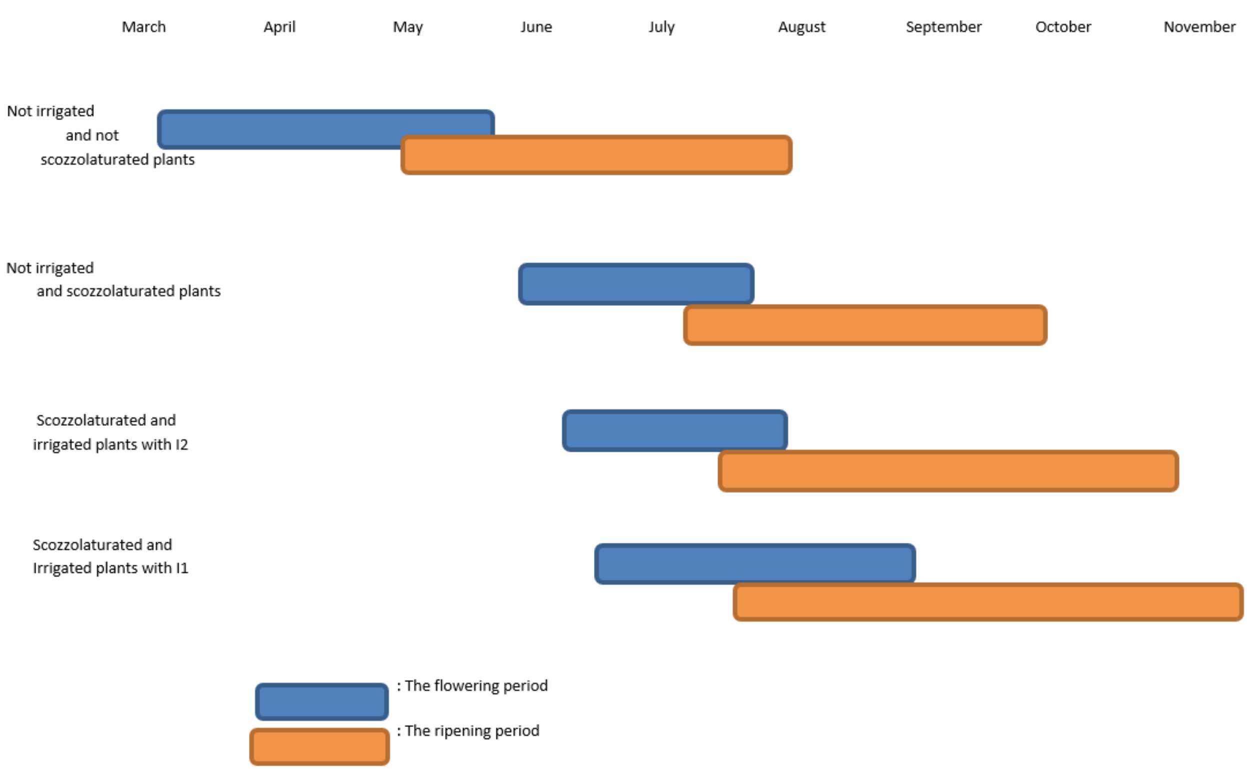

Figure 1: Effect of the scozzolatura practice and irrigation on the reflowering and late ripening periods of cactus pear (*Opuntia ficus-indica* (L.) Mill.) in Souss-Massa area

treatment (Table 1). The rate of reflowering in ISP is for 69 % for I0. 33 % for I1 and 47 % for I2. According to Barbera et al. (1992) and Anonymous (2015), this rate of reflowering may be sufficient for I0 (69 %) and I2 (47 %) and insufficient for I1 (33 %). In comparison with not irrigated plants, irrigation of cactus pear after the scozzolatura practice disadvantages the initiation of flowers. Interaction of the two factors irrigation and scozzolatura practice had significant effect ($p \leq 0{,}001$) on the reflowering of cactus pear. In NINSP, the average number of flowers was 159 per plant, whereas in ISP the average number was 61 to 85 per plant (Table 1). Our results on IP with I2 (which is a moderate amount of irrigation in comparison with I1), and NSP, are similar to those of Arba et al. (2018) who reported that irrigation of not scozzolaturated plants with a moderate amount of water in spring (30 mm) had increased the emission of floral buds.

The scozzolatura practice significantly affected the late ripening of cactus pear. The ripening period of NINSP extended from May 20th to August 15th 2017, and the ripening period of NISP extended from June 15th to October 10th 2017. Whereas the ripening period of ISP extended from July 28th to November 20th 2017 for I1 and from July 20st to November 12th 2017 for I2 (Figure 1). Our results are similar to those of Ochoa et al. (2009) who reported that in SP the reflowering and fruiting periods are more lately in irrigated plants than in not irrigated ones. According to several authors (Barbera et al., 1991; Brutsch & Scott, 1991), the period when the scozzolatura practice is applied also affect the ripening period. Boujghagh & Bouharroud (2015) indicated that removing the spring flush before flowering led to early ripening for 15 to 20 days compared to removing applied in full flowering, and for 30 to 40 days in comparison to removing applied in end flowering.

3.2 EFFECT OF REMOVING THE SPRING FLUSH AND IRRIGATION ON THE INITIATION OF GROWTHS

The scozzolatura practice had significant effect on the initiation of growths. In not irrigated plants, the number of growths was higher in NSP (81 per plant) than in SP (21 per plant). Whereas in irrigated plants, the initiation of growths was higher in SP (103 per plant for I1 and 92 for I2) than in NSP (72 per plant for I1 and 80 for I2) (Table 1). Interaction of the two factors had a very significant effect ($p \leq 0.01$) on the initiation of growths. Our results are similar to those of Inglese (1995) who reported that in NIP, the number of growths was lower in SP (10 to 40 %) than in NSP (more than 50 %), but in IP the number of growths was higher in SP than in NSP. Arba (2017) and Arba et al. (2018) also reported that irrigation increased the initiation of growths in cactus pear.

3.3 EFFECT OF REMOVING THE SPRING FLUSH AND IRRIGATION ON FRUIT YIELD AND QUALITY

3.3.1 Effect on fruit yield and fruit size

The scozzolatura practice had no significant effect ($p \geq 0.05$) on the peel mass and skin thickness. SP and NSP have a similar peel mass of 52 g and a similar skin thickness of 4.5 mm. Therefore, irrigation had a significant effect ($p \leq 0{,}001$) on the peel mass and skin thickness. The fruits of IP, scozzolaturated or not, have higher skin mass (53 to 57 g) and skin thickness (4.52 to 4.92 mm) than the fruits of NIP, scozzolaturated or not (skin mass of 47 g and skin thickness of 4.12 mm). Interaction of the two factors had significant effect ($p \leq 0{,}001$) on the

Table 1 : Effect of the scozzolatura practice and irrigation on the initiation of flowers and growths in cactus pear *Opuntia ficus-indica* (L.) Mill. in Souss-Massa area

	Not scozzolaturated plants (NSP)	Scozzolaturated plants (SP)
Traitments of irrigation	Number of emitted flowers per plant	
I0	159 ± 3 a	111 ± 2,5 b
I1	183 ± 4 ab	61 ± 1,5 d
I2	179 ± 4 a	85 ± 1,5 c
	Number of emitted growths per plant	
I0	81 ± 3,5 ab	21 ± 1,3 c
I1	72 ± 3 b	103 ± 4 d
I2	80 ± 3,5 b	92 ± 4 ad

I0: Without irrigation; I1: Saturation of the ground with water; I2: Half saturation of the ground with water
a, b, c and d: Comparison groups according to Tukey test with a confiance level of 95 %

Table 2 : Effect of the scozzolatura practice and irrigation on fruit yield and fruit size of cactus pear *Opuntia ficus-indica* (L.) Mill. in Souss Massa area.

	Not scozzolaturated plants			Scozzolaturated plants		
	Treatments of irrigation					
	I0	I1	I2	I0	I1	I2
Fruit yield (t ha^{-1})	56,2 ± 2,5	62,5 ± 3	71,5 ± 3,5	44,1± 1,5	26,2 ± 1	36,5 ± 1,5
Fruit lenght (cm)	7,46	7,52	7,57	7,72	7,83	7,92
Fruit diameter (cm)	5,23	5,41	5,56	5,17	5,46	5,83
Fruit mass (g)	103,4 ± 3 a	120,9 ± 3,5 bc	119,3 ± 3,5 c	106,9 ± 3 d	130,9 ± 4 a	122,9 ± 3,5 b
Pulp mass (g)	55,99 ± 3 e	68,59 ± 3,5 b	62,39 ± 3 c	59,67 ± 3 d	75,10 ± 4 a	69,74 ± 3,5 b
Peel mass (Effect of removing the spring flush and irrigation on the reflowering and late ripening of cactus pear *Opuntia ficus-indica* (L.) Mill.)	47,38 ± 2,5 c	52,33 ± 3 b	56,96 ± 3 a	47,21 ± 2,5 c	55,76 ± 3 ab	53,15 ± 3 b
Peel thikness (mm)	4,10 d	4,86 b	4,87 bcd	4,13 cd	5,10 a	4,56 bc
Number of seeds / 10 g pulp	47,41 ± 2,5 a	45,79 ±2 ab	46,37 ±2,5 a	45,96 ±2,5 a	41,93 ± 2 c	44,10 ± 2 b

I0: Without irrigation; I1: Saturation of the ground with water ; I2: Half saturation of the ground with water
a, b, c and d: Comparison groups according to Tukey test with a confiance level of 95 %

skin mass, skin thickness, and on the number of seeds per fruit. Average number of seeds per fruit was lower in SP (44 per 10 g pulp) than in NSP (47 per 10 g pulp) and average number of seeds per fruit in IP, scozzolaturated or not, was lower (42 to 46 per 10 g pulp) than that of NIP, scozzolaturated or not (46 to 48 seeds per 10 g pulp). Barbera & Inglese (1993) and Mulas (1997) also have reported that the fruits of SP contain fewer seeds than those of NSP.

3.3.2 Effect on the organoleptic parameters of the fruits

The scozzolatura practice and irrigation, and the interaction of the 2 factors have a significant effect ($p \leq 0{,}001$) on the content of juice in the fruits. The rate of juice in the fruits of ISP was higher (49 to 55 %) than that of NINSP (43 %) (Table 3).

The scozzolatura practice and irrigation also have a significant effect ($p \leq 0{,}001$) on the content of sugars in the fruits. The rate of sugars was higher (15.20 °Brix) in the fruits of NISP than in those of NINSP (14.13 °Brix), the fruits of ISP (13.83 to14.56 °Brix) and those of INSP (12.91 to 13.11 °Brix) (Table 3). Interaction of the two factors had no significant effect ($p \geq 0.05$) on the content of sugars in the fruits. Several authors also reported that the fruits of SP are tastier and sweeter than the fruits of NSP (Barbera & Inglese, 1993; Mulas, 1997; Ochoa et al., 2009; Anonymous, 2015), and other authors indicated that irrigation decreases the rate of sugars in the juice due to dilution and increases the content of juice in the fruits (Felker et al., 2002; Barbara, 2007; Arba, 2017; Arba et al., 2021). The scozzolatura practice and rrigation also have significant effect ($p \leq 0{,}001$) on the content of citric acid in the fruits and on the pH of juice. The rate of citric acid in the fruits (15.20 g l^{-1} of juice) and the pH of juice (6.28) were higher in NISP than in NINSP (14.13 g/l for the citric acid and 5.98 for the pH), and the content of acidity and the pH of juice (13.83 to 14.56 g l^{-1} for the citric acid and 6.37 to 6.49 for the pH) were also higher in ISP than in INSP (12.91 to13.11 g l^{-1} for the citric acid and 6.23 to 6.35 for the pH) (Table 3). Interaction of the two factors also had significant effect ($p \leq 0{,}001$) on the rate of acidity and the pH of juice. Our results are similar to those of several authors (Barbera & Inglese, 1993; Mulas, 1997; Anonymous 2015) who reported that the fruits of SP are tastier than those of NSP since the acidity rate in the fruits is an essential criterion in the fruit flavour.

4 CONCLUSIONS

The scozzolatura practice leads to the emission of the second flush of flowers and the rate of reflowering may be sufficient since it exceeded 50 % of the spring flush. In SP, irrigation favored the emission of growths than flowering. The scozzolatura practice and irrigation significantly affect the late ripening of cactus pear in Souss-Massa area. The late ripening was at least one

Table 3: Effect of the scozzolatura practice and irrigation on the content of juice and sugars in the fruits, and on the titratable acidity and the pH of juice of *O. ficus-indica* (L.) Mill. in Souss-Massa area

	Not scozzolaturated plants			Scozzolaturated plants		
	Traitments of irrigation					
	I0	I1	I2	I0	I1	I2
The content of juice n the fruits (%)	43,20 ± 2 f	51,33 ± 4 b	46,46 ± 3 d	44,96 ± 4 e	55,41 ± 3 a	49,09 ± 3 c
The content of sugars in the fruits (°Brix)	14,13 ± 0,4 b	12,91 ± 0,3 d	13,11 ± 0,5 cd	15,20 ± 0,3 a	13,83 ± 0,5 bc	14,56 ± 0,4 ab
The content of citric acide in the fruits (g l^{-1} juice)	1,337 a	1,100 cd	1,217 b	1,289 a	1,071 d	1,141 c
pH of the juice	5,98 d	6,35 b	6,23 c	6,28 ab	6,49 a	6,37 b

I0: Without irrigation; I1: Saturation of the ground in water ; I2: Half saturation of the ground in water
a, b, c, d, e and f: Comparison groups according to Tukey test with a confiance level of 95 %

month later in ISP than in NISP and NINSP, and the ripening of NISP was two months later than that of NINSP. Fruit yield was lower in ISP than in NISP and NINSP. Therefore, fruit size was larger in ISP than in NISP and NINSP. Irrigation increased the skin mass and thickness in the fruits of SP and NSP, but the scozzolatura practice did not affect these parameters. Irrigation and the scozzolatura practice improved the juice rate in the fruits, but they decreased the rate of sugars, the acidity and the pH of juice, and the number of seeds in the fruits.

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doi:10.14720/aas.2023.119.1.2380 Original research article / izvirni znanstveni članek

Comparison of shoot and root regeneration of miniature potted rose (*Rosa* x *hybrida* L.) and Damask rose (*R. damascena* Mill.) in microculture system

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Received October 16, 2021; accepted December 29, 2022.
Delo je prispelo 16. oktobra 2021, sprejeto 29. decembra 2022

Comparison of shoot and root regeneration of miniature potted rose (*Rosa* x *hybrida* L.) and Damask rose (*R. damascena* Mill.) in microculture system

Abstract: Miniature potted rose and Damask rose are important commercial plant cultivars in ornamental horticulture. Root suckers are common rose propagation method, but it is slow and seasonally dependent. In this survey, the propagation of nodal explants of these two species was studied through *in vitro* regeneration system. 16 and 24 different media were used for study of shoot and root regeneration respectively. The axillary buds were sprouted earlier in miniature rose than *R. damascena*. Shoot induction and proliferation (shoot ramification and growth) were observed 5 and 17 days after planting in miniature rose and 16 and 38 days in *R. damascena* respectively. The highest shoot proliferation obtained in media 3 and 7 in miniature rose, and medium 16 for *R. damascena*. These three media were recorded as optimal media with 100 % shoot proliferation. In these media, root initiation and growth of miniature rose (respectively after 78 and 92 days) was earlier than Damask rose (respectively 125 and 138 days). The successful rooting occurred in three and two media for miniature and Damask rose respectively. Rooting frequency was higher in the half strength MS liquid media than the others. Thus, cultivar potted rose as a modern species is propagated easier than old rose (*R. damascena*).

Key words: micropropagation; proliferation; rooting rate

Primerjava regeneracije poganjkov in korenin pri miniaturni ločni vrtnici (*Rosa hybrida* L.) in damaščanski vrtnici (*R. damascena* Mill.) v mikrokulturi

Izvleček: Miniaturna lončna vrtnica in damaščanska vrtnica sta pomembni komercialni sorti med okrasnimi rastlinami. Za razmnoževanje vrtnic se najbolj pogosto uporabljajo poganjki iz korenin, a je njihova rast počasna in sezonsko odvisna. V raziskavi je bilo preučevano razmnoževanje teh dveh sort z izsečki nodijev v *in vitro* kulturah. Za regeneracijo poganjkov in korenin je bilo uporabljeno 16 in 24 različnih medijev. Zalistni brsti so odgnali prej pri miniaturni kot pri damaščanski vrtnici. Zasnova in rast poganjkov sta se pri miniaturni vrtnici pojavili 5 in 7 dni po sadnji in 16 ter 38 pri damaščanski vrtnici. Največje število poganjkov je bilo pri miniaturni vrtnici v medijih 3 in 7 in v mediju 16 pri damaščanski vrtnici. Ti trije mediji so bili prepoznani kot optimalni, sa je bila v njih dosežena 100 % tvorba poganjkov. V teh medijih sta bili zasnova in rast korenin pri miniaturni vrtnici zgodnejši (po 78 in 92 dneh) kot pri damaščanski vrtnici (po 125 in 138 dneh). Uspešno ukoreninjenje se je za miniaturno vrtnico pojavilo v treh medijih in le v dveh za damaščansko vrtnico. Pogostnost ukoreninjenja je bila večja v polovičnih tekočih MS medijih kot v drugih. Zaključimo lahko, da se miniaturna lončna vrtnica kot moderna vrsta razmnožuje lažje kot starinska damaščanska vrtnica.

Ključne besede: mikropropagacija; proliferacija; hitrost ukoreninjanja

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1 INTRODUCTION

There are more than 18,000 cultivars of roses, which collectively are based on only eight of the approximately 200 wild species in Rosa: *R. damascena*, *R. chinensis*, *R. wichuraiana* Crép., *R. odorata* (Andrews) Sweet, *R. moschata* Herrm., *R. multiflora* Thunb., *R. foetida* Herrm., and *R. rugose* Thunb.. The cultivation of roses for many purposes has been widespread in temperate climates throughout the world (Ma et al., 1996; Khaleghi and Khadivi, 2020). Roses are one of the most important commercial plants, and as the queen of flowers are very important due to their usage in high value essential oil production and as garden rose, potted plants and cut flowers. The rose essential oil is used in perfumes for their sweet and long lasting fragrance (Muiruri et al., 2011).

Rosa damascena is one of the most important species of *Rosa* genus, commonly known as Damask rose (known as Gole Mohammdi in Iran), which is cultivated in Bulgaria, France, Italy, Turkey, Iran, Morocco, USA, and India. *R. damascena*, a beautiful aromatic flower with immense horticultural importance, is one of the oldest and most valuable species. In addition, it has many applications in the perfume, cosmetic, and food industries including production of rosewater, jam, jellies, conserves. This species is also used worldwide for manufacture of products with diverse applications such as aromatherapeutic, anti-HIV, antibacterial, antioxidant, antidepressant, antimicrobial, antiseptic, astringent agent, antispasmodic, sedative and blood cholesterol altering (Ozkan et al., 2004; Khaleghi and Khadivi, 2020).

Miniature roses are a type of roses that are smaller in mass than the others. Miniature roses are characterized with a prolonged flowering and could be used for creation of borders, flower beds, dwarf rose trees as well as a pot culture (Younis et al., 2015; Brailko et al., 2017).

Roses are generally propagated by vegetative methods like cutting, layering, budding and grafting. In addition, seeds are used for propagation of species, new cultivars and production of rootstocks. Root suckers are the traditional and the most common rose propagation method, but this method is very low as it is seasonally dependent. Currently, *in vitro* micropropagation methods save time as well as can produce large numbers of plants within a small physical space (Pierik, 1991). Further, tissue culture permits manufacturing genetically similar and without disease plant material (Kadhimi et al., 2014; Cai et al., 2015). Micropropagation of rose species and their hybrids ranged from easy to difficult. Generally, plants with higher secondary metabolite contents are less suitable for growing in *in vitro* culture. It has been reported that a cytokinin such as 6- benzylamino purine (BAP) and an auxin, mostly 1-naphthalene acetic acid (NAA), or 2,4-dichlorophenoxyacetic acid (2,4-D) are normally included in the primary culture medium and have essential role on shoot proliferation in roses (Pati et al., 2010, Ahmadian et al., 2013). Indole-3 acetic acid (IAA) causes enlargement of plant cells, cell division, lateral branching of shoots and roots and vascular differentiation (Hobbie et al., 2000). Successful micro-propagation of some rose cultivars has been reported previously (Pati et al., 2010; Mahmoudi Noodezh et al., 2012). However, the success of these methods is dependent on the cultivar and genetic background of the plant. Some cultivars do not response to *in vitro* conditions; their proliferation and rooting rate is slow and many plantlets die during acclimatization (Alsemaan, 2013). Most studies on rose propagation have been carried out in *Rosa damascena* and only few studies on miniature rose. There are reports showing that rose micropropagation depends on cultivar genotype, the type and age of explant and type of culture media. The objective of the current study was to inves-

Fig. 1A-C: Rose species. A: miniature rose, B, C: Damask rose tigate and compare the direct *in vitro* regeneration and proliferation of two rose species (Damask rose and miniature rose). Further, the effect of different combinations of plant growth regulators and different strengths of MS media (full and half) in solid and liquid media were studied and compared.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

Explants are one of the primary factors for the efficient regeneration of *in vitro* plant cultures. In this study, nodal segments of *Rosa damascene* (Damask rose) and miniature rose (cultivar Modern Hybrid) were used as the explants for *in vitro* culture establishment (Fig. 1). Newly sprouted and actively growing young branches were collected from 3–4 years old stock plants growing in Lalehzar area in Keman province, Iran. Foliar parts were removed and branches were cut into segments with 1–2 nodes per segment. These nodal shoot segments were surface-sterilized by washing in detergent for 10 min. Then, they were kept under tap water for 30 min, dipped in 70 % ethanol for 1 min, immersed in 10 % sodium hypochlorite plus 1 % Tween 20 for 3 min, and embedded in with 0.1 % $HgCl_2$ plus 1 % Tween 20 for 3 min, followed by 3 rinses with sterile distilled water. The last stage was embedding in antibiotics (100 mg l^{-1} ampicillin and tetracycline) for 20 min each (Tarrahi and Rezanejad, 2013).

2.2 MS MEDIUM PREPARATION AND SHOOT INITIATION AND MULTIPLICATION

The rate of tissue growth and morphogenetic responses are highly affected by media features. The medium consisted of Murashige and Skoog (1962) basal salts and vitamins supplemented with different concentrations of growth regulators including different combinations of BAP, 2,4-D, NAA and gibberellic acid (GA_3), sucrose (30 g l^{-1}), and agar (8 g l^{-1}) (16 different media, Tab. 1). The pH was adjusted to 5.7-5.8 before autoclaving. All media were sterilized by autoclaving for 20 min at 121 °C (1.5 kg cm^{-2} pressure). 15 sterilized explants were cultured on each petri dish containing autoclaved medium. Three petri dishes (3 repetitions) were used for each treatment and kept under a temperature of 25 ± 2°C and 16/8 h (light/dark) photoperiod. Light intensity of 23 µmol m^{-2} s^{-1} provided by cool white fluorescent tubes was used for shoot induction and proliferation and 11.5 µmol m^{-2} s^{-1} for root induction and growth. Explants were then routinely subcultured onto medium of the same composition in two weeks intervals.

2.3 ROOT INDUCTION AND GROWTH

Root formation and growth of healthy shoots (1.5-2 cm long) were studied and compared in both species using different strengths (full and half) of solid and liquid basal MS media supplemented with various concentrations of IAA (24 media, Tab. 2). For the highest root formation frequency, before transferring 1.5-2 cm shoots into rooting media, two pretreatments were utilized: one set of shoots were floated in 500 mg l^{-1} IAA for 1 min, and other set were cultured on solid MS containing 3 mgl $^{-1}$ 2,4-D for 2 weeks. Thus, 24 various media were used for root initiation and growth (Tab. 2).

2.4 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The experiments were conducted as a completely randomized design. Data are expressed as mean ± standard error (SE). Analysis of variance (ANOVA) was used

Tab. 1: Different types of culture media used in shoot induction and proliferation of Damask rose and miniature rose

	Plant growth regulators (mg l^{-1})			
Media	NAA	BAP	2, 4-D	GA_3
1	0.1	1	0	0.1
2	0.1	1.5	0	0.1
3	0.1	2	0	0.1
4	0.1	2.5	0	0.1
5	0.05	1	0	0.1
6	0.05	1.5	0	0.1
7	0.05	2	0	0.1
8	0.05	2.5	0	0.1
9	0	1	0.1	0.1
10	0	1.5	0.1	0.1
11	0	2	0.1	0.1
12	0	2.5	0.1	0.1
13	0	1	0.05	0.1
14	0	1.5	0.05	0.1
15	0	2	0.05	0.1
16	0	2.5	0.05	0.1

Each experiment had three replicates containing at least eight explants in each culture vessel

Tab. 2: Different types of culture media used in rooting of Damask rose and miniature rose

Medium number	Pretreatments	Medium type	Strength of MS medium	IAA (mg l^{-1})
1	Floating explants in 500 mg l^{-1} IAA for 1 min as pretreatment	Solid	Full	0.10
2		Solid	Full	0.05
3		Solid	Full	0.00
4		Solid	Half	0.10
5		Solid	Half	0.05
6		Solid	Half	0.00
7		Liquid	Full	0.10
8		Liquid	Full	0.05
9		Liquid	Full	0.00
10		Liquid	Half	0.10
11		Liquid	Half	0.05
12		Liquid	Half	0.00
13	Culturing explants on solid MS containing 3 mg l^{-1} 2,4-D for two weeks as pretreatment	Solid	Full	0.10
14		Solid	Full	0.05
15		Solid	Full	0.00
15		Solid	Half	0.10
17		Solid	Half	0.05
18		Solid	Half	0.00
19		Liquid	Full	0.10
20		Liquid	Full	0.05
21		Liquid	Full	0.00
22		Liquid	Half	0.10
23		Liquid	Half	0.05
24		Liquid	Half	0.00

Each experiment had three replicates containing at least eight explants in each culture vessel

to compare the means. Duncan's test ($p < 0.05$) was employed to determine significant differences between means. Statistical analysis was conducted using the SPSS software. Each experiment had three replicates containing minimum eight explants in each culture vessel.

3 RESULTS

3.1 SHOOT INDUCTION AND PROLIFERATION IN DIFFERENT CULTURE MEDIA

The sterilized nodal explants were inoculated on different media containing different hormonal combinations and their shoot induction and proliferation were compared (Tabs. 3, 4, Figs. 2, 3). In miniature rose, the highest shoot proliferation (100 %) was obtained in the presence of 2 mg l^{-1} BAP and concentrations of 0.05 and 0.1 mg l^{-1} NAA (media 3 and 7) whereas in *R. damascena*, the highest levels of proliferation were observed in the presence of 2.5 mg l^{-1} BAP and 0.05 mg l^{-1} of 2, 4-D (medium 16) (Tab. 4). Also, shoot proliferation in medium 6 for miniature rose and media 4, 7, 8, 12 for *R. damascena,* was higher than 90 % (Tab. 4). In these optimal media (more than 90% proliferation), the axillary buds were sprouted earlier in miniature rose compared with *R. damascena*. In miniature rose, shoot induction and proliferation were observed about 5 and 17 days after planting of single nodes respectively while these two phases occurred in *R. damascena* about 16 and 38 days after culturing respectively (Tab. 3 and Figs. 2, 3).

3.2 ROOT FORMATION AND GROWTH

Root formation and growth of healthy shoots were studied on various combinations of IAA under different strengths of MS media (full and half) in solid and liquid

Tab. 3: The comparison of shoot and root regeneration of miniature rose and Damask rose in optimal medium

	Days				
Species	Shoot induction	Shoot proliferation (1.5-2 cm long)	Transfer to the rooting medium	Root Initiation	Root length 2 cm
miniature rose	5.33	15.67	35.33	77.67	91.67
Damask rose	15.67	37.67	57.67	124.67	137.67

Tab. 4: The comparison of the effects of different combinations of plant growth regulators on the proliferation of Damask rose and miniature rose

	Growth regulators (m/l)				Proliferation (%)	
Media number	BAP	2, 4-D	NAA	GA_3	miniature rose	Damask rose
1	1.00	-	0.10	0.10	0.88d±57.78	57.78 ± 1.20cd
2	1.50	-	0.10	0.10	1.53abcd ±73.33	62.22 ± 1.45cd
3	2.00	-	0.10	0.10	0.34a0±100	± 1.53 cd66.67
4	2.50	-	0.10	0.10	0.88d±57.78	0.33a ±97.78
5	1.00	-	0.05	0.10	0.88abc±88.89	2.20cd±62.22
6	1.50	-	0.05	0.10	0.57ab±93.33	1.15cd±60
7	2.00	-	0.05	0.10	0.00a ±100	0.67ab ±91.11
8	2.50	-	0.05	0.10	1.20bcd±71.11	1.00ab ±93.33
9	1.00	0.10	-	0.10	0.88cd±62.22	11.15cd ±60
10	1.50	0.10	-	0.10	2.33bcd±71.11	1.20cd ±57.78
11	2.00	0.10	-	0.10	1.33d ±55.56	1.53cd ±53.33
12	2.50	0.10	-	0.10	0.53d ±53.33	0.33ab ±95.56
13	1.00	0.05	-	0.10	0.57 d ±6	0.88bc ±75.56
14	1.50	0.05	-	0.10	1.76cd ±64.44	1.00cd ±53.33
15	2.00	0.05	-	0.10	1.56abcd ±8	0.33d ±51.11
16	2.50	0.05	-	0.10	1.86abcd±77.78	0.41a ±100

Each experiment had three replicates containing at least eight explants in each culture vessel. Data are expressed as mean ± standard error, values with same letters in the same column are not significantly different ($p \leq 0.05$) using Duncan's multiple range test

culture media. Further, two pretreatments were utilized for best efficiency. In optimal media, root initiation and growth of miniature potted rose was observed earlier than *R. damascena*. In miniature rose, root initiation and growth were observed about 78 and 92 days after culturing respectively while in *R. damascena*, root emergence and growth were recorded 125 and 138 days after transferring to first medium for shoot induction respectively. Therefore, roots were initiated 42 and 67 days after transferring to rooting media respectively in miniature rose and Damask rose (Tab. 3 and Figs. 2, 3).

The results of rooting rate in various media revealed that the successful root formation just occurred in three media for miniature rose and two media for *R. damascena*. In both species, rooting frequency was higher in the half strength MS liquid medium than half strength MS solid medium (Tab. 5).

In miniature rose, these three media are as follows: 1- the half strength MS liquid medium containing 0.05 mg l^{-1} IAA floated in 500 mg l^{-1} IAA (for one minute) as pretreatment with rooting frequency of 60 %. 2- half strength MS liquid medium without IAA, pretreatment in solid MS medium containing 3 mg l^{-1} 2, 4-D for 2 weeks, with a rooting rate 62 %). 3- the half strength MS solid medium containing 0.05 mg l^{-1} IAA pretreated in solid MS medium containing 3 mgl^{-1} 2, 4-D for 2 weeks, with root formation at a rate of 29% (Tab. 5, the underlined values).

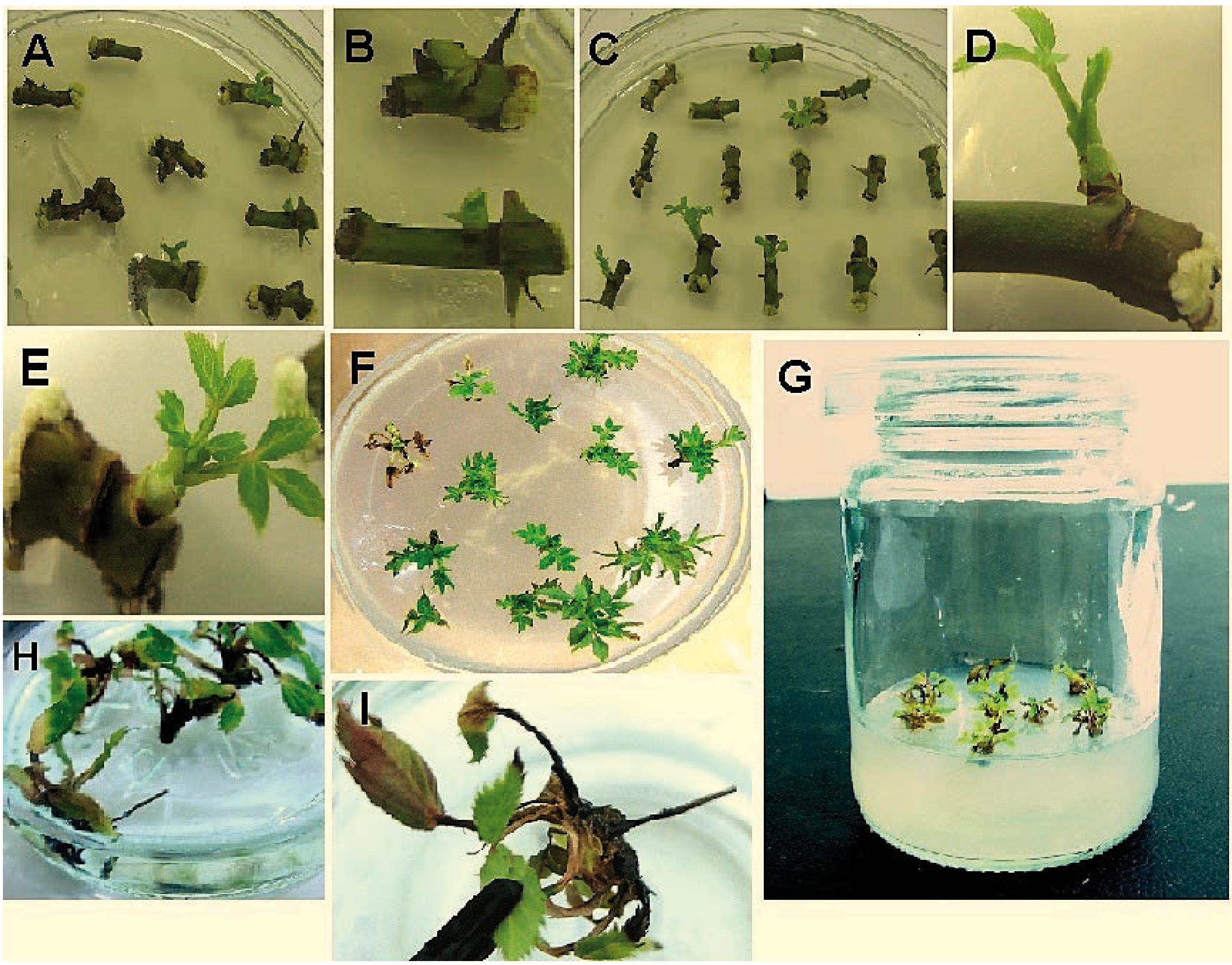

Fig. 2: A-I: Different stages of miniature rose propagation in optimal medium. A, B: The first stage of shoot induction and growth (swelling); C-F: Shoot proliferation and formation of the multi-leaf shoots (1.5-2 cm long); G-I: Transfer to the rooting medium and shoot and root growth (I, 78 days after initial culture)

In *R. damascena*, rooting percentage obtained about 53 % in half strength MS liquid medium containing 0.05 mg l^{-1} IAA (pretreated onto solid MS medium containing 3 mg l^{-1} 2,4-D for 2 weeks). The other optimal medium with 32 % rooting percentage was recorded in half strength MS solid medium containing 0.1 mg l^{-1} IAA (floated in 500 mg l^{-1} IAA for 1 min as pretreatment) (Tab. 5, the underlined values).

4 DISCUSSION

MS medium has been reported as the most common basal medium used for rose micro-propagation. In addition, modified MS and ½MS media have been used successfully in various studies of rose species. Mahmoudi Noodezh et al. (2012) utilized a modified MS medium with higher levels of nitrates, calcium, and iron supplemented with 4 mg l^{-1} 6-benzylaminopurine and 0.25 mg l^{-1} indole-3-acetic acid for shoot initiation and proliferation in *R. damascena*. Also, their results showed that a liquid half-strength medium supplemented with 1 mg l^{-1} IBA is the most successful medium for *in vitro* rooting in this cultivar. Badzian et al. (1991) reported that medium containing ½MS and 1g l^{-1} activated charcoal was appropriate for root formation in miniature rose cultivars (Badzian et al., 1991).

In this study, the induction and growth of shoots and roots of two rose species were investigated in 16 different media for shoot initiation and growth and 24 different media for rooting. Proliferation is the most important stage of micropropagation and hence a successful protocol with high efficiency is needed to increase its quality. Cytokinins are the main growth regulators during proliferation. The induction and growth rate of explants depends upon many factors like season of sampling, age

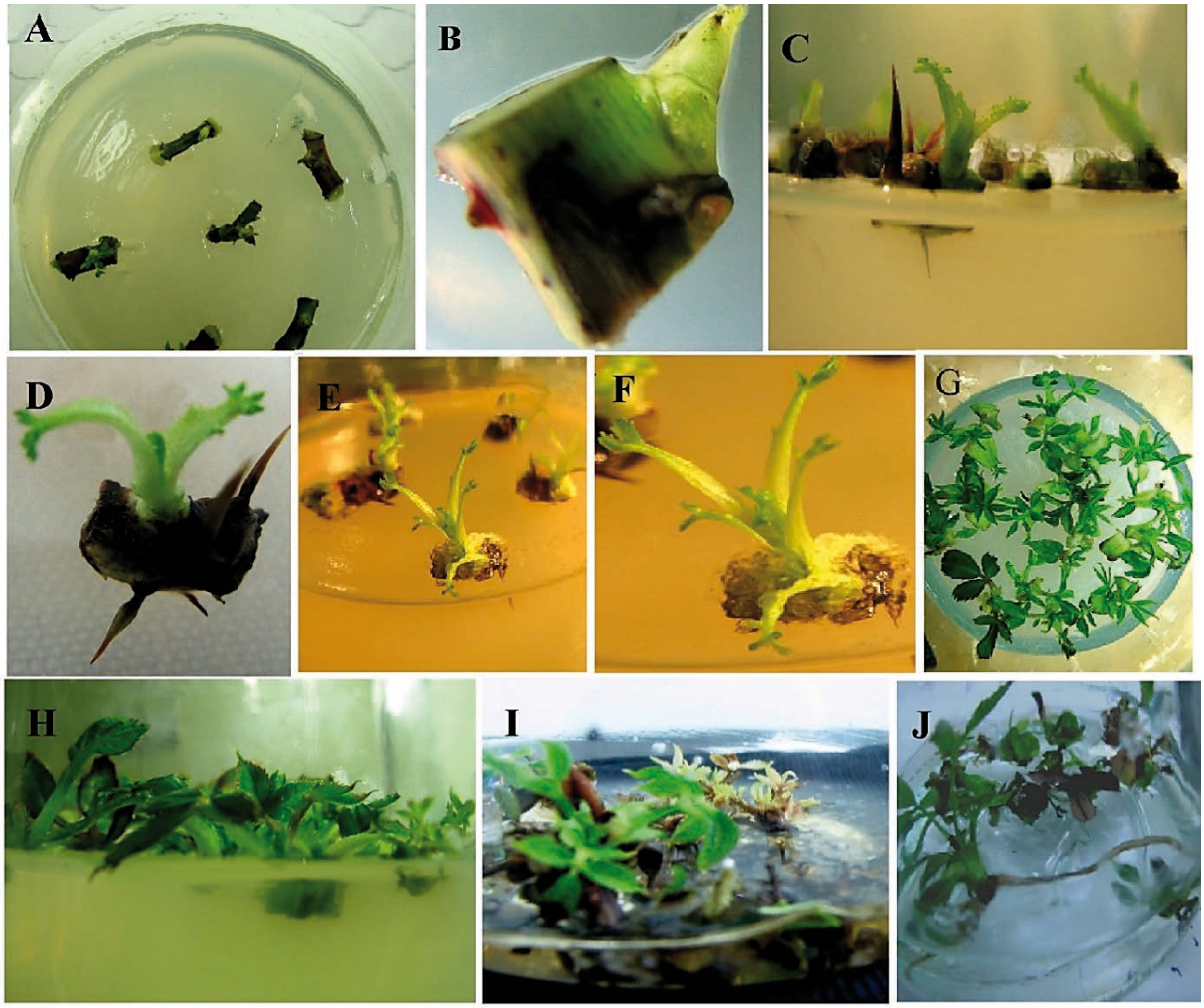

Fig.3: A-J: Different stages of *R. damacesna* propagation in optimal medium. A, B: Initial stage of shoot induction (swelling); C, F: The stage of appearance and initial growth of shoots; E-F: Proliferation or the multi-leaf stage of shoots (1.5-2 cm long), G-J: Transfer to the rooting medium and root growth (J, 125 days after initial culture)

and portion of the branch, culture media, cultivar type, growth regulators, moisture and nutrient status (Pati et al., 2006). The concentrations of 2-2.5 mg l^{-1} BAP, 0.1 mg l^{-1} GA3 and low levels of NAA, were appropriate for the highest proliferation rate in two studied species. The highest rate (100 %) of shoot proliferation was obtained in media 3 and 7 for miniature rose and medium 16 for *R. damascena*. The studies have been shown that concentrations 1-10 mg l^{-1} BAP are required for bud break, proliferation and growth of shoots (Pati et al., 2006).

Davoudi Pahnekolayi et al (2015) reported that the highest shoot proliferation in *Rosa canina* L. was obtained on Van der Salm (VS) medium containing 2 mg l^{-1} BAP compared with MS medium. Furthermore, the highest root induction obtained in ½ VS containing 0.6–0.9 mg l^{-1} of NAA or IBA. BAP is necessary for proliferation, although the auxins particularly NAA, IAA and IBA in combination with BAP simultaneously improve the formation of the shoots. They indicated that NAA was more effective than other auxins (Davoudi Pahnekolayi et al., 2015). Kim et al. (2003) reported the highest rate of shoot proliferation in the presence of 2 mg l^{-1} BAP and 0.01 mg l^{-1} NAA in full-strength MS medium (Kim et al., 2003). Thi et al. (2008) demonstrated that the most suitable concentration for shoot initiation and multiplication of roses was observed on MS medium supplemented with 3 mg l^{-1} BAP (Thi et al., 2008). The highest number of shoots in rose 'Morrasia' was produced in 3 mg l^{-1} BAP (Asadi et al., 2009). In *Rosa chinensis*, different concentrations of BAP (0, 0.5, 1, 1.5, 2 mg l^{-1}) (BA) and Thidiazuron (1.5 mg l-1)) induced shoot production with a percentage of 100 % (Tibkwang et al., 2018). Quick

Tab. 5: The rooting rate (%) of miniature rose and Damask rose under two pretreatments, different combinations of IAA (0, 0.1, 0.05 mgl^{-1}) and different strengths of MS media (full and half) in solid and liquid media (24 various media)

		PGR	Root induction (%)			
			Solid MS medium		Liquid MS medium	
Species	Pretreatments	IAA	Full strength	Half strength	Full strength	Half strength
Miniature rose	Dipping (floating) in 500 mg l $^{-1}$ IAA	0	-	-	-	-
		0.05	-	-	-	1.5a ±60
		0.1	-	-	-	-
	culturing on solid MS containing 3 mg l^{-1}2,4-D	0	-	-	-	a1.2±62.2
		0.05	-	28.8 ± 0.67b	-	-
		0.1	-	-	-	-
Damask rose	Dipping (floating) in 500 mg l^{-1} IAA	0	-	-	-	-
		0.05	-	-	-	-
		0.1	-	0.9b ±31.1	-	-
	culturing on solid MS containing 3 mg l^{-1}2,4-D	0	-	-	-	-
		0.05	-	-	-	1.8a± 53.3
		0.1	-	-	-	-

Each experiment had three replicates containing at least eight explants in each culture vessel. Data are expressed as mean ± standard error, values with the same letters in the same column are not significantly different ($p \leq 0.05$) using Duncan's multiple range test

deep treatment of microshoots in auxin compounds have been reported frequently (Kumar et al., 2000; Nikbakht et al., 2005). In this study, the highest proliferation rate of shoots in studied species was obtained in higher concentrations of BAP and lower amount of auxins and GA_3. In optimal medium, the axillary buds were sprouted earlier in miniature rose than *R. damascena*. Cultivar, explant type and medium composition are considered as three main factors affecting *in vitro* plant regeneration in many plant species (Gubis et al., 2003, Bidabadi and Jain, 2020).

In present study, the significant differences were observed in regeneration capacity between two species as well as between different combinations of culture media. Root induction is affected by different external and internal factors, among them the height and age of shoots are important factors (Pati et al., 2006). Exogenous auxins were shown to increase the availability of carbohydrates at the site of root development (Abidin and Metali, 2015). According to a study by Jabbarzadeh and Khosh-Khui (2005) in roses, the best treatment for rooting of shoots was 2.5 mg l^{-1} of 2,4-D for 2 weeks in MS medium (as pretreatment) and then transferring the explants to hormone free MS medium (Jabbarzadeh and Khosh-Khui, 2005). In current study, the 2, 4-D induced root induction successfully. It has been reported that 2, 4-D prevent the failure and degradation of the endogenous auxins through the oxidase enzymes and lead to root induction (Jabbarzadeh and Khosh-Khui, 2005). Root induction with 2,4 -D has also been reported in roses and other plants (Edwin and Paul, 1984). Although rooting occurs in both solid and liquid media but there are significant differences in the rooting potential of the two media. In the two species studied, rooting frequency was higher in the half strength MS media than in the full strength media. Moreover, root formation and growth was higher in MS liquid medium than solid one. The highest rooting frequencies were 62 % and 53 % in miniature rose and Damask rose respectively. Similarly, Pati et al. (2006) reported the highest percentage of rooting in liquid medium (85 %) compared with solid media (5 %) suggesting that low osmotic potential in solid medium reduces the root induction (Nikbakht et al., 2005; Pati et al., 2006). According to the results, similar to shoot induction and proliferation, rooting in miniature rose was faster than in *R. damascena*. A lower rooting ability was also cited in old garden roses (*R. damascena* and *R. canina*) compared with modern (new) ones (*R. hybrid*) (Pati et al., 2006). Kirichenko et al. (1991) reported that micro shoots of the essential oil bearing roses have higher rooting problems and are rooted worse than the ornamental and modern varieties (Kirichenko et al., 1991). Nikbakht et al. (2005) reported that in vitro rooting of old roses including Damask rose is much more difficult than modern roses (Nikbakht et al., 2005). Similarly, in current study, *R. damascena* as an old species with the highest potential essential oils revealed the lower percentage of rooting compared with miniature rose. Further, it has been reported that there are genes that are involved in shoot and root formation and growth. Also, the possible involvement of the gene in modulating hormone levels has also been reported (Ginova, 2012).

In conclusion, *in vitro* culture methods of roses are important procedures in production of new and adaptable cultivars, eliminating incompatible rootstocks and fast formation of superior cultivars and rootstocks. In present study, the significant differences were observed in regeneration capacity between two species as well as between different combinations of culture media. The results showed that MS medium supplemented with low concentrations of plant growth regulators were resulted in 100 % shoot proliferation in both species. The 2, 4-D induced root induction successfully. The rooting frequency was higher in the half strength MS liquid medium than the others. The highest rooting frequency was 62 % and 53 % respectively in miniature rose and Damask rose. The shoot and root formation were faster and higher in miniature potted rose compared with *R. damascena* as an old species with highest potential essential oils.

5 ACKNOWLEDGEMENTS

This work was financially supported by Shahid Bahonar University of Kerman, Iran. The authors acknowledge financial support from the University of Kerman.

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doi:10.14720/aas.2023.119.1.2707 Original research article / izvirni znanstveni članek

Bags impregnated with garlic (*Allium sativum* L.) and parsley (*Petroselinum crispum* (Mill.) Fuss) essential oils as a new biopesticide tool for *Trogoderma granarium* Everts, 1898 pest control

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Received May 28, 2022; accepted January 11, 2023.
Delo je prispelo 28. maja 2022, sprejeto 11. januarja 2023

Bags impregnated with garlic (*Allium sativum* L.) and parsley (*Petroselinum crispum* (Mill.) Fuss) essential oils as a new biopesticide tool for *Trogoderma granarium* Everts, 1898 pest control

Abstract: Stored product pests can cause significant damages and great economic problems in stored commodities and grain cereal. Using synthetic pesticides in the storage pest control has adverse effects on human health. In our study, the toxicity of garlic (*Allium sativum*) and parsley (*Petroselinum crispum*) essential oils (EOs) impregnating with three types of bags were assessed against *Trogoderma granarium* Everts, 1898 adults after different exposure intervals. GC-MS analysis of the investigated EOs revealed that the major components of parsley and garlic were 1, 3, 8-p-menthatriene (23.34 %) and di-allyl disulfide (27.9 %), with (1.40 %) alpha-terpinene and (1 %) of di-alyl tetra-sulfide as minor components respectively. Additionally, comparison the toxicity among the treated bags was assessed based on the LC_{50} values and a persistence efficiency of the tested EOs was carried out by the LC_{90} values for each bag type. In all bag types, garlic and parsley had mortality by 100 % for clothes, and 80 % for both plastic and paper bags after 7 days of exposure, respectively. After two and five days of garlic treatment, plastic bags were the most effective, but after seven days of exposure paper bags was more effective than the other two types. Finally, cloth bags treated with EOs were the most effective packaging for insect control, indicating that this approach could be considered as an additional tool to the concept of stored product management.

Key words: essential oils; stored grain; bags; *Trogoderma granarium*

Vrečke, impregnirane z eteričnimi olji česna (*Allium sativum* L.) in peteršilja (*Petroselinum crispum* (Mill.) Fuss) kot novo biopesticidno orodje za nadzorovanje indijskega žitnika (*Trogoderma granarium* Everts, 1898)

Izvleček: Skladiščni škodljivci lahko povzročijo znatno škodo in velike ekonomske probleme v skladiščih žit .Uporaba sintetičnih pesticidov pri nadzoru skladiščnih škodljivcev ima škodljive učinke na zdravje ljudi. V raziskavi so bili preučevani toksični učinki eteričnih olj česna in peteršilja na odrasle osebke indijskega žitnika s tremi tipi vrečk (plastične, papirnate in iz blaga), impregniranimi z eteričnimi olji obeh rastlin v različnih časovnih izpostavitvah. GC-MS analiza preučevanih eteričnih olj je pokazala, da sta bili njihovi glavni sestavini 1, 3, 8-p-mentatrien (23, 34 %) in dialil disulfid (27,9 %), z (1,40 %) alfa-terpinenom in (1 %) dialil tetrasulfidom v manjšem deležu. Dodatno je bila ocenjena primerjava strupenosti impregniranih vrečk na osnovi LC_{50} vrednosti. Trajnost učinka eteričnih olj je bila preiskušena na osnovi LC_{90} vrednosti za vsak tip vrečke. Primerjalno je impregniranost vrečk z eteričnimi olji česna in peteršilja povzročila 100 % smrtnost pri vrečkah iz blaga in 80 % smrtnost pri plastičnih in papirnatih vrečkah po sedemdnevni izpostavitvi. Po izpostavitvi za dva in pet dni v vrečkah impregniranih s česnom so bile najbolj učinkovite plastične vrečke, po sedmih dneh izpostavitve so bile najbolj učinkovite papirnate vrečke. Zaključimo lahko, da so vrečke iz blaga, impregnirane z eteričnimi olji najbolj učinkovite za uravnavanje škodljivih žuželk in bi jih lahko uporabili kot dodatno orodje pri ohranjanju shranjenih pridelkov.

Ključne besede: eterična olja; shranjena zrna; vrečke; *Trogoderma granarium*

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1 INTRODUCTION

Stored commodities are vulnerable to insect attack. Major stored grain cereal crops can be attacked by more than 600 species of beetles, moths, and other insect pests and mites, causing a great economic problem either qualitatively or quantitatively deteriorating (Rajendra and Sriranjini, 2008). According to estimates, more than one-third of food products are lost during post-harvest storage due to pest infestation (Tripathi et al., 2009). *Trogoderma granarium* Everts (Coleoptera: Dermestidae), the khapra beetle, is one of the most important storage pests, which has economic importance under severe phytosanitary worries (Myers and Hagstrum 2012; EPPO 2013; Athanassiou et al., 2019). This species has been classified among the most harmful and intrusive outsider species on the planet (Lowe et al., 2000). The potential expansion of *T. granarium* is due to its ability to attack numerous stored cereals or related products and non-grain commodities. It feeds on 96 commodities and infests very dry food in a very dry environment with 2 % relative humidity (r. h.). In addition to its ability to shuttle from one place to another according to worldwide exchange and temperature tolerance, which ranged between (21 and 40 °C) with an optimum at 35 °C (Degri and Zainab 2013; Athanassiou et al., 2016; Kavallieratos et al., 2019). Their larvae may fall under selective diapause for a long and show resistance to insecticides (Edde et al., 2012; Myers and Hagstrum, 2012; Athanassiou et al., 2015). Due to the widespread overuse of synthetic pesticides in managing insect pests, including those attacking stored products, and the development of resistances, efforts to reduce environmental pollution represent a major issue of concern for environmental and health issues (Desneux et al., 2007).

Due to expansion instances of khapra beetle resistance against traditional insecticides such as phosphine, malathion and pyrethroids have more featured its monetary importance and raised another errand for the researchers to seek a new way to control it (Myers and Hagstrum, 2012; Honey et al., 2017; Khalique et al., 2018). In this way, the European Union supports a considerable decrease in the utilization of insecticides, by looking for less harmful substances under the Integrated Pest Management (IPM) models (Hillocks, 2012; Lucchi and Benelli 2018). During the last years, botanicals such as plant powder and essential oils have been evaluated as promising alternatives for the control of a huge range of pests on different-stored commodities (Athanassiou et al., 2014; Bohinc et al., 2020). Plant essential oils are complex natural mixtures of volatile organic compounds resulting as secondary metabolites in plants, constituted by terpenes, terpenoids, and phenol-derived aromatic components and, aliphatic components (Bakkali et al., 2008). They have been reported by many studies that essential oils have toxic effects such, as a repellent, antifeedant, antifungal, antibacterial, reproduction, and inhibitory development effects against varied insect pests (Ebadollahi and Jalali Sendi 2015; Tu et al., 2018; Hu et al., 2019).

Packing is one of the most common methodologies for the development of bioactive preservation tools. It is confirmed that packaging offers a basic point in food quality conservation and a definitive defense against insect pests. Active packaging is considered a promising innovation for food packaging. It consists of incorporating into the packaging material active compounds useful for food protection. Earlier studies mentioned that packages are one of the possible ways to protect stored products during storage until they reach consumers (Campbell et al., 2004). Furthermore, naturally derived compounds such as essential oils and plant extracts consolidated in active packaging can be used in place of commonly used chemical preservatives by consumers who prefer natural preservatives (Bazargani-Gilani et al., 2015). However, insect pests can infest packaged products in many ways (Mullen et al., 2012; Costa, 2014; Stejskal et al., 2017). Endeavors ought to be made not just towards the planning of successful frameworks, which might hinder the food quality rot, but also towards the improvement of insect-proof packages, ready to stand up against insect penetration and/or to repel their presence from the food package's environment.

Recently, many essential oils have been employed as promising natural preservatives in packaging. A lot of exhaustive research has developed numerous packaging material types that can offer different levels of protection for stored products at a low cost for either individuals or companies (Stejskal et al., 2017). Treated packages protect the stored grain commodities by blocking insect infestations resulting from the storage environmental conditions (Paudyal et al., 2017a). Similarly, a covering containing citronella oil is applied to the containerboard for insect repellence (Wong et al., 2005). Consequently, insecticides or other protective materials can be impregnated into the synthesis of the storage bags or treated straightforwardly on the packaging material (Scheff et al., 2016; Kavallieratos et al., 2017a, b; Paudyal et al., 2017a, b; Scheff et al., 2017; Scheff and Arthur 2018). Active agents consolidated in packing materials could be released through evaporation of the headspace or can migrate from the package to the product by diffusion through the packaging material prolonging the specific activity and reducing the migration to the preserved material. For instance, antimicrobial cellulose packaging was achieved through the laccase-interceded joining of phenolic compounds (Elegir et al., 2008). Therefore, the objectives of this study were to evaluate the immediate and delayed mortality of *T. granarium* adults exposed to three types of available and low-cost storage bags made of plastic, paper, and clothes material impregnated with two natural essential oils garlic and parsley addition to evaluating their storage long activity.

2 MATERIALS AND METHODS

2.1 INSECT

Trogoderma granarium Everts, 1898 (Coleoptera: Dermestidae) used in the bioassays were obtained from stock colonies maintained in the laboratory of Stored Product Insects of the Sakha Agricultural Research Station, Agriculture Research Center (ARC) Egypt. Adults of *T. granarium* < 24 h old and larvae having 2–4 mm long (Athanassiou et al., 2016) were used in the experiments, which were cultured on wheat at 35 °C, 65 % relative humidity (RH), and continuous darkness.

2.2 ESSENTIAL OIL

Garlic (*Allium sativum* L.) and parsley (*Petroselinum crispum* [P. Mill.] Nyman ex A.W. Hill) EOs are widely available in Egypt, and they were supplied by Hashem Brothers Company for Essential Oils and Aromatic Products (Kafr-Elsohby, Kalyoubeya, Egypt).

2.3 GC/MS ANALYSIS OF THE ESSENTIAL OIL

The chemical composition of the essential oils was analyzed by gas chromatography-mass spectrometry (GC/MS) using the model (HP5890- USA) provided by an HP column (60 m × 0.25 mm, 0.25 µm film thickness) (HP-5 ms). The initial temperature was 60 °C and the maximum temperature was 250 °C for 65.3 min. The injector temperature was 240 °C. Relative percentage amounts were calculated from the peaks' total area by apparatus software. The compounds were identified by matching the mass spectra data with those held in a computer library (Wiley 275. L), according to Swigar and Silverstein (1981) and Adams (1995). All analysis steps were carried out at the laboratory of Hashem Brothers Company, Egypt.

2.4 STORAGE BAGS

Three different types of bags were used in the experiments: polypropylene (BOPP) bags that are utilized for containing and moving products like food sources, produce, powders, ice, magazines, chemicals, and waste it is a common form of packaging. Kraft paper (KP) which are some of the most popular forms of packaging for consumers and finally clothes bags, which are considered a popular alternative to plastic and paper shopping bags, because cloth bags do not cause the environmental harm as plastic bags.

2.5 BIOASSAYS

2.5.1 Contact

The standard solution of each tested essential oil (EO) was obtained by diluting 1 ml of crude oil in 100 ml acetone then, four concentrations of each essential oil, (1.25, 2.5, 5, and 7.5 mg ml^{-1}) of garlic, & (7.5, 10, 12.5, and 15 mg ml^{-1}) of parsley were prepared from the standard solution of garlic and parsley then impregnated into tested bags. Each concentration was sprayed into the tested bags with 1 ml as a fine mist that contained the appropriate concentration of each oil. Bags were splashed with a brush on both sides. After treatment with each EOs, the brush was carefully cleaned with acetone and subsequently, the following treatment was applied to the other type. An additional series of bags were prepared without any treatment and served as controls. The sprayed bags were left to become dry for 24 h (h) at 30 °C and 65 % r. h. Then, in each type of bag, 20 g of wheat was placed, and ten adults of *Trogoderma granarium* were put in each bag and then closed with stapler staples. Each concentration was replicated three times for each type and placed in incubators set at 30 C and 65 % r. h. Accumulative mortality counts were recorded at 2, 5, and 7 days after treatment corrected by Abbott's formula (1925).

2.6 LONG-TERM EXPERIMENT

Long–term experiment was conducted to evaluate the persistence and long-active effectiveness of various concentrations of testing essential oils loaded with testes bags, this experiment was carried out as follows. After calculating the LC_{90} value of testing essential oil for each type of bag individually, 9 bags of each type were treated with the 2 days calculated LC_{90} value of garlic and parsley and stored treated until further investigation. After one month of storage three bags of each treated type (paper, plastic, and cloth) were infected with 10 adults of *T. granarium* insect and another three untreated bags served as a control, mortality was observed after 2 days. This experiment was repeated after three months as mentioned above and recorded mortality rate.

2.7 STATISTICAL ANALYSIS

The results were analyzed by one-way analysis of variance ANOVA followed by the Least Significant Difference test for mean separation. *p* values of ≤ 0. 05 were considered significant. The experiments were performed in triplicate, the data presented are the mean ± SE. The lethal concentration for 50 % mortality (LC_{50}) was determined by log-probit analysis (Finney, 1971), and the data were analyzed by determining chi-square values and degrees of freedom. The analysis of data was performed with SPSS program version 24.0 for Windows (SPSS Inc., IBM Corp.)

3 RESULTS AND DISCUSSION

3.1 CHEMICAL COMPOSITION TESTED ESSENTIAL OILS

The major components of testing essential oils are summarized in Tables 1 & 2. A total of 33 components were identified for parsley essential oil according to their retention indices in Table 1, the major constituents representing 84.63 % of the total component were trimethyl bicylo (13.01 %), beta pinene (8.28 %), beta myrcene (3.93 %), beta phellandrene (3.81 %),1,3,8-p-menthatriene (23.34 %), benzodioxole (11.67 %), apiol (12.72 %) and benzofuran (7.87 %). Whereas the minor constituents were chavicol (2.51 %), benzene-methyl (2.06 %), and alpha-terpinene (1.40 %). Where in Table 2 a total of 42 components were identified for garlic essential oil according to their retention indices, the major constituents representing 94.4 % of the total component were dimethyl disulfide (1.4 %), diallyl sulfide (9.5 %), allyl methyl disulfide (8.3 %), dimethyl trisulfide (2.9 %), diallyl disulfide (27.9 %), allyl (Z)-1 propenyl disulfide (2.2 %), allyl (E)-1-propenyl disulfide (3.7 %), allyl methyl trisulfide (17.7 %), 4-methyl-1,2,3-trithiolane (1.2 %), 2-vinyl-4H-1,3-dithiine (1.8 %), diallyl trisulfide (16.8 %) and diallyl tetrasulfide (1 %).

Essential oils from various plant species could represent an alternative way to existing synthetic pesticide agents, either by direct application or by loading on a carrier.

In our study chemical composition of parsley (EO) *P. crispum* agrees with earlier studies from different regions all over the world (Zhang et al., 2006; Soher et al., 2014) with some differences in component percentage due to climate change and environmental factors. Also, the chemical composition of garlic (EO) *A. sativum* is in agreement with those (Satyal et al., 2017; Mossa et al., 2018**),** who reported that garlic oil from different topographical areas has shown subjective similitudes, yet quantitative contrasts in the groupings of the organosulfur compound.

3.2 TOXICITY BIOASSAY

In the current study *A. sativum* and *P. crispum,* essential oils exhibited strong insecticidal activity against *T. granarium* adults with a significant difference between treated concentrations along the test period and within bag types. Where the results demonstrate that *A. sativum* oil showed high efficiency over *P. crispum* oil against khapra beetle with all tested bags. Mortality percentage for garlic increased as a function of time exposure, where the highest concentration of garlic recorded 100 % mortality in clothes bags, 86.66 % for plastic bags, and 96.66 % for paper bags after 7 d of exposure (Fig 1c). On the other hand, parsley recorded mortality by 80 % for plastic

Table 1: Main components of parsley (*Petroselinum crispum*) essential oil analyzed by gas chromatography-mass spectrometry (GC-MS)

Compounds	Percent Composition %	Molecular formula	Retention time (min)
Trimethyl bicylo	13.01	$C_{10}H_{16}$	6.20
Beta pienen	8.28	$C_{10}H_{16}$	7.52
Beta myrcene	3.93	$C_{10}H_{16}$	7.97
Beta phellandrene	3.81	$C_{10}H_{16}$	9.31
1,3,8-p-menthatriene	23.34	$C_{10}H_{14}$	12.85
Benzodioxole	11.67	$C_7H_6O_2$	30.05
Apiol	12.72	$C_{12}H_{14}O_4$	36.02
Benzofuran	7.87	C_8H_6O	12.08

Table 2: Main components of garlic *(Allium sativum)* essential oil, analyzed by gas chromatography-mass spectrometry (GC-MS)

Compounds	Percent Composition %	Molecular formula	Retention time(min)
Dimethyl disulfide	1.4	$C_2H_6S_2$	12.32
Diallyl sulfide	9.5	$C_6H_{10}S_2$	14.25
Allyl methyl disulfide	8.3	$C_4H_8S_2$	15.26
Dimethyl trisulfide	2.9	$C_2H_6S_3$	16.13
Diallyl disulfide	27.9	C6H10S2	18
Allyl (Z)-1-propenyl disulfide	2.2	$C_4H_8S_2$	18.21
Allyl (E)-1-propenyl disulfide	3.7	$C_6H_{10}S_2$	18.33
Allyl methyl trisulfide	17.7	$C_4H_8S_3$	18.96
4-Methyl-1,2,3-trithiolane	1.2	$C_3H_6S_3$	19.21
2-Vinyl-4H-1,3-dithiine	1.8	$C_6H_8S_2$	20.23
Diallyl trisulfide	16.8	$C_6H_{10}S_3$	21.68
Diallyl tetrasulfide	1	C6H10S4	25.66

Table 3: Toxicity assessment of garlic and parsley essential oils against *Trogoderma granarium* adults after different exposure periods in plastic bags

Tested oil	Time (d)	LC_{50} Value (mg kg^{-1})	Confidence Interval 95 %		Slope value	Chi-Square (X^2)
			Lower	Upper		
	2 days	19.26 a	24.06	15.39	1.165	0.035
Garlic	5 days	9.86 d	7.88	12.33	0.599	0.238
	7 days	1.240 f	0.99	1.55	0.107	0.874
	2 days	17.98 b	15.00	22.48	1.575	0.598
parsley	5 days	12.01 c	7.508	15.02	1.022	0.483
	7 days	3.65 e	2.92	4.56	0.257	0.325

Values in the row followed by the same letters are not significantly different ($p > 0.05$) according to ANOVA and Duncan multiple-comparison tests

Table 4: Toxicity assessment of garlic and parsley essential oils against *Trogoderma granarium* adults after different exposure periods in paper bags

Tested oil	Time (d)	LC_{50} Value (mg kg^{-1})	Confidence Interval 95 %		Slope value	Chi-Square (X^2)
			Lower	Upper		
Garlic	2 days	6.31 d	5.05	7.89	0.504	0.600
	5 days	5.21 e	4.17	8.08	0.641	2.085
	7 days	4.33 f	3.46	7.07	0.717	0.087
parsley	2 days	18.89 a	14.94	23.61	1.869	0.347
	5 days	16.14 a	12.91	20.18	1.22	0.086
	7 days	8.34 c	3.54	10.08	1.187	0.505

Values in the row followed by the same letters are not significantly different ($p > 0.05$) according to ANOVA and Duncan multiple-comparison tests

Table 5: Toxicity assessment of garlic and parsley essential oils against *Trogoderma granarium* adults after different exposure periods in clothes bags

Tested oil	Time (d)	LC_{50} Value (mg kg^{-1})	Confidence Interval 95 % Lower	Confidence Interval 95 % Upper	Slope value	Chi-Square (X^2)
Garlic	2 days	6.13 b	4.68	9.65	1.013	0.214
	5 days	2.24 d	0.144	3.47	0.438	1.95
	7 days	1.211 f	0.76	2.18	0.366	2.685
parsley	2 days	6.63 a	4.52	8.29	0.732	0.828
	5 days	3.32 c	2.66	4.15	0.189	0.055
	7 days	1.47 e	1.17	3.59	0.162	0.183

Values in the row followed by the same letters are not significantly different ($p > 0.05$) according to ANOVA and Duncan multiple-comparison tests

bags, 80 % for paper bags, and 73.33 % for clothes bags at the highest concentration after 7 d of exposure as shown in Fig. 2 c.

In Table 3, *A. sativum* essential oil had the lowest LC_{50} values by 19.26, 9.86, and 1.24 mg kg^{-1} after two, five, and seven days respectively, opposite to 17.98, 12.01, and 3.65 mg kg^{-1} for *P. crispum* for plastic bags treatment. The same was in both paper and clothes bags (Tables 4 & 5), where LC_{50} values were 6.31, 5.21, and 4.33 mg kg^{-1} for *A. sativum* compared to 18.89, 16.14, and 8.34 mg kg^{-1} for *P. crispum* after two, five and seven days in paper bags treatment, while clothes bags recorded LC_{50} values by 6.13, 2.24, and 1.211 mg kg^{-1} for *A. sativum* oil compared to 6.63, 3.32, and 1.47 mg kg^{-1} for *P. crispum* after the same investigation period.

In this framework, our findings indicate that essential oils impregnated into different types of bags can contribute to managing one of the stored-product insect species with economic importance *Trogoderma granarium.* The EO of *A. sativum* was the most effective among the tested EOs against *T. granarium*, causing a higher mortality rate than *P. crispum* oil with all types of testing bags. Botanicals incorporate a wide scope of promising compounds used for developing novel, effective, and ecologically economical pesticides (Stevenson et al., 2017; Pavela et al., 2019a). Extracts and oils of garlic have already been advertised as pest control products. Yang et al. (2009) found that garlic essential oil loaded on coated polyethylene glycol (PEG) in the size of the nanoparticles could effectively control *Tribolium castaneum* (Herbst) insect. In addition, George et al. (2010) found that the garlic oil was highly toxic to the red poultry mite, *Dermanyssus gallinae* (De Geer, 1778). On the other side, the insecticidal activity of parsley has been demonstrated in some studies on arthropods (Bortolucci et al., 2015; Mansour et al., 2015). Whereat, Massango et al. (2016) reported that the essential oil of parsley showed low fumigant toxicity than phosphine against *Callosobruchus maculatus* (Fabricius, 1775)**.** In addition, Kavallieratos et al. (2020) mentioned that eight essential oils could be considered grain protectants to manage adults and larvae of *T. granarium*. Janaki et al. (2018) showed that *T. granarium* adults were highly susceptible to the *Cyperus rotundus* L. EO when applied to filter paper at 0.04 µl cm^{-2}, causing 94 % repellence after 2 h of exposure.

A comparison between tested bags to illustrate the most powerful type of bags results in Fig. 3, showed that paper and clothes bags impregnated with garlic oil was the most effective one after two and five days post-treatment, but with increasing the investigation period to seven days clothes bags showed high effectiveness than the other two types. However, in parsley treatment, Fig. 4 clothes bags were the most effective one than the other two bag types during the tested period.

Coating is the most common methodology for the advancement of bioactive packing, our bioassay was established for the evaluation of insect control by oil-impregnated packaging through three types of bags. Bags are a trustworthy method of storage, due to the expansion of the assurance they can give against insect invasions is of high significance (Paudyal et al., 2017a). Previous studies have demonstrated the efficiency of loading different toxic agents against stored product insects. Wong et al. (2005) indicated that commercial citronella could reduce beetle infestation, initially by approximately 50 % when applied at 0.2 g m^{-2} of carton board in addition to its repellent effect for 16 weeks when applied directly as a coating into carton board in ethanol solution. Our results are in line with Kavallieratos et al. (2017a) who documented that deltamethrin impregnated into ZeroFly storage bags was very effective against larger grain borer *Prostephanus truncatus* (Horn, 1878) by 94.4 % after 5 d of exposure, the rice weevil *Sitophilus oryzae* (L., 1763) by 100 % after 1 d of exposure, and by 96.7 % on the ware-

(a)

(b)

(c)

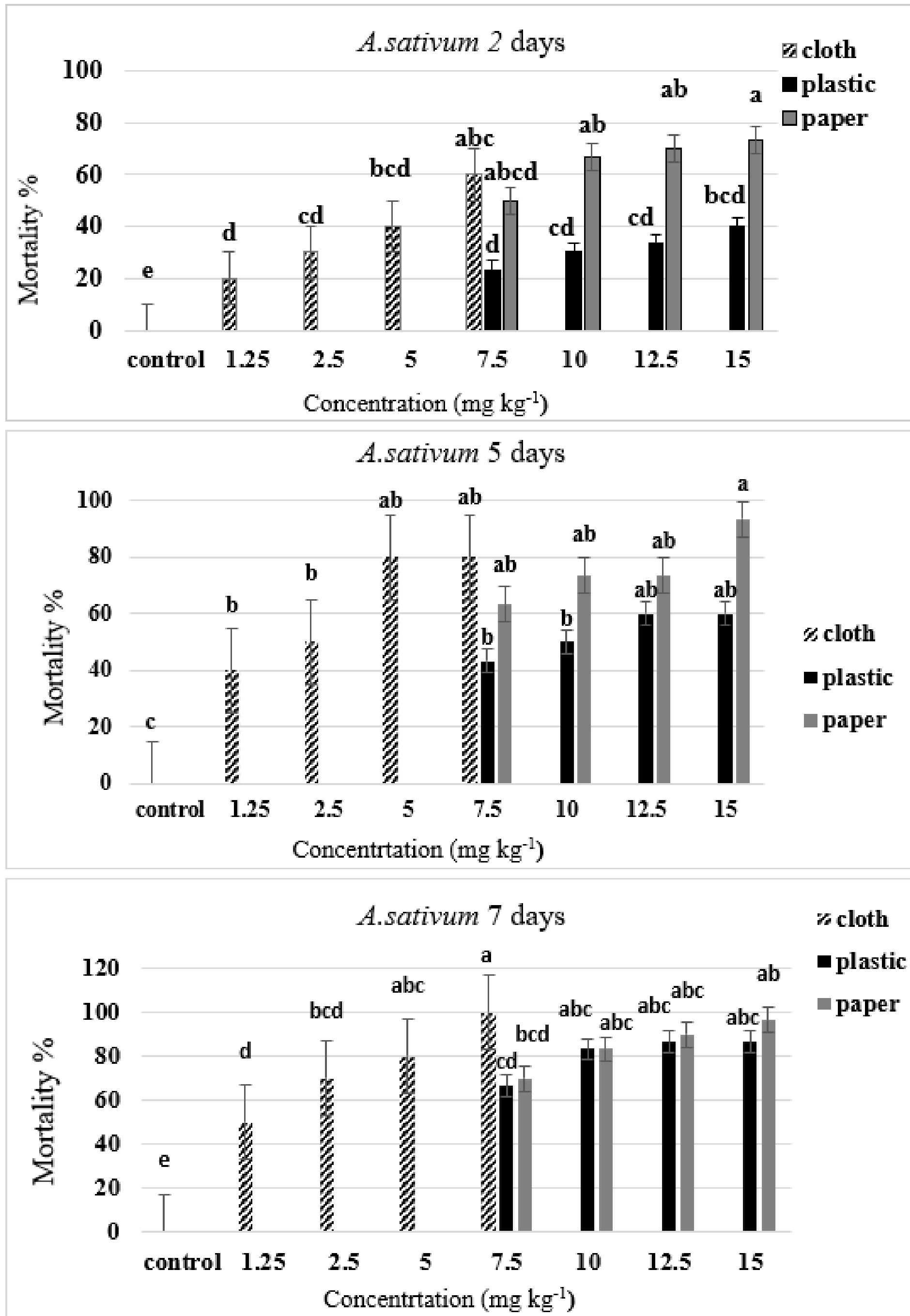

Fig 1: Mortality percentage of *Trogoderma granarium* adults in bags treated with garlic (*Allium sativum*) essential oil after different exposure periods (a, b and c)

house beetle, *Trogoderma variabile* (Ballion, 1878) after 5 d of exposure. In the same trend, Kavallieratos et al. (2017b) found that treated bags with chlorfenapyr and pirimiphos-methyl were effective to kill all adults of *P. trunactus* and the lesser grain borer, *Rhyzopertha dominica* (F., 1792) (Coleoptera: Bostrychidae) after 3 d of exposure. The same was reported by Paudyal et al. (2017a) on S. *oryzae* and the red flour beetle, *T. castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) adults. Our results showed that all bag types had a mortality efficiency against tested insects, whereas Kavallieratos and Boukouvala (2018) in their studies reported that three tested types of bags showed equal mortality levels of *T. granarium*. In addition, Herrera et al. (2021) reported that the

(a)

(b)

(c)

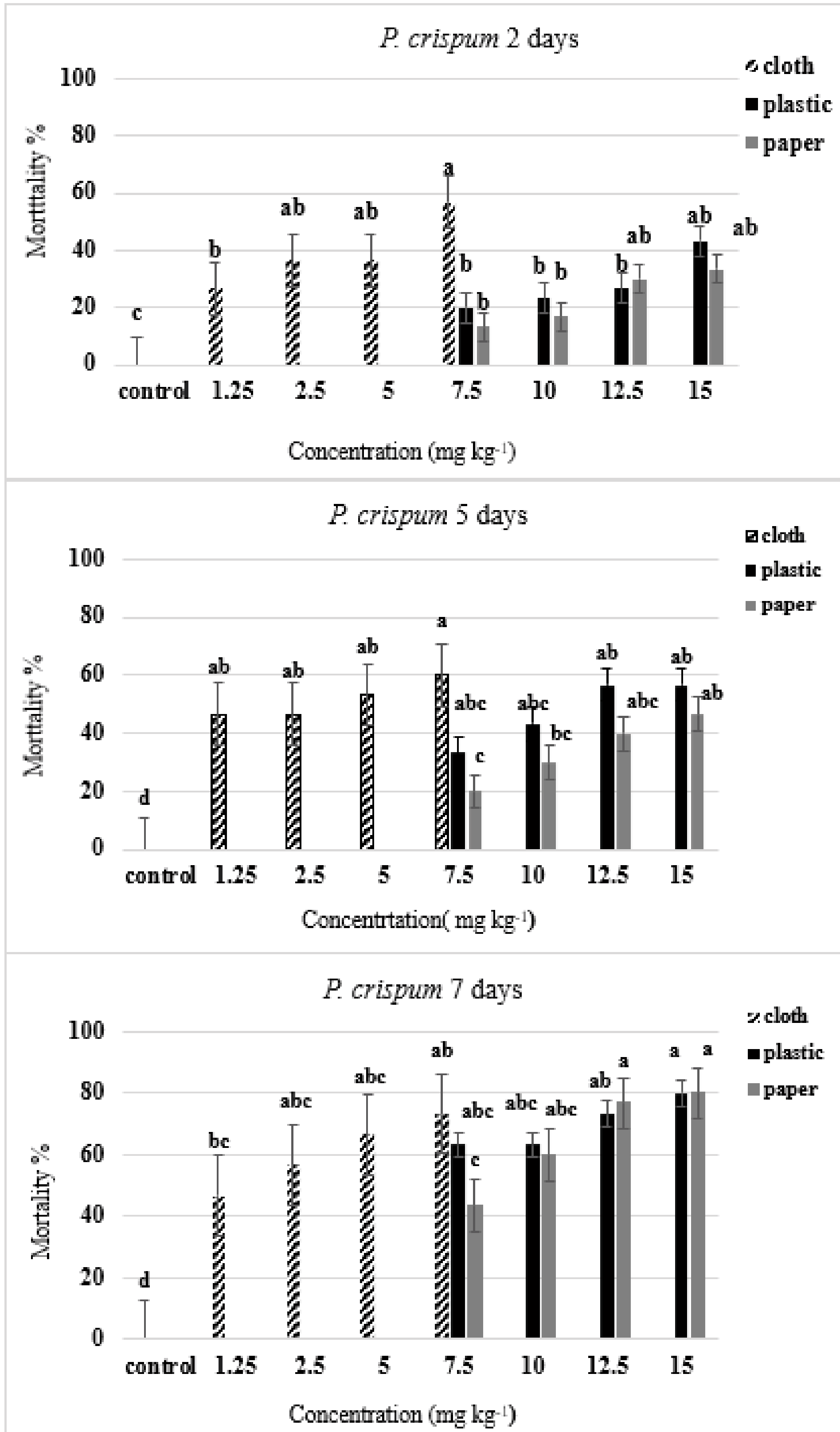

Fig 2: Mortality percentage of *Trogoderma granarium* adults in bags treated with parsley (*Petroselinum crispum*) essential oil after different exposure periods (a, b and c)

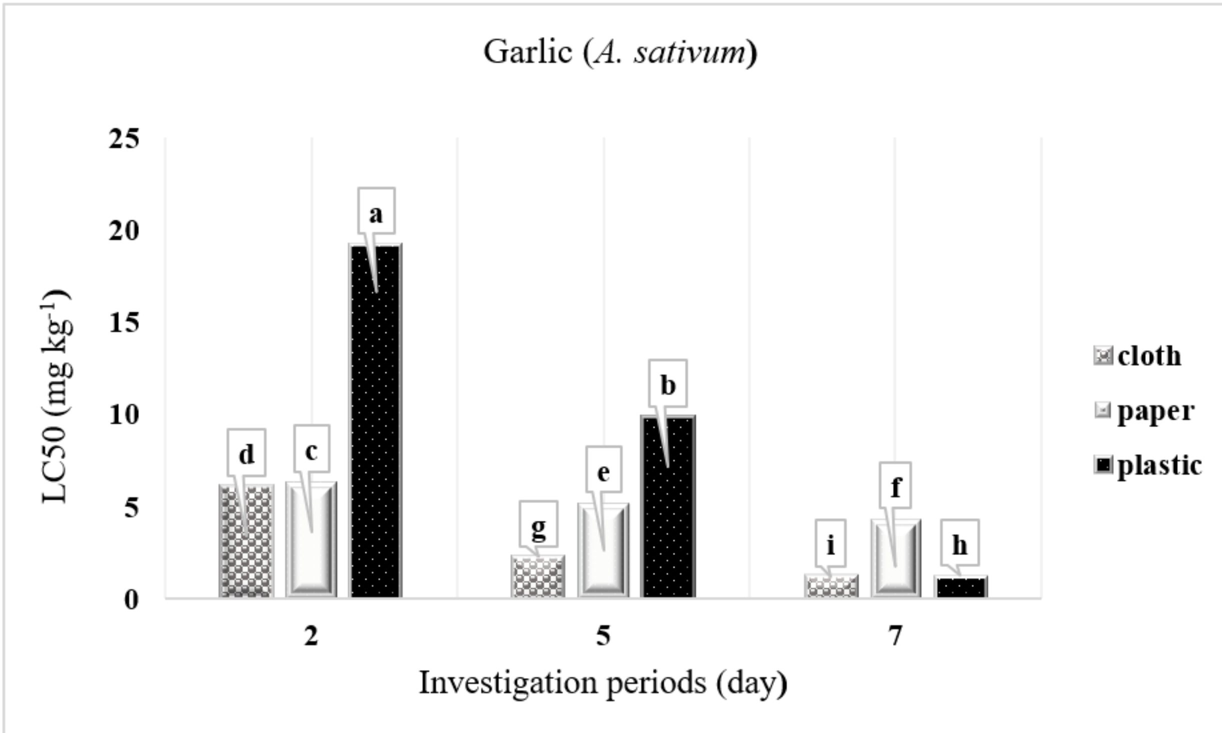

Fig. 3: Comparison between bags types treated with garlic oil (*Allium sativum*) according to LC_{50} value

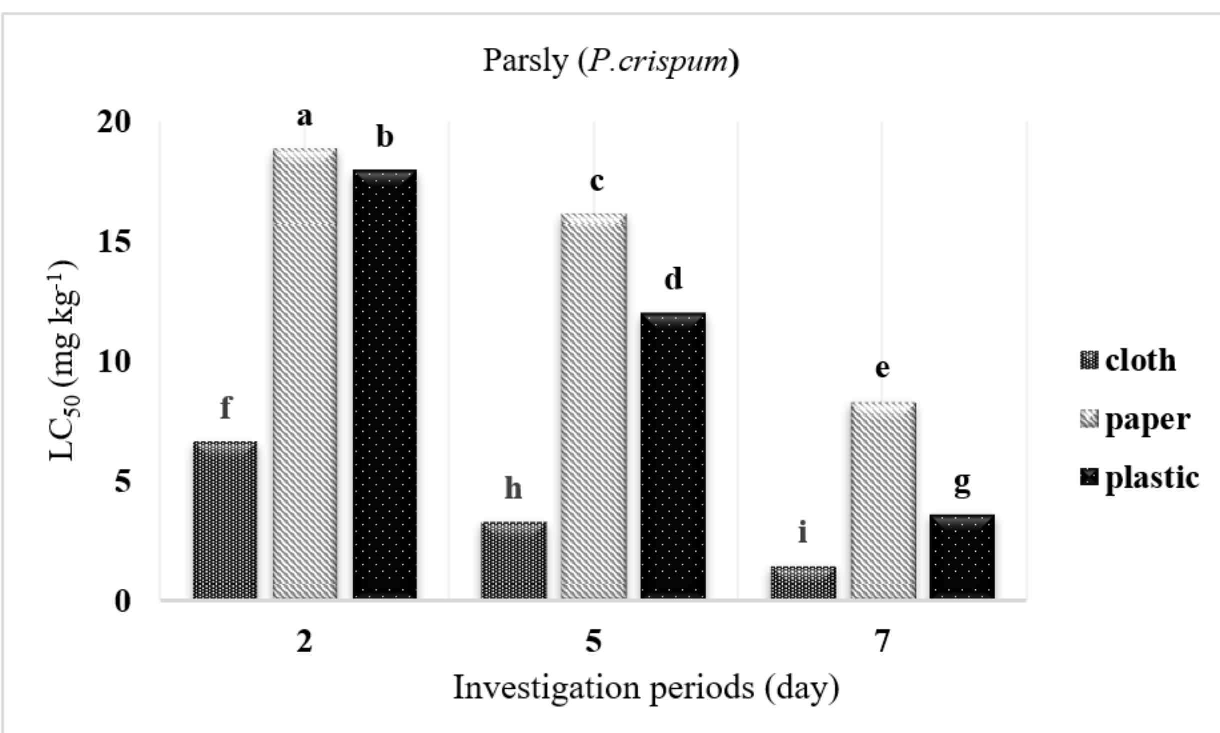

Fig. 4: Comparison between bags types treated with parsley oil (*Petroselinum crispum*) according to LC_{50} value

Table 6: Mortality percentage of *Trogoderma granarium* adults exposed to bags treated with LC_{90} value of garlic and parsley essential oils after different storage times

	Mortality (%) after 1 month of storage		Mortality (%) after 3 months of storage	
Bags	Garlic	Parsley	Garlic	Parsley
Paper	90 b	86.66 b	20 bc	16.66 bc
Plastic	73.33 b	70 b	40 b	30 b
Cloth	76.66 b	76.66 b	33.33 b	26.66 bc
Control	0 a	0 a	0 a	0 a

Values in the row followed by the same letters are not significantly different ($p > 0.05$) according to ANOVA and Duncan multiple-comparison tests

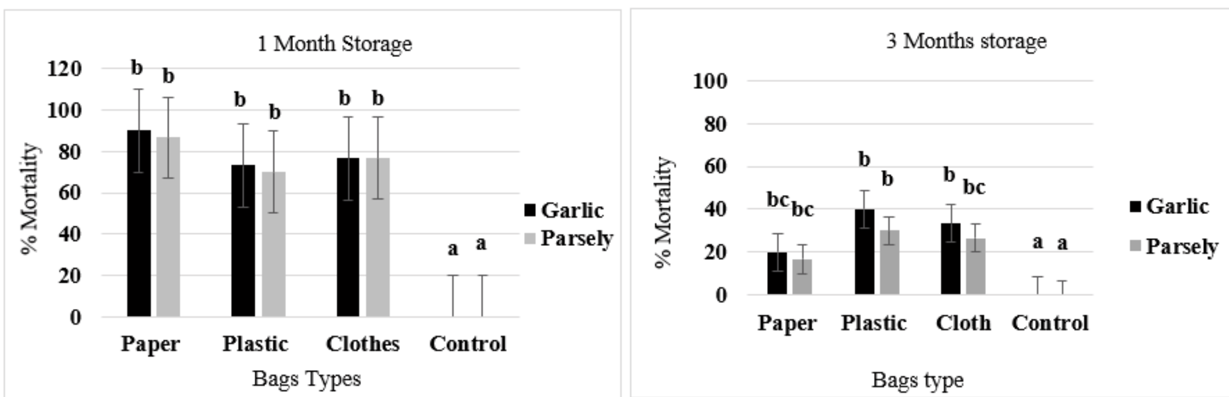

Fig 5: Storage efficiency of garlic oil (*Allium sativum*) and parsley (*Petroselinum crispum*) oil impregenated in different bags types after one and three months

insecticidal effect of the biopesticide of the silo bag with the added essential oil of *Mentha* x *piperita* L. released from the silo bag against *R. dominica* (F.) showed 100 % mortality during the time examined therefore silo bag could be used to control different pests in stored grain. Also, Vendl et al. (2021) mentioned that the treatment of different pieces of food packaging, cardboard spacer, and pallet with bergamot oil had critical repellent activity against *Sitophilus granarius* and *Tribolium confusum* Jacquelin du Val, 1863.

The storage experiment summarized in Table 6 showed that all tested bags had lasting toxic efficiency against adult *T. granarium* along the storage period that extended for three months. Where after one month of storage mortality percentage ranged from 90 to 70 % with the superiorly of paper bags over clothes and plastic bags for both tested essential oils respectively. On the other side, garlic oil was significantly more effective than parsley with all bag types after 3 months of storage, where the mortality rate was 20, 40, and 33.3 % for paper, plastic, and clothes bags respectively compared to 16.6, 30, and 26.66 in parsley.

In the long-term experiment, results indicate that LC_{90} concentration of garlic and parsley essential oils applied to tested bag types could produce protection to stored grains against *T. granarium* infection for a while lasting for three months. This technique offers the possibility of developing a new approach with many advantages. Amalraj et al. (2020) reported that black pepper and ginger essential oils consolidated in chitosan- (CS), gum arabic– (GA), and polyethylene glycol (PEG) showed high antimicrobial activity against a wide range of bacteria such as *Bacillus cereus* Frankland & Frankland 1887*, Staphylococcus aureus* Rosenbach 1884*, Escherichia coli* Migula 1895*, and Salmonella typhimurium* Lignières 1900, as a promising option in food packaging and wound dressing materials. Also, Mapossa et al. (2021) reported that studies searched for controlled-release formulations could be effective against mosquito vectors of malaria parasites. Moreover, plant powder from *Xylopia aethiopica* (Dunal) A. Rich. can serve as a carrier when mixed with its essential oil giving longer-term protection for grain against *Callosobruchus maculatus* insect due to lipids or other components present in the fruit powder may adsorb the terpenes, slowing down their release in the flask as reported by Habiba et al. (2010).

4 CONCLUSION

Controlling *T. granarium* adults could be attainable by impregnating both garlic and parsley essential oil in three types of storage bags polypropylene (BOPP), kraft paper (KP), and clothes. Garlic oil had a greater mortality effect on *T. granarium* than parsley oil. The BOPP and clothes bags showed higher mortality than (KP) bags with both garlic and parsley oil after 7 days of exposure. It was revealed that the treatment of clothes bags is the most effective approach for protecting grain against storage pests, which might have significant ramifications for the utilization under industrial conditions. For long-term storage results indicated that all tested bags had lasting toxicity effectiveness extended for 3 months varying from one type to another, whereas paper bags (KP) was the most lasting one over the other two types. Finally, the impregnation of essential oils as toxicants in different storage bag types mentioned above can offer protection against stored insects indicating that this approach could be considered as an additional tool to the concept of stored product management.

4.1 DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

4.2 FUNDING

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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doi:10.14720/aas.2023.119.1.2682 Original research article / izvirni znanstveni članek

Standardization of detached leaf assay to screen chickpeas for resistance to beet armyworm, *Spodoptera exigua* (Hübner, 1808)

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Received May 12, 2022; accepted January 19, 2023.
Delo je prispelo 12. maja 2022, sprejeto 19. januarja 2023

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Abstract: The beet armyworm, *Spodoptera exigua* (Hübner, 1808) is an important pest of several economically important crops, and recently emerged as a serious pest of chickpea in South Central India. We standardized a detached leaf assay technique to evaluate chickpea germplasm and segregating populations for resistance to this pest under laboratory conditions. Two chickpea genotypes ICCL 86111 and ICC 3137 grown under field and greenhouse conditions were used for the detached leaf assay at the vegetative and flowering stages. The terminal branches were infested with 5, 10, 15, and 20 neonate larvae of *S. exigua*. The test genotypes were also infested with 2, 4, 6 and 8 third-instar larvae at the podding stage. At the vegetative stage, ICCL 86111 suffered less damage than ICC 3137 across infestation levels. The differences in larval survival between the genotypes were significant, and larval survival was lower on ICCL 86111 than on ICC 3137 across infestation levels. The results suggested that infesting the chickpea terminal branches with 10–15 neonate larvae per branch at the vegetative stage or six third-instar larvae at the podding stage can be used to evaluate chickpea genotypes for resistance to *S. exigua*.

Key words: *Spodoptera exigua*; chickpea; host plant resistance; screening technique; detached leaf assay

Standardizacija preiskusa z odtrganimi listi za preiskus odpornosti čičerike na pesno sovko (*Spodoptera exigua* (Hübner, 1808))

Izvleček: Pesna sovka (*Spodoptera exigua* (Hübner, 1808)) je pomemben škodljivec številnih ekonomsko pomebnih kulturnih rastlin in se zadnje čase pojavlja kot resen škodljivec čičerike v južni osrednji Indiji. Standardiziran je bil preiskus z odtrganimi listi za ovrednotenje genotipov čičerike na odpornost proti temu škodljivcu in razdelitev njenih populacij glede na odpornost v laboratorijskih razmerah. Dva genotipa čičerike, ICCL 86111 in ICC 3137, rastoča na prostem in v rastlinjaku sta bila uporabljena v poskusu z odtrganimi listi v vegetativni in reproduktivni fazi razvoja. Vršni poganjki so bili okuženi s 5, 10, 15, in 20 mladimi ličinkami pesne sovke. Preiskušani genotipi so bili okuženi še z 2, 4, 6 in 8 ličinkami tretje razvojne stopnje v razvojni fazi tvorbe strokov. V vegetativni razvojni fazi je imel genotip ICCL 86111 manj poškodb kot genotip ICC 3137 pri vseh jakostih okužbe. Razlike v preživetju ličink med genotipi so bile značilne in njihovo preživetje je bilo manjše na genotipu ICCL 86111 kot na genotipu ICC 3137 pri vseh jakostih okužbe. Rezultati nakazujejo, da bi se okužba vršnih poganjkov čičerike z 10–15 mladimi ličinkami na poganjek v vegetativni fazi ali s 6 ličinkami tretje razvojne stopnje v fazi tvorbe strokov lahko uporabila za ovrednotenje odpornosti genotipov na pesno sovko.

Ključne besede: *Spodoptera exigua*; čičerika; odpornost gostiteljske rastline; preiskus ugotavljanja; preiskus na odtrganih listih

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1 INTRODUCTION

Chickpea, *Cicer arietinum* L., is the third most important grain legume in the world, after dry beans and peas. It is cultivated in more than 42 countries in Asia, Eastern and Northern Africa, North and Central America, Mediterranean Europe and Australia. Chickpea yields have shown only a marginal increase over the past 50 years because of the heavy losses due to biotic and abiotic stress factors. Besides *Helicoverpa armigera* (Hübner, 1808), which is the major constraint to chickpea production in the Indian sub-continent (Sharma, 2005a), the beet armyworm, *Spodoptera exigua* (Hübner, 1808) (Lepidoptera: Noctuidae) is emerging as an important pest especially in South Central India. The young larvae of *S. exigua* initially feed gregariously on chickpea foliage and reproductive parts of the plant (Gutierrez et al., 1986; Sharma et al., 2007). As the larvae mature, they become solitary and continue to eat, producing large, irregular holes on the foliage (Ahmed et al., 1990; Sharma et al., 2007).

S. exigua is a cosmopolitan species infesting >90 plant species in North America, many of which are crop plants (Pearson, 1982). Insecticides directed against the larvae are the primary method of control, but its high tolerance to most insecticides and associated environmental problems may jeopardize their continued use (Mascarenhas et al., 1996). Development of crop cultivars with resistance or tolerance to *H. armigera* (Hübner [1808]) and *S. exigua* has a major potential for use in integrated pest management (Fitt, 1989; Sharma and Ortiz, 2002), but there is very little information on identification and utilization of resistance to control *S. exigua*. However, interspecific derivatives of *Cicer reticulatam* Ladiz. (FLIP 84-92C - susceptible) x *Cicer arietinum* (PI 599072 - resistant) have shown high levels of resistance to this pest (Clement et al., 2010).

Large-scale screenings of *C. arietinum* germplasm accessions have not resulted in identification of high levels of resistance to insects (Clement et al., 1999). It is important to screen the test material for resistance to the target insect under optimum and uniform level of insect infestation at the most susceptible stage of the crop (Sharma et al., 1992; Smith et al., 1994). Of the several techniques used to screen for insect resistance, detached leaf assay is quite fast, precise, and requires minimum resources. It can be used to screen a large number of germplasm lines, mapping populations and segregating breeding material (Sharma et al., 2005b). Therefore, the present studies were undertaken to standardize detached leaf assay to screen for resistance to beet armyworm, *S. exigua* under uniform insect pressure.

2 MATERIALS AND METHODS

The test genotypes, ICCL 86111 and ICC 3137, which had shown resistant and susceptible reaction under field conditions, respectively (Shankar et al., 2013), were grown under field and greenhouse conditions at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana, India (latitude 17.53 ^{0}N, longitude 78.27 ^{0}E and an altitude of 545 m).

2.1 REARING *S. EXIGUA* ON ARTIFICIAL DIET

The larvae of *S. exigua* were reared on a chickpea flour based artificial diet, which was developed for rearing *H. armigera* (Armes et al., 1992). The egg masses and larvae of *S. exigua* were collected from chickpea plants from farmer's fields in Andhra Pradesh, India. The *S. exigua* culture was maintained under controlled environmental conditions (27 ± 2 ˚C and 65 to 75% RH) (Chitti babu et al., 2014). The neonates were reared in groups of 300-400 in 250 mL plastic cups (having 2 to 3 mm layer of artificial diet on the bottom and sides) for 7 days or up to third-instar. After seven days, the larvae were transferred individually to cell-well plates containing six cells (each cell with 3.5 cm diameter, and 2 cm in depth) or small plastic cups (3.5 cm diameter and 2.5 cm in depth) to avoid cannibalism. The pupae were removed from cell wells, sterilized with 2% sodium hypochlorite solution, and kept in groups of 50 in plastic jars containing moist vermiculite. After adult emergence, 25 pairs were released inside an oviposition cage (L x B x H: 30 x 30 x 30 cm). Adults were provided with 15% sucrose solution in a cotton swab for feeding. The adults laid eggs on the nappy liners hung inside the cage. The liners were removed daily and the eggs were sterilized with 10% formalin. The liners were then washed with tap water, dried under a fan, and placed inside the plastic cups (250 mL). After egg hatching, the larvae were transferred on to the artificial diet.

2.2 RAISING CHICKPEA PLANTS UNDER FIELD AND GREENHOUSE CONDITIONS

2.2.1 Field conditions

Two chickpea genotypes (ICCL 86111 - resistant, and ICC 3137 - susceptible) were sown in the field (latitude 17.53^{0}N, longitude 78.27^{0}E and an altitude of 545 m) during the post-rainy seasons of 2010-11 and 2011-12.

The experiment was laid in a randomized complete block design (RCBD) with five replications. The plot size was two rows, 2 m long, and row-to-row spacing of 60 cm and plant-to-plant spacing was 10 cm. Normal agronomic practices were followed for raising the crop. A basal dose of di-ammonium phosphate (100 kg ha^{-1}) was applied before sowing. There was no pesticide application in the field. The chickpea genotypes were evaluated for resistance to *S. exigua* using neonate and third-instar larvae in the detached leaf assay and pod bioassay, respectively. The plants were evaluated for resistance to *S. exigua* at the vegetative (30 days after seedling emergence, DAE) and flowering (45 days after seedling emergence) stages.

2.2.2 Glasshouse conditions

Two chickpea genotypes (ICCL 86111 - resistant, and ICC 3137 - susceptible) were grown under glasshouse conditions (27 ± 5°C and 65 - 90% RH). The seeds were sown in a sterilized mixture of black soil (Vertisols), sand and farmyard manure (2:1:1) filled in medium sized plastic pots (30 cm in diameter and 30 cm in depth). The plants were watered as and when required. Six seeds were sown in each pot and three plants with uniform growth were retained at 10 days after seedling emergence. Di-ammonium phosphate (DAP) was applied at 15 days after seedling emergence (20 g per pot). There were five replications for each treatment in a completely randomized design (CRD). The chickpea genotypes were evaluated for resistance to *S. exigua* using neonate or third-instar larvae in the detached leaf assay at the vegetative (30 DAE) and flowering (45 DAE) stages, respectively.

2.2.3 Detached leaf assay to evaluate chickpea genotypes for resistance to *S. exigua*

The chickpea plants grown in the field and greenhouse were bioassayed under controlled conditions in the laboratory [27 ± 2°C temperature; 65 - 75% RH, and photoperiod of 12: 12 h (L: D)] to screen for resistance to *S. exigua* using detached leaf assay. Terminal branches of chickpea (three to four fully expanded leaves and a bud) were excised from the plants and inserted in 3% agar-agar in plastic cups (4.5 x 11.5 cm diameter) (Sharma et al., 2005b). The solidified agar-agar served as a substratum for maintaining the chickpea branches in a turgid condition for 5-7 days. The terminal branches were infested with 5, 10, 15 and 20 neonate larvae of *S. exigua* using a camel hairbrush, and then covered with a lid to keep the chickpea terminals in a turgid condition. The experiment was conducted in a CRD, and there were five replications for each treatment. The experiments were terminated when >80% of the leaf area was consumed in the susceptible genotype or when there were maximum differences between the resistant and susceptible genotypes (generally at 5 days after releasing the larvae on the leaves). The plants were scored for leaf feeding visually on a 1-9 damage rating scale (1 = <10 %, and 9 = >80 % leaf area consumed). Data were also recorded on larval survival and mass of the larvae 4 h after terminating the experiment.

At the podding stage, the plants raised under field conditions were used for the bioassays. The terminal branches (10 cm long) with pods (6 - 8 pods) were excised with a sharp knife and placed in agar-agar as described above in a 500 mL plastic jar. The terminal branches were infested with 2, 4, 6 and 8 third-instar (8 days old) larvae per branch. There were five replications for each treatment, and the cups were arranged in a completely randomized design (CRD). The experiment was terminated when >80% of the pods were damaged in susceptible control. Data were also recorded on pod damage rating (DR) on a 1-9 scale (1 = <10% and 9 = >80% pods consumed), larval survival, and larval mass.

2.2.4 Statistical analysis

Data were subjected to analysis of variance using GenStat version 14.0, (GenStat, 2010). The data on detached leaf assays were analyzed by factorial analysis with genotypes as the main treatment, and the infestation levels as the sub treatment. Significance of differences between the genotypes was tested by F-test, while the treatment means were compared by least significant differences (LSD) at p 0.05.

3 RESULTS

3.1 RESPONSE OF CHICKPEA GENOTYPES TO DIFFERENT LEVELS OF INFESTATION WITH NEONATE LARVAE OF *S. EXIGUA* IN PLANTS GROWN UNDER FIELD CONDITIONS

3.1.1 Leaf damage

The differences in leaf feeding across infestation levels at the vegetative stage, the differences between the genotypes and the interaction effects between the genotypes and the infestation levels were significant. Maximum differences in leaf feeding between ICCL 86111 and ICC 3137 were observed in branches infested with 10 (DR 2.6 in ICCL 86111 compared to 5.2 in ICC 3137) and 15 larvae (DR 3.9 in ICCL 86111 compared to 6.7 in ICC 3137) per branch (Fig. 1a).

At the flowering stage, the differences in leaf feeding across infestation and the interaction effects between the genotypes and the infestation levels were significant. However, the differences between the genotypes were non-significant. Maximum differences in leaf feeding were observed when the terminal branches were infested with 20 neonate larvae per branch (DR 5.0 in ICCL 86111 compared to 6.8 in ICC 3137) (Fig. 1a).

3.1.2 Larval survival

The differences in larval survival across infestation levels at the vegetative stage were non-significant while the differences between the genotypes and the interaction effects between the genotypes and the infestation levels were significant. Maximum differences in larval survival were observed when terminals were infested with 10 larvae (28% on ICCL 86111 and 62% on ICC 3137) (Fig. 1b).

At the flowering stage, the differences in larval survival across infestation levels and the interaction effects between the genotypes and the infestation levels were non-significant. However, the differences between the genotypes were significant (Fig. 1b).

3.1.3 Larval mass gain

The differences in larval mass across infestation levels at the vegetative stage, differences between the genotypes, and the interaction effects were non-significant. Maximum differences in larval mass were recorded when the chickpea terminal branches were infested with 10 neonate larvae. (Fig. 1c).

At the flowering stage, the differences in larval mass across infestation levels and the differences between the genotypes were non-significant. However, the interaction effects were significant (Fig. 1c).

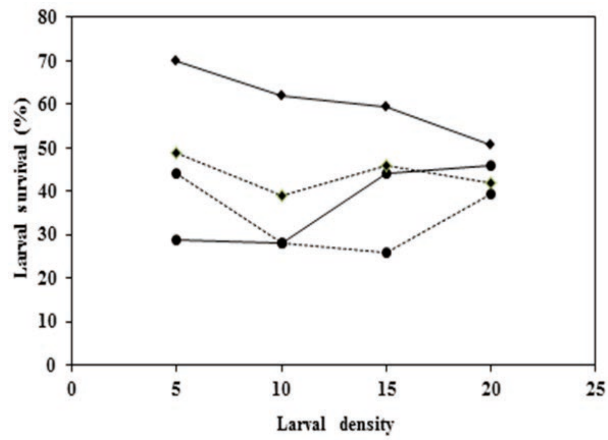

Fig. 1b: Survival of neonate larvae of *S. exigua* on two chickpea genotypes (ICC 3137 (♦) and ICCL 86111(●)) at the vegetative (solid line) and flowering (dotted line) stages in plants grown under field conditions using detached leaf assay (Vegetative stage SE ± 3.35, Flowering stage: SE ± 2.60)

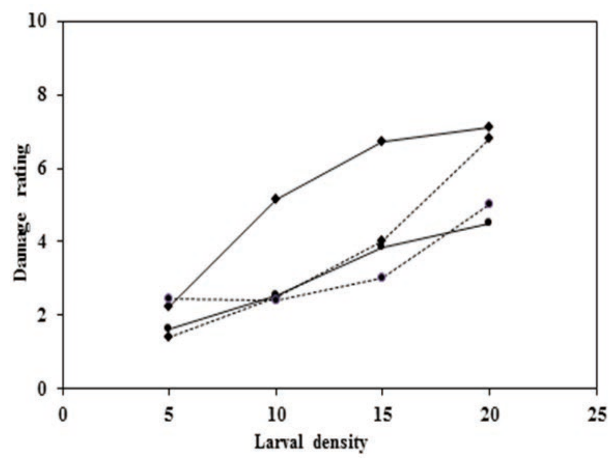

Fig. 1a: Leaf damage ratings in two chickpea genotypes (ICC 3137 (♦) and ICCL 86111(●)) infested with different densities of neonate larvae of *S. exigua* at the vegetative (solid line) and flowering (dotted line) stages in plants grown under field conditions using detached leaf assay (Vegetative stage SE ± 0.19, Flowering stage SE ± 0.17). Damage rating (1 = <10% leaf area, and 8 = > 90 % leaf area damaged)

3.2 RESPONSE OF CHICKPEA GENOTYPES TO DIFFERENT LEVELS OF INFESTATION WITH NEONATE LARVAE OF *S. EXIGUA* IN PLANTS GROWN UNDER GREENHOUSE CONDITIONS

3.2.1 Leaf damage rating

The differences in leaf feeding across infestation levels and between the genotypes were significant (Fig. 2a). However, the interaction effects between infestation levels and the genotypes were non-significant. Maximum differences in leaf feeding were observed when the terminals were infested with 20 neonates (Fig. 2a).

At the flowering stage, the differences in leaf feeding across infestation levels and the differences in leaf feeding between the genotypes were significant (Fig. 2a). The interaction effects between the genotypes and the infestation levels were non-significant. Maximum differences in

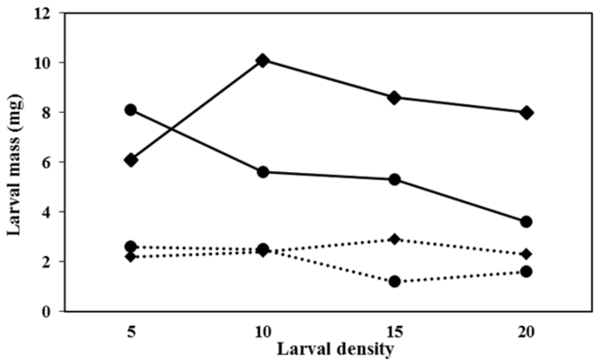

Fig. 1c: Mass gain by neonate larvae of *S. exigua* on two chickpea genotypes (ICC 3137 (♦) and ICCL 86111(●)) at the vegetative (solid line) and flowering (dotted line) stages in plants grown under field conditions using detached leaf assay (Vegetative stage SE ± 0.97, Flowering stage SE ± 0.19)

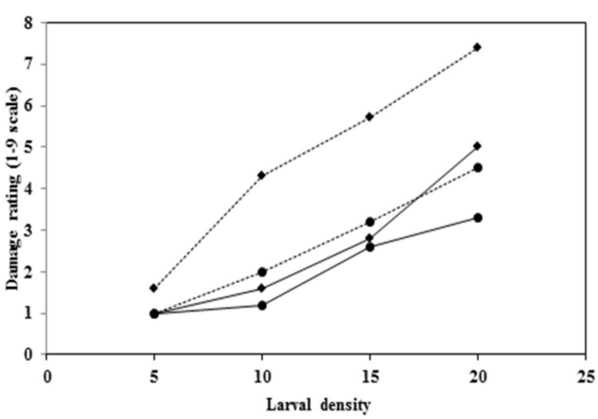

Fig. 2a: Leaf damage ratings in two chickpea genotypes (ICC 3137 (♦) and ICCL 86111(●)) infested with different densities of neonate larvae of *S. exigua* at the vegetative (solid line) and flowering (dotted line) stages in plants grown under greenhouse conditions using detached leaf assay (Vegetative stage SE ± 0.16, Flowering stage SE ± 0.41). Damage rating (1 = < 10 % leaf area, and 8 = > % leaf area damaged)

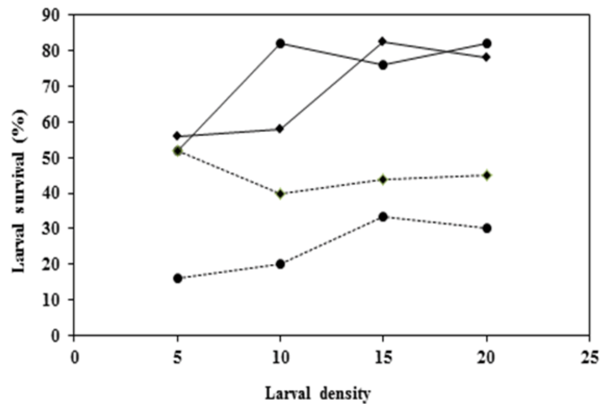

Fig. 2b: Survival of neonate larvae of *S. exigua* on two chickpea genotypes (ICC 3137 (♦) and ICCL 86111(●)) at the vegetative (solid line) and flowering (dotted line) stages in plants grown under greenhouse conditions using detached leaf assay (Vegetative stage SE ± 4.07, Flowering stage SE ± 3.94)

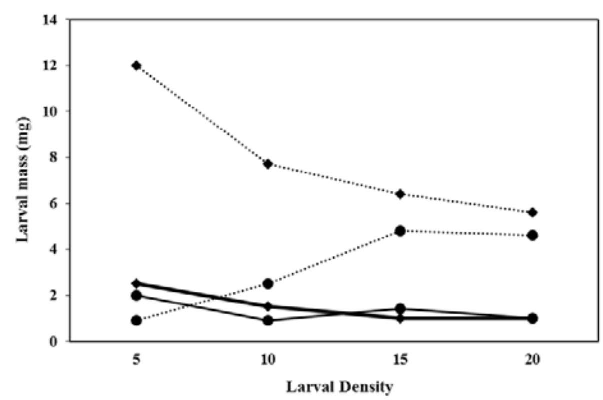

Fig. 2c: Mass gain by the neonate larvae of *S. exigua* on two chickpea genotypes (ICC 3137 (♦) and ICCL 86111(●)) infested at the vegetative (solid line) and flowering (dotted line) stages in plants grown under greenhouse conditions using detached leaf assay (Vegetative stage SE ± 0.11, Flowering stage SE ± 0.93)

leaf feeding were observed when the terminal branches were infested with 15 neonates (Fig. 2a).

3.2.2 Larval survival

Differences in larval survival across infestation levels at the vegetative stage were significant, but the interaction effects, differences between the genotypes and the interaction effects between the infestation levels and the genotypes were non-significant (Fig. 2b). Differences in larval survival across infestation levels were non-significant. However, the differences between the genotypes were significant.

3.2.3 Larval mass gain

Differences in larval mass across infestation levels at the vegetative stage were significant, but the interaction effects and the differences between the genotypes were non-significant (Fig. 2c).

At the flowering stage, the differences in larval mass across infestation levels were non-significant. However, the differences between the genotypes (Fig. 2c) and the interaction effects between the genotypes and the infestation levels were significant.

3.3 RESPONSE OF CHICKPEA GENOTYPES TO DIFFERENT LEVELS OF INFESTATION WITH THIRD-INSTAR LARVAE OF *S. EXIGUA* AT THE PODDING STAGE

At the podding stage, the differences in leaf feeding across infestation levels and the differences between the genotypes were significant (Fig. 3a). However, the interaction effects between the infestation levels and the genotypes were non-significant.

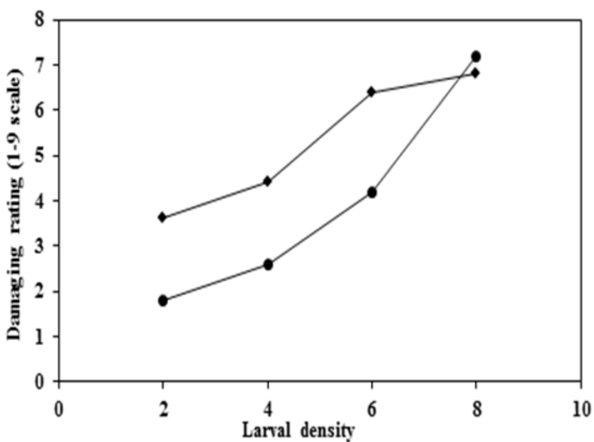

Fig. 3a: Leaf damage ratings in two chickpea genotypes (ICC 3137 (♦) and ICCL 86111(●)) infested with different densities of third-instar larvae of *S. exigua* at the podding stage in plants grown under field conditions using (SE ± 0.52). Damage rating (1 = < 10 % leaf area, and 8 = > 90 % leaf area damaged)

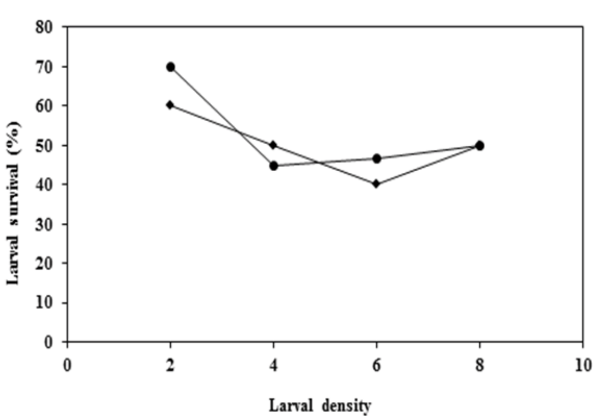

Fig. 3b: Survival of third-instar larvae of *S. exigua* on two chickpea genotypes (ICC 3137 (♦) and ICCL 86111(●)) at the podding stage in plants grown under field conditions using (SE ± 14.3)

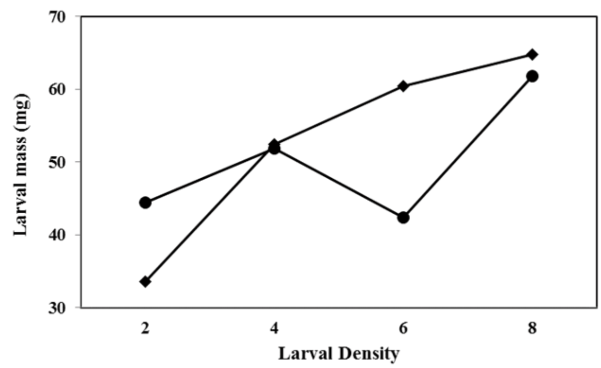

Fig. 3c: Mass gain by the third-instar larvae of *S. exigua* on two chickpea genotypes (ICC 3137 (♦) and ICCL 86111(●)) infested at the podding stage in plants grown under field conditions using (SE ± 9.29)

Differences in larval survival across infestation levels, genotypes, and the interaction effects were non-significant (Fig. 3b).

The differences in larval mass across infestation levels, genotypes, and the interaction effects were non-significant (Fig. 3c).

4 DISCUSSION

Screening for host plant resistance to insect pests under natural conditions is a long-term process because of variations in insect population in space and time. As a result, it is difficult to identify stable sources of resistance under natural infestation (Sharma et al., 1997, Devetak et al., 2014). Is important to develop techniques to screen for resistance to insects under uniform insect pressure. Therefore, careful consideration should be given to use an optimum insect density that results in maximum differences between the resistant and susceptible genotypes. The detached leaf assay not only gives an idea of the relative feeding by the larvae on different cultivars but also provides useful information on antibiosis component of resistance in terms of larval mass (Sharma et al., 2005c; Jaba et al., 2017). In this context, the detached leaf assay can be used to evaluate the test material under uniform insect pressure at the seedling, flowering and podding stages under laboratory conditions.

In the crop raised under field conditions, the differences in leaf feeding, larval survival, and larval mass between ICCL 86111 and ICC 3137 were greater at the vegetative stage than at the flowering stage. Maximum differences in leaf feeding between ICCL 86111 and ICC

3137 were observed in branches infested with 10 and 15 larvae per branch at vegetative stage. Across infestation levels, larval survival was lower on ICCL 86111 than on ICC 3137, maximum differences were observed when the terminal branches were infested with 10 neonates of *S. exigua* at vegetative stage and 15 neonates at flowering stage. Heavy rains in the 2010-11 post-rainy season possibly washed out the organic acids on the chickpea leaves (Sharma et al., 2010), resulting in reduced differences in leaf feeding and larval survival between the genotypes tested. Lower leaf feeding, larval survival and larval mass gain were recorded on EC 583260, EC 583264 and ICC 12475 (Shankar et al., 2014). Narayanamma et al., 2007 reported low larval survival and mass gain on ICC 12475. In other study (Jaba et al., 2017), significantly low *H. armigera* larval mass and maximum percent mass gain were recorded in chickpea genotypes, ICCV 097105 and ICCV 07306 respectively (101.9 mg (88.5%) and 382.3 mg (317.4%), respectively.

In plants raised under greenhouse conditions, leaf feeding was maximum when the plants were infested with 20 neonate larvae at vegetative and flowering stages. The differences in leaf feeding, larval survival and mass varied across plant growth stages and infestation levels possibly because of differences in plant growth and accumulation of secondary metabolites (Sharma et al., 2005b; War et al., 2013), that affect leaf feeding, growth and development of insects.

At the podding stage, the differences in leaf feeding between the genotypes were significant. Maximum differences in leaf feeding were recorded in branches infested with 4 and 6 larvae per branch. The results suggested that infesting the chickpea terminal branches with 10-15 neonate larvae per branch at the vegetative stage or six third-instar larvae at the podding stage could be used to evaluate chickpea genotypes for resistance to *S. exigua*.

5 ACKNOWLEDGEMENTS

We thank the Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India, for providing the financial support, and the staff of entomology for their help in carrying out these studies.

6 CONFLICTING INTEREST

The authors declare no conflict of interest

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doi:10.14720/aas.2023.119.1.2472 Original research article / izvirni znanstveni članek

Impact of coffee sustainability schemes on rural coffee producer households' living standard in Aceh province, Indonesia

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Received December 21, 2021; accepted January 24, 2023.
Delo je prispelo 21. decembra 2021, sprejeto 24. januarja 2023

Impact of coffee sustainability schemes on rural coffee producer households' living standard in Aceh province, Indonesia

Abstract: As the 3rd largest coffee producer globally, the gains from certified coffee trades have not significantly affected the farmers' economy. This study aims to re-examine the impacts of coffee certification on coffee smallholders' living standards. This study employs household survey data of 487 Aceh Gayo Arabica smallholder coffee farmers consisting of 205 fairtrade farmers, 116 organic farmers, and 166 non-certified farmers from 8 districts in Aceh Province, Indonesia, collected in 2020. A propensity score matching (PSM) approach was employed to evaluate coffee certification's impact on coffee price, per capita income, and per capita expenditure. This study found that the effect of certification was significant on the coffee price under fairtrade and organic schemes, in which fairtrade provided higher coffee prices than organic. This price improvement was also followed by an increase in the farmers' monthly per capita income. However, the application of the coffee standards has no impact on the daily per capita expenditures. This research suggests a deeper understanding to the certification scheme proponents to evaluate coffee farmers' living standard in the future.

Key words:coffee certification; smallholder farmer; living standards; propensity score matching; Indonesian Gayo Arabica

Vpliv trajnostnih shem pridelovanja kave na življenski standard kmečkih gospodinjstev v provinci Aceh, Indonezija

Izvleček: Kot tretjemu, globalno največjemu pridelovalcu kave, certificirana trgovina ni v večjem obsegu izboljšala ekonomskega položaja kmetov. Namen raziskave je bil ponovno preveriti vpliv certificiranja na življenski standard majhnih pridelovalcev kave. V raziskavi so bili uporabljeni podatki pregleda 487 gospodinjstev majhnih pridelovalcev kave (Aceh Gayo Arabica), katere je sestavljalo 205 "Fairtrade " kmetov, 116 kmetov z organsko pridelavo in 166 ne certificiranih kmetov iz 8 območij province Aceh, Indonesia, zbranih leta 2020. Za ovrednotenje vpliva certificiranja kave na njeno ceno, prihodek na pridelovalca in njegovo potrošnjo je bil uporabljen PSM pristop (propensity score matching). V raziskavi je bilo ugotovljeno, da je certifikacija značilno vplivala na ceno kave v primerih "prijazne" (Fairtrade) in organske sheme pridelave, kjer je prijazna shema omogočila višje cene kave kot organska shema. Izboljšanju cene je sledilo tudi povečanje mesečnega prihodka kmetov. Uporaba teh shem v pridelavi kave pa ni vplivala na dnevno potrošnjo posameznika. Raziskave napeljuje k poglobljenem razumevanju shem certifikacije v pridelavi kave pri vrednotenu življenskega standard kmetov v prihodnosti.

Ključne besede: certifikacija kave; majhni kmetje; življenski standard; PMS pristop; indonezijska 'Gayo Arabica'

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1 INTRODUCTION

Over the past several decades, the international food trades have undergone several significant changes. One of the reasons is based on the preference to introduce social, environmental dimensions and, most importantly, the sustainability of agricultural commodities or other market products (Helmsing & Vellema, 2012). To create this, food quality and safety standards have begun to emerge around the world. Over the last decades, these food standards have been developing in developed countries, where the population has a higher level of self-awareness of the importance of ethical and environmental aspects in the production process of agricultural products and the trade processes. In developing countries, this standard is starting to dominate urban markets, which have an increasing demand for food products of guaranteed quality and safety (Henson & Reardon, 2005; Maertens & Swinnen, 2009).

The transformation of the food market and the increasing consumer demand for safe and quality food products are influenced by several factors, including globalization, urbanization, changing consumer preferences resulting from increased living standards of the population, and increased awareness of healthy living resulting in changes in the dietary habit (Borsellino et al., 2020; Chiputwa et al., 2015). Among various agricultural commodities in the world, coffee is one of the first commodities to have standards on a sustainability basis (Ruben & Verkaart, 2012).

Most of the sustainable coffee standards are pioneered by non-governmental organizations (NGOs) that usually work with certification bodies and global retail businesses (Ibnu & Marlina, 2019; Kolk, 2013; Tran et al., 2013). Products with labels and certifications containing sustainable and ethical backgrounds attract consumers to pay prices higher than conventional products (Jena et al., 2012; Loureiro & Lotade, 2005; Meemken et al., 2017). This is supported by buyers' interest or motivation to pay extra for products that meet certain standards. The moral and personal values of a buyer also play a role in the willingness to pay for certified products (Kolk, 2013).

In Indonesia, approximately 75 percent of the total certified coffee is 'Arabica', while the rest is 'Robusta' (Ibnu et al., 2015). As the third-largest coffee-producing country in the world after Brazil and Vietnam (FAO, 2018), Indonesia still has the potential to develop its coffee production. In 2018, Indonesia's coffee production reached 756,051 tonnes (Directorate General of Plantation, 2018). It is recorded that the export figure increased to 45,360 kg in August 2020 (ICO, 2019). Domestic coffee consumption has also increased by 6 percent in December 2019 (Rahmanulloh and Mcdonald, 2020), associated with coffee outlets growth to balance the demand for coffee drink lifestyles from America and Europe.

In the late 1990s, coffee farmers in Aceh Province, Indonesia, began to adopt organic coffee intervention with assistance from an international development agency such as USAID (Arifin, 2010). The coffees from this region refer to the 'Aceh Gayo Arabica' based on geographical indication (GI) to distinguish among other coffee beans from other areas. Currently, most coffee cooperatives in Aceh possess Organic, Fairtrade, Starbucks C.A.F.E Practices certification as well as a collaborative program between Nespresso and the Rainforest Alliance since 2013, namely the AAA Sustainable Quality Program. The four certification programs have relatively similar goals in improving the welfare of coffee farmers while at the same time applying the principle of sustainability in its implementation. Fairtrade focuses on small farmers managed by cooperatives with democratic principles (Macdonald, 2007).

On the other hand, Organic emphasizes on environmental ecology. It is one of the strictest voluntary standards because farmers' land must pass a transition period of at least three years before obtaining certification (Blackman & Naranjo, 2012; Ibanez & Blackman, 2016). Both Starbucks Cafe Practices and Nespresso AAA promote sustainability and concentrate on improving producers' quality, production, and socioeconomic conditions (Niemuth et al., 2014; Renard, 2010).

The centers of certified 'Gayo Arabica' coffee in Aceh are in Aceh Tengah and Bener Meriah's districts. The physical environment and climatic conditions are suitable for agriculture and 'Arabica' coffee farmings. For that reason, coffee is the main livelihood and source of income for most of the population in these two areas. The smallholders consist of 124,236 hectares (100 percent), with coffee production reaching 70,774 tons in Aceh (Central Bureau of Statistics, 2019).

Even though it has received recognition in the domestic and international markets for its quality, assistance, and attention from all agencies or organizations are needed to improve the 'Aceh Gayo Arabica' coffee farmers' living standard. The fact that certified coffee has a higher selling price, the role of certification in improving household welfare is still questionable. Furthermore, the poverty rates in Aceh Tengah and Bener Meriah are above the national rate, as illustrated in Figure 1 (USD/IDR = 15,496.1 as per 30 December 2022). The monthly income per capita line (poverty line) in Aceh is higher than the national line, meaning that the locals require a higher standard of living. Moreover, coffee export gains have not been enjoyed by farmers living in the Bener Meriah district since their poverty rate is even higher than the poverty rate of Aceh Province and the national.

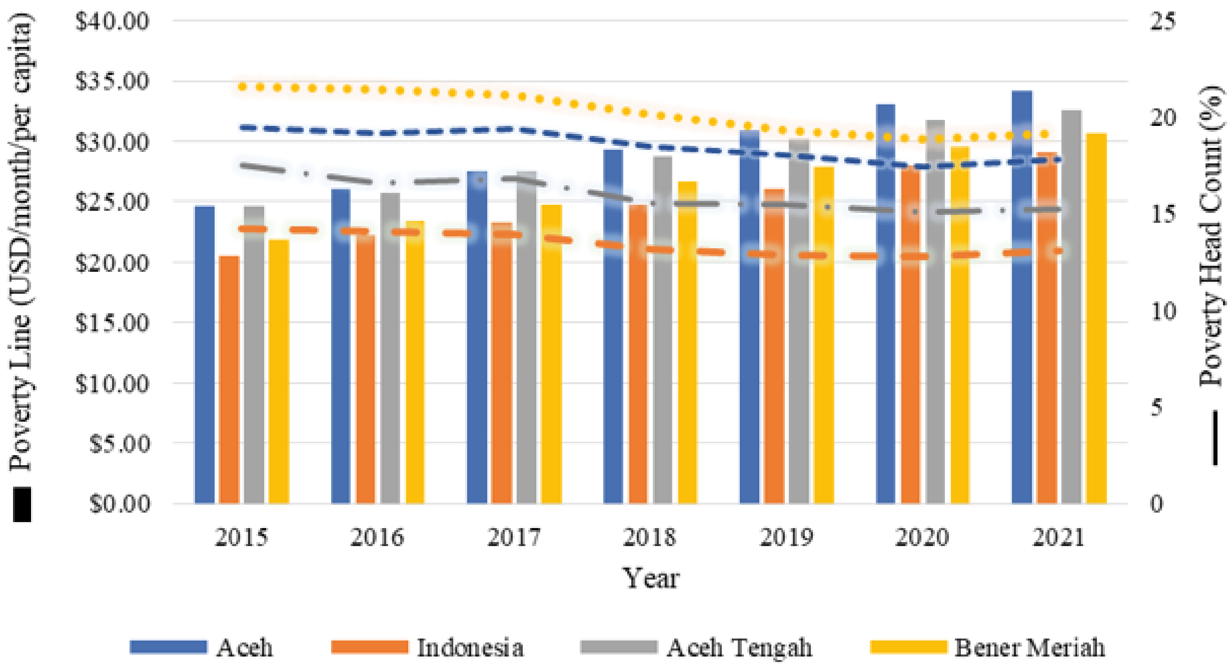

Figure 1. Poverty line and Headcount Poverty Ratio in Aceh

This study aims to assess the impacts of coffee certification schemes on smallholder 'Aceh Gayo Arabica' coffee farmers in Indonesia, highlighting the certification scheme that would give the best impacts on overall farmers' living standards. There are two research questions to be addressed: 1) What are the impacts of coffee certification on the coffee producer households' living standards?; 2) Which type of coffee certification has the greatest impact on smallholder coffee producer households?

2 METHOD

2.1 COFFEE PRODUCTION IN THE STUDY AREA

On average, Aceh Province produces roughly 156.39 thousand tons of 'Gayo Arabica' coffee during 2016-2020 (Directorate of Agriculture, 2020), mainly located in Aceh Tengah and Bener Meriah. Approximately, the coffee production areas in Aceh Tengah are 49,835 ha and in Bener Meriah are 48,950 accounting for 12 percent and 22 percent of total agricultural land in those two districts. These two districts are located in the Gayo highland with an altitude ranging from 800 to 2,600 meters above sea level and temperatures ranging from 18-20 °C, suitable for growing 'Arabica' coffee. This condition is very potential for the growth of crops, especially 'Arabica' coffee. Coffee estates in these two districts have existed since the 1900s. In 1980, coffee expansion was implemented through the transmigration program, inviting farmers from the island of Java and granting land ownership rights of 2 hectares per family, such as those in Jagong Jeged Sub-District, Aceh Tengah. Therefore, coffee plantations were characterized by similar patterns (planting year, size of the land, and coffee variety, especially in this area.

In general, Aceh Arabica Gayo coffee farmers are familiar with the traditional organic cultivation processes. The farmers applied coffee and livestock manure as natural fertilizers. Regarding pests and diseases, the farmers have different choices in dealing with them to avoid the production risks. Seeds are generally from the parents' plant. Certified seeds are rarely used due to being expensive. The common varieties are Ateng Super, Tim-Tim, Jember.

Generally, harvesting periods start from September to April annually. The coffee is harvested in the form of cherry, then is sold directly to local collectors at prices ranging from USD $0.448-$0.64 per kilogram (IDR/USD = 0.000064). Selling in the form of cherries is more convenient due to the absence of coffee processing machines. Moreover, most of the farmers demand immediate cash. In general, bean processing machines are owned by collectors. At these collectors, cherries are processed into unhulled or green beans to add value and obtain higher prices. It is recorded that the prices range from USD $0.96-$1.28 per kilogram for unhulled beans and USD $2.56-$3.2 for the green beans. The collectors sell these processed beans to local coffee cooperatives and exporters.

The certification scheme in the two districts is driven by the cooperative. In other words, certified 'Arabica' coffee exports are the core business of coffee cooperatives. There are two main certifications in Aceh Tengah and Bener Meriah districts, namely Fairtrade and Organic, demanded by importers. Through coffee cooperatives, contracts for certified coffees are created with the existing or newly established coffee farmer groups. The communication between cooperatives and the group leaders is connected by the cooperatives' agent.

Moreover, a coffee farmer group leader also acts as a coffee collector in the village. This relationship has been maintained since the first coffee certification scheme was introduced in Aceh. This relationship is dynamic. In Aceh Tengah and Bener Meriah, a farmer who has several lands can participate in many cooperatives. The farmers may decide to change or resign from a particular cooperative or farmer group. Therefore, Fairtrade or Organic label/signature in a particular coffee plot typically changes over time depending on the on-going contracts.

2.2 HOUSEHOLD SURVEY

We conducted structured interviews during June-July 2020 despite the Covid-19 pandemic and applied a multi-stage sampling procedure. At the first stage, we contacted the several coffee cooperatives and exporters in Aceh Tengah and Bener Meriah to obtain certification details, distribution of members, and coffee plantations' areas. Based on these lists, we purposely selected eight sub-districts in Aceh Tengah and another four sub-districts in Bener Meriah (Figure 2). All the selected locations produce 'Arabica' coffee.

At the time of the survey, most cooperatives joined Fairtrade and Organic certification, while the cooperative members possess only one specific certification, either Fairtrade or Organic. We could not identify cooperatives nor farmers that are under UTZ, C.A.F.E Practices, and Rainforest Alliance certifications.

Smallholder Aceh Gayo Arabica coffee producer households in Aceh Tengah and Bener Meriah Districts in Aceh Province, Indonesia, were selected for the population in this survey. There at least 60,000 farmers living

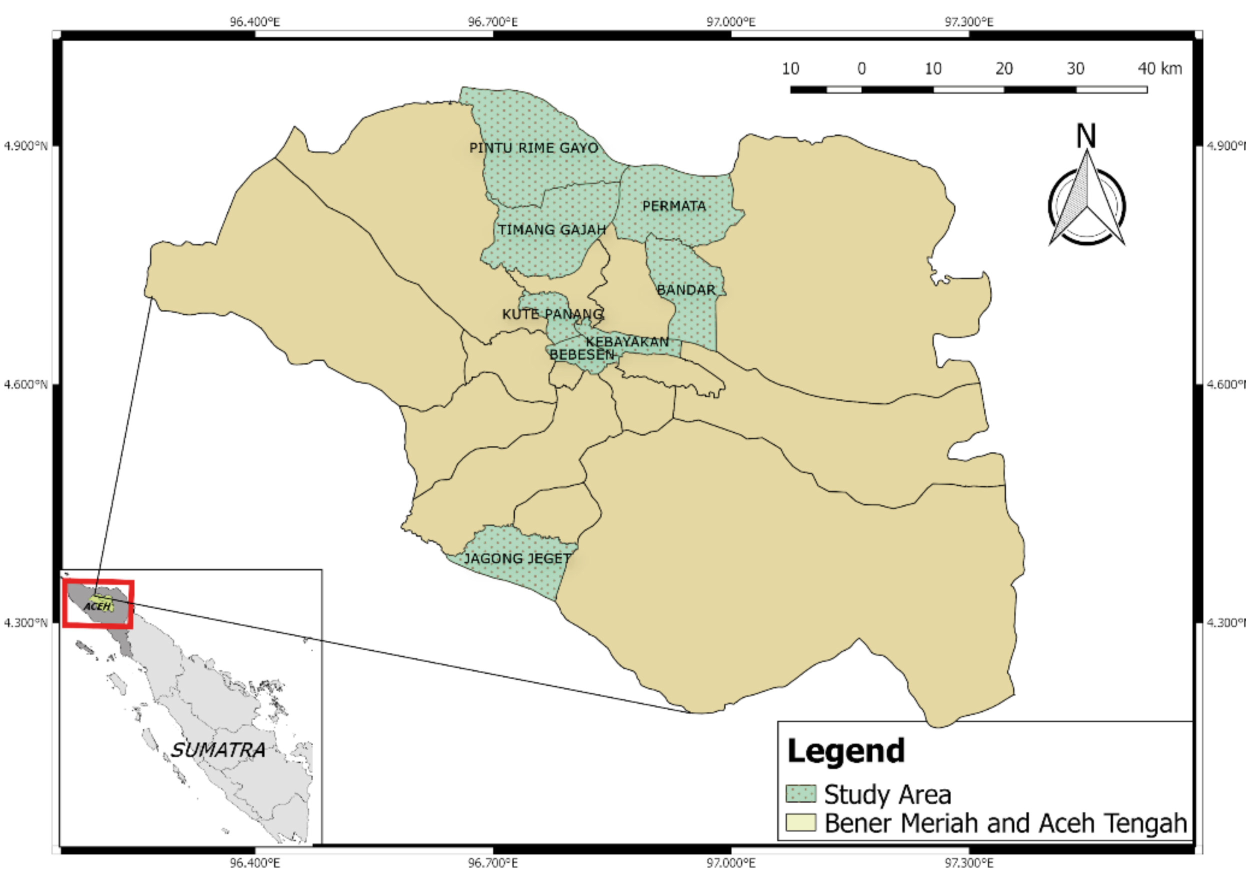

Figure 2:. Coffee household survey location

in those two regions (Central Bureau of Statistics, 2019). These farmers normally cultivate more than one commodities, a combination of coffee and horticulture. Based on eight sub-districts that were selected previously, certified and non-certified coffee farmer households were randomly selected for the survey. These certified coffee producer households will be treated as the treatment group in the impact analysis whereas the non-certified coffee farmer households will be the control group. Using a 95 % of confidence level and 5 % margin of error, the minimum size of samples needed will be around 382 farmers. In total, we interviewed 487 smallholder Aceh Gayo coffee farmers, consisting of 205 fairtrade farmers, 116 organic farmers, and 166 non-certified farmers (Table 1).

We used a structured questionnaire to interview all of the respondents in the research areas. The questionnaire includes most of the basic household demographics, income, food and non-food expenditures, and some aspects of coffee production and marketing. Field surveys and interviews were conducted during pre-harvest sessions. We believe that consumption spikes and money circulation are absent during these periods, and therefore it should not lead to any biases in the impact assessments.

2.3 METHOD ANALYSIS

As the first step, binomial logit is used to evaluate the factors that influence Aceh Gayo Arabica smallholder farmers' decision to participate in a coffee certification scheme. Later on, this binomial logit will be used to calculate the propensity scores for further impact analysis. The dependent variables in the logit regression model are based on the respondents' response to the question: "Are you joining a particular certification or not ?". The response will be given a score of "1" if the coffee household participates in a particular certification scheme; and a score of "0" if the coffee household does not participate in any certification schemes. In general, the logit model is written as follow (Azen & Walker, 2011)

$$\mathrm{Logit}(Px) = \log Px/(1 - Px) = \beta0 + \beta1x1 + \beta2x2 + \beta3x3 + \cdots + \beta jxj \tag{1}$$

where *Px* is the probability of participating the certification; *1 - Px* is the probability of not participating the certification; β0 is the constant; β1, · · , βj are the parameter coefficients; and *x1, · · · , xj* are the explanatory variables.

It is predicted that many individual characteristics might influence farmers' decision to participate in a certification scheme, such as male household head, age, education, and dependents. The length of stay in the village may also influence farmers' decisions because farmers usually observe their villages' certification participation process. The coffee plantation age may also have an effect because it is generally easier to enter certification for newly established coffee states. An active member of a coffee farmer group or cooperative may also be more willing to participate in a certification scheme. Several accesses to agriculture may also determine the farmers' decision, e.g., access to input markets, access to finance, and access to extension services. The variables included in the multinomial logit and their estimation results are shown in Table 2.

In the second stage, the Propensity Score Matching (PSM) is used to estimate the impact of certification on coffee farmer poverty. PSM uses information from a group of units that do not participate in the intervention to identify the participating units' outcome in the absence of the intervention. By comparing how different the participants' results relative to the non-participants, the effect of the intervention will be estimated (Heinrich

Table 1: The study area and sample size

		Type of certification / Number of coffee producer households			
District	Subdistrict	Non-certified	Fairtrade	Organic	Total Sample
Bener Meriah	Bandar	43	38	27	108
	Pintu Rime	11	14	-	25
	Timang Gajah	9	16	2	27
	Permata	28	21	12	61
Aceh Tengah	Kute Panang	19	35	22	76
	Bebesen	27	30	24	81
	Kebayakan	14	13	6	33
	Jagong Jeged	15	38	23	76
Total Sample		166	205	116	487

et al., 2010). Based on Rosenbaum & Rubin (1983) and Abdia et al. (2017)such as matching, regression, stratification, inverse probability weighting (IPW, the formula for estimating the impact of a program on the participating individuals or the Average Treatment Effect on the Treated (ATT) is:

$$ATT = E(Y_1|e(X), Z = 1) - E(Y_0|e(X), Z = 0) \qquad (2)$$

where Y_1 is the outcome under treatment, while Y_0 is the outcome under no treatment. Subjects with covariate *X (e (X))* in the treatment group will be compared with subjects in the comparison group with the same covariate *X (e (X))*. *Z = 1* indicates the treated group while *Z = 0* is the control group.

To match control households with treatment households, the Nearest Neighbour Matching (NNM) method is used. NNM is one of the most frequently chosen matching methods (Austin, 2011; Stuart, 2010). Participants from the control group will be paired with participants from the group that received the treatment based on the closest propensity score. There are several variations of the NNM matching method, namely NNM "with substitutes" and NNM "without substitutes". The use of NNM "with substitutes" is when cases are found where the distribution of trend score data in the treatment and control groups is very different. For example, many participants in the treatment group have high propensity scores, but only a few participants with high scores in the control group. Under these circumstances, the matching process and quality will decrease, and bias will increase. This can be overcome by doing a "replacement", which will reduce the number of different non-participants, increasing the variance (Caliendo & Kopeinig, 2008).

3 RESULT AND DISCUSSION

3.1 DATA AND STATISTICS

During the interview, we collected data consisting four aspects of coffee producer households. First of all, this study identified socio-economic household characteristics that may including age and gender of the farmers, education level, number of family members, period of stay in village, housing structure and respondent's main occupation. Age, gender and educational level show the human resources as the main labor forces in Aceh coffee production. Number of family members indicates the households' dependency level and economic power in the family. Period of stay in the village indicates the interaction periods between the famers and coffee environment in the study areas. Housing structure and main occupation show the economic status in the community.

The certification scheme is also strongly related with coffee farming characteristics. The scheme normally required data on land size and productivity, labor capacity, and land status during the feasibility study phase. Experience in coffee cultivation is also important since it may reflect the farmers' ability to digest the benefit and cost of joining the certification. Price of coffee (cherry) is purposively in a unit of can (1 can = 1.2 kg) so that the farmers can easily detect the price difference using their selling tradition method.

We also measure the connectedness between coffee farming and supporting access. The access consisted of physical (distance to main road and agricultural inputs market), technological (internet access and financial digitalization), and services (credit and agricultural extension). We expect that the more connected between coffee producers and the supporting access, the more influence in the coffee certification decision making.

Lastly, we identified variables to measure the living standards including income, expenditure, and asset ownership. It was a challenge to measure the coffee producers' income since their revenue from selling depended on harvesting periods per year. The amount received from selling also varied from time to time. Similarly, the expenditure was also sensitive case since most the households might refuse to answer. We were also aware of the respondents' trap that the total expenditure might be higher than the total income within the same period.

There are several fundamental differences between the characteristics of the certified and the non-certified coffee farmers, as well as among the certified coffee farmers, as shown in Table 2. Certified coffee farmers are generally male with the position of head of household who have lived longer periods in their village and have a longer coffee farming experience. Certified farms also have larger sizes of coffee areas but lower productivity. Furthermore, coffee prices, labor capacity, access to input markets, and access to an agricultural extension are significantly different for the certified coffee than the non-certified.

On average, Fairtrade farmers possessed larger sizes of coffee areas and received higher prices on their coffees. On the other hand, organic farmers have slightly higher productivity, are closer to extension services access, and have higher labor capacity.

3.2 ESTIMATION RESULT

(a) Factors influencing the certification decision

We begin this analysis by examining the factors

Table 2: Summary statistics by certification scheme

	Pooled sample		By certification scheme	
	non-certified (N = 166)	certified (N = 321)	Fairtrade (N = 205)	Organic (N = 116)
Household Characteristics				
Age (years)	42.373 (11.44)	44.289* (11.52)	44.160 (12.18)	44.517 (10.27)
Education (years)	11.102 (3.86)	10.704 (3.70)	10.565 (3.72)	10.948 (3.66)
Gender (female = 0;male = 1)	0.385 (0.48)	0.542*** (0.49)	0.492 (0.50)	0.629*** (0.48)
Status of Respondent (dummy)	0.373 (0.48)	0.551*** (0.49)	0.512 (0.50)	0.620*** (0.48)
Household size (members)	4.186 (1.29)	4.242 (1.43)	4.219 (1.34)	4.284 (1.59)
Years staying in village	26.253 (17.07)	30.647*** (15.50)	30.414 (15.95)	31.060 (14.74)
House structure (dummy)	0.349 (0.47)	0.398 (0.49)	0.414 (0.49)	0.370 (0.48)
Main job (dummy)	0.891 (0.31)	0.878 (0.32)	0.882 (0.32)	0.870 (0.33)
Farm characteristics				
Land size (ha)	0.984 (0.74)	1.237*** (1.03)	1.248* (0.95)	1.217 (1.15)
Productivity (kg ha^{-1})	1374.12 (385.53)	1284.28** (364.09)	1297.26 (373.18)	1261.35* (347.84)
Price (IDR/can)	6849.39 (1131.46)	8688.47*** (1707.38)	8946.34*** (1657.44)	8232.759 (1706.03)
Experience in coffee farming (years)	18.243 (12.42)	21.52*** (10.71)	21.292 (11.14)	21.948* (9.94)
Land status (dummy)	0.963 (0.18)	0.968 (0.17)	0.960 (0.19)	0.982 (0.13)
Labor capacity	2.926 (0.86)	3.111* (1.04)	3.052 (0.96)	3.217** (1.17)
Access supports				
Access to extension (times/year)	1.680 (2.26)	2.305* (3.90)	1.873 (2.28)	3.068*** (5.67)
Access to credit (dummy)	0.349 (0.47)	0.389 (0.48)	0.375 (0.48)	0.413 (0 .49)
Access to input market (km)	3.385 (2.38)	4.074** (3.77)	4.048 (3.66)	4.120 (3.97)
Access to main road (km)	2.077 (3.01)	2.136 (2.95)	2.078 (2.40)	2.238 (3.74)
Number of family member with internet access (persons)	1.632 (1.26)	1.660 (1.39)	1.624 (1.33)	1.724 (1.49)
Access to financial digitalization (dummy)	0.174 (0.38)	0.183 (0.38)	0.156 (0.36)	0.232* (0.42)
Access to internet (dummy)	0.771 (0.42)	0.788 (0.40)	0.795 (0.40)	0.775 (0.41)

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Living Standard Characteristics				
Income per capita (IDR/ month)	1668464 (619936.6)	1758879 (800831.5)	1770000 (708584.4)	1739224 (945143.3)
Expenditure per capita (IDR/day)	34266.98 (15051.79)	32111.81 (15588.03)	32658.45 (14543.95)	31145.75 (17304.14)
Cattle ownership (dummy)	0.439 (0.49)	0.492 (0.50)	0.482 (0.50)	0.508 (0.50)
Motorcycle ownership (dummy)	0.951 (0.21)	0.953 (0.21)	0.946 (0.22)	0.965 (0.18)
Fridge ownership (dummy)	0.801 (0.40)	0.937*** (0.24)	0.921* (0.26)	0.965*** (0.18)

Notes: Mean values are shown with standard deviations in parentheses. Mean values across schemes are tested for statistically significant differences; $^{*}p < 0.1$, $^{**}p < 0.05$, $^{***}p < 0.01$ when compared to non-certified farmers

Table 3: Logit model estimate for participants in the certification scheme

	Pooled sample	Fairtrade	Organic
Household Characteristics			
Age (years)	-0.038 (0.03)	-0.120** (0.05)	0.040 (0.05)
Education (years)	-0.124 (0.09)	-0.223 (0.13)	-0.033 (0.13)
Gender (female = 0;male = 1)	0.425 (1.32)	0.820 (1.68)	-0.633 (1.78)
Status of Respondent (dummy)	1.016 (1.38)	0.866 (1.74)	2.123 (0.98)
Household size (members)	0.006 (0.28)	-0.031 (0.37)	0.145 (0.44)
Years staying in village	0.063** (0.02)	0.140*** (0.04)	0.057* (0.03)
House structure (dummy)	-0.518 (0.66)	-0.833 (0.99)	-0.171 (1.04)
Main job (dummy)	-0.491 (1.04)	-1.539 (1.52)	-1.057 (1.50)
Farm characteristics			
Land size (ha)	-0.198 (0.36)	-0.344 (0.417)	-0.652 (0.47)
Productivity (kg ha^{-1})	-0.001 (0.00)	-0.000 (0.00)	-0.002* (0.00)
Price (IDR/can)	0.003*** (0.00)	0.004*** (0.00)	0.003*** (0.00)
Experience in coffee farming (years)	0.026 (0.03)	0.029 (0.04)	0.016 (0.05)
Land status (dummy)	-1.056 (1.35)	-2.048 (1.63)	-0.101 (1.98)
Labor capacity	0.054 (0.43)	-0.417 (0.56)	0.318 (0.57)

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Access supports			
Access to extension (times/year)	0.186 (0.12)	0.027 (0.45)	0.264* (0.15)
Access to credit (dummy)	0.993 (0.65)	2.063** (0.94)	0.260 (1.03)
Access to input market (km)	0.263* (0.14)	0.461** (0.22)	0.472* (0.26)
Access to main road (km)	-0.501*** (0.15)	-1.156*** (0.35)	-0.684** (0.27)
Number of family member with internet access (persons)	-0.059 (0.35)	-0.234 (0.55)	-0.239 (0.50)
Access to financial digitalization (dummy)	1.174 (0.91)	0.349 (1.26)	1.923 (1.35)
Access to internet (dummy)	-0.142 (1.02)	-0.166 (1.60)	-0.676 (1.49)
Living Standard Characteristics			
Income per capita (IDR/ month)	-6.64e (5.07e)	-1.27e* (7.50e-)	-3.26e- (7.92e-)
Expenditure per capita (IDR/day)	0.000 (0.00)	0.000 (0.00)	0.000 (0.00)
Cattle ownership (dummy)	-0.411 (0.62)	0.375 (0.88)	-1.180 (0.95)
Motorcycle ownership (dummy)	-0.473 (1.58)	0.354 (2.32)	-0.572 (2.29)
Fridge ownership (dummy)	1.660 (1.00)	2.526* (1.37)	2.040 (1.55)

Notes: Coefficient estimates are shown with standard errors in parentheses. The base category consists of farmers without any certification. * $p < 0.1$; ** $p < 0.05$; *** $p < 0.01$

that influence farmer participation in coffee certification schemes and the estimation results are shown in Table 3

Under pooled data, farmers' tendency to participate in coffee certification schemes is influenced by the period of stay in the village (inheritance factor), coffee prices, access to input markets, and access to main roads. The longer the period of stay in the village, the more likely they are to participate in a coffee certification scheme. The price of certified coffee also increases the likelihood of certification participation. Proximity to the main road will facilitate access while minimizing the costs of marketing, increasing the likelihood of farmers participating in certification. The need for special inputs in sustainable coffee cultivation affects the tendency to join the certification.

Prices remain consistent in increasing farmers' likelihood to join fairtrade or organic. Similarly, it applies to input market access and main roads. Access to agricultural extension seems essential for organic farmers, while access to credit affects farmers' likelihood to participate in fairtrade.

(b) Impact of certification

The average treatment effect on the treated is estimated into several models. We applied the certification schemes as the treatment (T = 1 if coffee is certified; T = 0 if coffee is not certified). In the case of fairtrade vs. organic, we applied T = 1 for fairtrade and T = 0 for organic. Here, the outcomes include coffee price, daily per capita expenditure, and monthly per capita income. The estimation result is illustrated in Table 4.

First, we compared certified coffee farmers with non-certified farmers. The result shows that coffee certification impacts coffee prices, which is IDR 1,654.20 per can (24.15 %) higher than the average price of non-certified coffee. In general, the monthly per capita income of certified coffee farmers is also higher at IDR. 384,112.1 or about 23.02 % higher than the average per capita income of non-certified farmers. However, the impact on per capita expenditure is insignificant.

This study found that certification was significant on coffee price variables, both in fairtrade and organic schemes, when comparing non-certified farmers to each certification scheme. Participation in fairtrade increases the price by IDR. 1,878.04 per can (27.41 % higher than

Table 4: Average Treatment effect for household coffee producer certification

Treatment	Output	ATT	S.E	t-values
all certified vs non-certified	coffee price (IDR/can)	1654.20***	351.86	4.70
	income per capita (IDR/month)	384112.1**	119953.4	3.20
	expenditure per capita (IDR/day)	1268.17	5611.49	0.23
Fairtrade vs non-certified	coffee price (IDR/can)	1878.04***	349.25	5.38
	income per capita (IDR/month)	144390.2	184934	0.78
	expenditure per capita (IDR/day)	3638.27	5110.95	0.71
Organic vs non-certified	coffee price (IDR/can)	1405.17**	394.44	3.56
	income per capita (IDR/month)	-173706.9	653086.7	-0.27
	expenditure per capita (IDR/day)	-5908.77	11393.96	-0.52
Fairtrade vs Organic	coffee price (IDR/can)	78.53	110.44	0.71
	income per capita (IDR/month)	209816.8**	82527.45	2.54
	expenditure per capita (IDR/day)	3610.39	1913.07	1.89

Notes: Coefficient estimates are shown with standard errors in parentheses. The base category consists of farmers without any certification. *$p < 0.1$; **$p < 0.05$; ***$p < 0.01$

the average price of non-certified coffee), while participation in the organic scheme increases the coffee price by IDR. 1,405.17 (20.51 % higher than the average price of non-certified coffee). When we compared fairtrade and organic, participation in fairtrade increases per capita income by IDR. 209,816.8 per month or 12.06 % higher than the average per capita income of organic coffee farmers. However, no significant impact was found on prices and expenditure per day.

3.3 DISCUSSION

Participation in the coffee certification scheme is mainly correlated with cooperative membership. Unlike in other coffee producing countries, coffee certification participation in Indonesia is a collective decision. Participation in coffee certification is mainly influenced by local collectors. Collectors are closely connected with the farmers and understand each coffee farmer's socio-demographic living under their territory. The producer-collectors relationship in Indonesia is not limited to coffee trades but includes a capital provision, non-financial support, and market information dissemination.

Additionally, a collector also acts as a cooperatives' right hand and is placed to each village or sub-district to deal with new certification member recruitments, extending cooperatives' networks, and manage existing coffee production under cooperatives. However, collectors may not always sell their coffee to the cooperatives. Cooperatives provide exclusive services for their members. However, non-certified farmers who do not belong to the cooperative can also sell their coffee.

Traditional coffee practices in Aceh rely on natural fertilizer such as coffee compost. Therefore, the conversion process to certification (especially organic) was relatively straightforward because the pre-existence condition has already been fulfilled. However, recent trends show discontentment on their production results, forcing traditional farmers to apply extra inputs, e.g., chemical fertilizers and pesticides, to boost production. This production change is mainly caused by external factors (e.g., pests/diseases, climate change). This phenomenon leads to cases where coffee farmers resign from certification schemes, either voluntarily or due to certification requirements violation.

External or hired labor in Aceh Tengah and Bener Meriah increases dramatically during the harvest through September-April. Each sub-district has its distinct characteristic on the intensity of labor use. In most cases, additional labor comes from relatives or neighboring dwellers. Unlike other coffee-producing countries, Aceh Gayo coffee landowners manage contacts of the designated labor from neighboring provinces. The landowners treat the laborers as part of families by providing living spaces to live in, referring to a kinship. These additional laborers typically experienced in other plantations, e.g., rubber or palm oil. They willingly migrate and stay for an extended period to harvest coffee because coffee provides higher earnings compared to their original crops. However, the Covid-19 pandemic has raised concern about these external labor demands. However, no strict policy was found in the surveyed areas, and the decision for accepting the external laborers differs from each coffee farmer.

Female coffee farmers played important roles in the coffee cultivation especially during the harvesting periods. However, traditional norms in study areas direct female farmers to give authority for decision making to their spouses. Many female farmers were reluctant to give their opinion during the interview. Although female farmers were closely involved in the coffee production, they were willing to represent their opinion represented by the male farmers. We found a solid coffee cooperative/exporter that fully consisted of female members in the study area. This female coffee cooperative managed the whole coffee production and the decision making was made through collective discussions by female representatives. However the number of this female cooperative is still limited.

The food standards application in the global coffee trade leads to an increasing trend in the certified coffee market (Nugroho, 2014). This attraction is then enforced in various ways into coffee-producing countries, where most of the agricultural structure consists of 80-90 percent of smallholder coffee farmers. Hopefully, the application of coffee standards will be able to increase their living standards. For that, we have analyzed the impact of coffee standards implementation and certification schemes on the Aceh Gayo Arabica coffee farmers' living standards in Aceh Province, Indonesia. The results showed that, in general, certified coffee farmers received more promising prices than non-certified farmers. This price improvement was also followed by an increase in the farmers' monthly per capita income. However, the application of the coffee standards has no impact on the daily per capita expenditures.

This study also has analyzed the impact of each certification separately. The results remain consistent that fairtrade provided higher coffee prices than the organic scheme. However, this study could not find significant impacts on improving per capita income or consumption expenditure per capita of coffee farmers. We argue that this weakness is due to the limitation of the sample size.

Several reasons may explain the strong relationship between the impact of coffee certification and coffee price. First, smallholder coffee farmers, who own limited coffee areas, seem difficult to increase coffee yields or productivities. The productivity of non-certified coffee is 90 kg ha^{-1} higher than certified coffee. Therefore, a higher selling price of coffee under the certification schemes allows smallholder coffee farmers to obtain equal or even better revenues than non-certified coffee. Generally, the average price of fairtrade coffee is reported to be higher than non-certified coffee prices (Arnould et al., 2009; Bacon, 2005; Raynolds et al., 2004; Ruben & Zuniga, 2011). Second, the smallholder coffee farmers prefer immediate cash payments rather than turning into further coffee processing stages. Thereby, certified coffee farmers have been satisfied by the guaranteed price. Moreover, these farmers perceived that either coffee processing costs or the investment in equipment are greater than the benefits. Normally, only large-scale farmers or collectors own coffee processing equipment and sell the processed coffees. Third, the financial relationship between smallholder coffee farmers and collectors along the coffee chain is based on kinship. However, both coffee farmers (borrowers) and collectors (lenders) prefer immediate returns on their capital to manage the capital turnover. Thus, cash received by the coffee farmers can be used directly to repay for loans/debts.

The conflicts between coffee quality and yield, as they were discussed in Chiputwa (2015) as well as in Barham and Weber (2012), indicate that coffee certification has failed to formulate the promises of coffee standards into the improvement of farmers' living standards. In the case of coffee in Indonesia, farmers mostly sell coffees in the form of coffee cherries. Therefore, coffee quality is often neglected, and farmers end up with prices that are not significantly different. Considering the tremendous role of certification, there should be efforts to improve the smallholder coffee quality. In coffee farming, individual farmers are responsible for managing the quality of their coffees. Therefore, as indicated by this study, the improvement in coffee prices may not reflect either the improvement of coffee quality or the improvement of smallholder coffee farmers' income and welfare.

4 CONCLUSION

To conclude, two valuable lessons can be taken from this research. First, the environmental conditions of Aceh Gayo Arabica coffee farming have been suitable with the requirements of global coffee certification standards. Minimized transformation efforts are needed to change the traditional cultivation system into a more sustainable coffee production system. Second, smallholder coffee farmers view the certification scheme as an opportunity to improve prices. The impact of certification schemes on price improvements has been validly proven in the estimation results. However, the role of certification schemes in collectively improving coffee quality is still questionable. An increase in coffee price allowing for improvements in certified farmers' income and living standards is still not clear. Therefore, it is expected that the participation of smallholder coffee farmers in the certification scheme is temporary. Most of the farmers perceived that the overall benefits have little impact on their economy.

This research provides a better understanding to certification scheme proponents that the role of certification in improving smallholder coffee farmers' living standards may still need to be improved. The current relationship between coffee certification regimes and local coffee cooperatives disregarded the role of local government and higher education institution. This study recommends that local government as a policy maker may develop interventions and regulation to protect the vulnerable farmers from being excessively exploited by the certification regime. The certification regimes were failed to provide reasonable price during the Covid-19 pandemic due to export restriction and cross border trade closure. Rural coffee producer households have less bargaining power during this situation and were forced to sell their coffee under the market.

The certification scheme also disregarded the coffee farmers' capacity to deal with future challenges due to climate change. There are two difficult options available for Aceh Gayo coffee producers: moving toward higher altitude, or changing the variety which more reliable to climate change. Both options might seem beyond the local coffee producer household's capacity. Therefore, local universities and research institutes are recommended to work together with the coffee certification bodies to mitigate future climatic risks on coffee production in Indonesia.

4.1 ACKNOWLEDGEMENTS

This work was supported by the Universitas Syiah Kuala, the Ministry of Education, Culture, Research and Technology [grant number 268/UN11/SPK/PNBP/2020].

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doi:10.14720/aas.2023.119.1.2940 Original research article / izvirni znanstveni članek

Tolerance to Zn toxicity in the halophyte *Lepidium latifolium* L. and the effect of salt on Zn tolerance and accumulation

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Received November 28, 2022; accepted January 24, 2023.
Delo je prispelo 28. novembra 2022, sprejeto 24. januarja 2023

Tolerance to Zn toxicity in the halophyte *Lepidium latifolium* L. and the effect of salt on Zn tolerance and accumulation

Abstract: Halophytes exhibit a high cross-tolerance to multiple stresses that enable them to survive under harsh environmental conditions. We hypothesized that salt treatment in halophytes improves their tolerance against other stressors. To investigate the salt-mediated heavy metal tolerance in halophytes, *Lepidium latifolium* (Brassicaceae) was cultivated in the absence or presence of salt (100 mM NaCl) and excess Zn (200 µM $ZnSO_4$), alone or in combination, for four weeks in the hydroponic medium. Salt treatment ameliorated the reduction of photosynthetic pigments in Zn-stressed plants and decreased Zn accumulation in the young leaves. The activity of peroxidase increased by both Zn toxicity and salt treatments; its maximum activity was achieved under the combination of both treatments associated with a significant reduction in malondialdehyde concentration. The activity of polyphenol oxidase increased by Zn stress alone or in combination with salt, accompanied by accumulation of free and cell wall-bound phenolics and enhanced lignin deposition in the leaves. Our results showed a mitigating effect of salt treatment in Zn-stressed plants through the activation of antioxidant defense and accumulation of phenolic compounds including flavonoids. Our results suggest *L. latifolium* as suitable species for revegetation and rehabilitation of saline soils contaminated with heavy metals.

Key words: halophytes; Zn toxicity; *Lepidium latifolium*; antioxidant defense; phenolics; lignin

Toleranca na strupenost Zn pri halofitu *Lepidium latifolium* L. in učinek soli na toleranco in kopičenje cinka

Izvleček: Halofiti imajo veliko navskrižno toleranco na multipli stres, kar jim omogoča preživetje v neugodnih okoljskih razmerah. Predpostavljamo, da obravnavanje s soljo pri halofitih izboljša njihovo toleranco na druge stresorje. Preučevali smo s soljo vzpodbujeno tolerenco na težke kovine pri halofitu *Lepidium latifolium* (Brassicaceae), gojenem v prisotnosti ali odsotnosti soli (100 mM NaCl) in pribitku cinka (200 µM $ZnSO_4$), posamično ali v kombinaciji, štiri tedne v hidroponskem gojišču. Obravnavanje s soljo je zmanjšalo upad vsebnosti fotosinteznih barvil v rastlinah v stresu zaradi cinka in zmanjšalo njegovo akumulacijo v mladih listih. Aktivnost peroksidaze se je povečala v obeh primerih, zaradi toksičnosti zinka in obravnavanja s soljo, in je dosegla največjo aktivnost v kombinaciji obeh obravnavanj, kar je bilo povezano z značilnim upadom koncentracije malondialdehida. Aktivnost polifenol oksidaze se je povečala v stresu zaradi cinka samega ali v kombinaciji z obravnavanjem s soljo, kar je bilo povezano z akumalacijo prostih ali na celično steno vezanih fenolov in pospešilo odlaganje lignina v listih. Ti rezultati so pokazali blažilni učinek obravnavanja s soljo v rastlinah v stresu zaradi cinka z aktiviranjem antioksidacijske obrambe in akumulacijo polifenolov. Rezultati tudi nakazujejo, da je halofit *L. latifolium* primerna vrsta za ozelenitev in izboljšanje slanih tal onesnaženih s težkimi kovinami.

Ključne besede: halofiti; strupenost Zn; *Lepidium latifolium*; antioksidacoijska obramba; fenoli; lignin

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1 INTRODUCTION

Environmental pollutants are rising progressively due to enormous economic development and the rapid growth of agriculture, urbanization, and industrial activities. Heavy metals are the most prevalent contaminants released from natural and anthropogenic sources into the environment and cause soil, air, and water pollution (Tchounwou et al., 2012). Heavy metals may accumulate in high concentrations in the edible part of crop plants, which are considered the primary cause of some diseases in humans and animals (Manara, 2012). Cadmium (Cd), lead (Pb), chromium (Cr), copper (Cu), nickel (Ni), and zinc (Zn) are the most common heavy metals that their accumulation in the environment causes an alarming situation of health problems (Tchounwou et al., 2012). DNA damage, inactivation of enzymes, and carcinogenic effects are predominant complications in humans after exposure to high concentrations of heavy metals (Manara, 2012).

Heavy metal toxicity in plants generally occurs through four principal mechanisms: 1) induction of oxidative stress through excess generation of reactive oxygen species (ROS) and changes in the permeability and integrity of the membranes, 2) changes in folding and activities of some proteins and enzymes due to the binding of heavy metals to their sulfhydryl groups, 3) competition with micronutrients to participate in cellular functions due to having similar physicochemical properties with them and 4) displacement of essential metal ion cofactors in the active sites of enzymes (Dal Corso, 2012).

Plants have some mechanisms for coping with heavy metal stress such as avoidance of metal uptake, prevention of their transport into the shoots, activation of defense mechanisms against ROS, and sequestration of heavy metals in the aerial parts through chelation by some organic compounds (Viehweger, 2014).

Zinc is an essential micronutrient for higher plants (Hafeez et al., 2013). However, similar to other heavy metals, it is toxic under excess concentrations (Küpper & Andresen, 2016). The toxic effects of Zn depend on its external bioavailable concentration, exposure time, and developmental stage of plants (Balaferj et al., 2020). Inhibition of shoot growth, reduction of root elongation, chlorosis of young leaves, and in some cases, cell death are the most obvious symptoms of Zn toxicity (Küpper & Andresen, 2016). Internal detoxification of Zn is achieved through its sequestration in the cytoplasmic compartments particularly in the vacuoles as chelated form with organic molecules, or as free ions (Balaferj et al., 2020).

Halophytes adapt to and grow under salinity conditions, consequently, they are interesting model species for the study of adaptation and tolerance mechanisms in harsh environments. Numerous physiological and molecular adaptive mechanisms, e.g. the ability to limit the entry of ions into the transpiration stream, ion compartmentation, and synthesis of compatible osmolytes, have developed in the halophytes, that may confer also tolerance to toxic concentrations of heavy metals (Van Oosten & Maggio, 2014). Halophytes are considered potent candidates for removing heavy metals from soils due to their higher ability for accumulation and phytoremediation (Peng et al., 2022).

Lepidium L. is a genus belonging to Brassicaceae and encompasses over 175 species (Mummenhoff et al., 2009). *L. latifolium*, known as perennial pepperweed or tall white top, is a perennial facultative halophyte native to Asia and part of southeastern Europe (Spenst, 2006). The plants spread through small and large numbers of seeds or vegetative reproduction and grow in a wide range of habitats. Because of its invasive and near-ubiquitous nature, this species is gaining more attention and is recognized as a global invader (Francis & Warwick, 2007). *L. latifolium* has also been widely used in traditional medicine as a diuretic and to reduce prostate hyperplasia, is a rich source of antioxidant compounds including phenolics, and is used as a whole herb for bacterial dysentery, enteritis, and other diseases (Kaur et al., 2013). Physiological studies on *L. latifolium* have mainly focused on drought resistance, salt tolerance, mineral elements, proteins, and amino acids (Hajiboland et al., 2020). There is no information on heavy metal accumulation or tolerance in this species.

Priming is a useful strategy for the improvement of the defense responses of plants against stressors. Various chemical compounds are used for priming; however, the effect of low concentrations of salt, as a priming agent has been relatively less investigated (Sako et al., 2020). In most studies, priming agents are applied at the seed germination stage, which may have a short-term effect on the tolerance of plants against stress. The impact of priming treatments in mature plants, however, has attracted much less attention from researchers.

Studies on the effect of low concentrations of salt in adult plants on their tolerance to heavy metal toxicity are still scarce. In addition, the effect of salt priming on heavy metal accumulation and tolerance in the halophytes has not been sufficiently addressed. A high cross-tolerance in the halophytes to multiple stresses that enables them to survive under extreme environmental conditions could be, at least partly, mediated by the salt-mediated induction of tolerance mechanisms. Our working hypothesis was that tolerance to toxic concentrations of Zn is enhanced through exposure to salt in the halophyte *L. latifolium*. For evaluation of the physiological response of plants, the antioxidant defense system, activity of phenolics metabolizing enzymes, and lignin deposition were analyzed in addition to the elemental composition under Zn stress in the absence or presence of low salt concentration as priming treatment in this species.

2 MATERIALS AND METHODS

2.1 PLANTS CULTURE AND TREATMENTS

Seeds of *L. latifolium* were collected in 2016 from a wild-grown population in Meghan Playa in north central Iran. To obtain sufficient seeds with a high germination rate, the collected seeds were germinated and the young seedlings were cultivated in a private garden until flowering and seed set stage. The seeds from these plants were used for this work.

Seeds were surface-sterilized with 10 % sodium hypochlorite and sown in plastic containers filled with washed perlite and irrigated with distilled water. After three weeks, the young seedlings were transferred to the light and irrigated with 50 % Hoagland nutrient solution (pH 5.8). Four-week-old seedlings were cultivated in plastic containers filled with aerated 100 % Hoagland solution, and after one week, the seedlings were transplanted in 2-liter hydroponic pots for starting the treatments. Plants were grown in a growth chamber with 16/8 h of light/dark photoperiod at 25/17 °C, relative humidity of 50–60 %, and at a photon flux density of about 400 μmol m^{-2} s^{-1} provided by fluorescent lamps. The nutrient solutions were refreshed weekly.

To evaluate the tolerance level of plants to salt and Zn toxicity, a preliminary experiment was designed with 400 mM NaCl and 400 μM $ZnSO_4$ separately in the hydroponic medium. The concentration of salt and Zn in the culture media was gradually increased by adding 50 mM NaCl and 50 μM $ZnSO_4$ every day. One week after reaching the final concentration of salt and Zn, plants were harvested, and their biomass was determined.

In the main experiment, the five-week-old plants were pretreated with 100 mM salt (NaCl) for one week and then Zn (as $ZnSO_4$) treatment at 200 μM was used simultaneously with salt treatment. Both NaCl and Zn were applied increasingly, by 50 mM and 50 μM steps on daily basis, respectively. Plants were grown for four weeks under treatment conditions and then harvested. At harvest, plants were washed with distilled water, blotted dry on filter paper, and their fresh mass (FM) was determined.

2.2 BIOCHEMICAL AND ELEMENTAL ANALYSES

Shoot parts were separated into young (the second youngest leaf) and old leaves (the second oldest leaf), then were subjected to biochemical and elemental analyses. The activity of enzymes was determined in fresh samples immediately after harvest. Other biochemical analyses were carried out after storage at –20 °C for a maximum of six days. Oven-dried samples were weighed and then used for elemental analyses.

2.2.1 Pigments concentration

Photosynthetic pigments including chlorophylls (Chl) and carotenoids were extracted from the leaves in ice-cold 80 % acetone in the dark at 4 °C. The absorbance of extracts was determined at 470, 645, and 662 nm, and the concentration of pigments was calculated and expressed based on leaf fresh weight (Lichtenthaler & Welburn, 1983). The flavonoids content was determined in the leaf homogenate prepared in an extracting solution containing 2 % $AlCl_3$ in methanol. After centrifugation, the absorbance of supernatants was measured at 415 nm. The total flavonoids concentration was expressed as μg quercetin equivalent per g of FW by drawing a calibration curve with 0-16 mg l^{-1} concentration of authentic quercetin (Arvouet-Grand et al., 1994).

2.2.2 Antioxidant enzymes assay

The total activity of superoxide dismutase (SOD, EC 1.15.1.1) in the plant samples was assayed using the mono-formazan formation test. One unit of SOD activity was defined as the amount of enzyme required for a 50 % inhibition in NBT (ρ-nitro blue tetrazolium chloride) reduction through the monitoring of the changes in the absorbance at 560 nm, compared to the control samples without the enzyme aliquot (Giannopolitis & Ries, 1977). Ascorbate peroxidase (APX, EC 1.11.1.11) activity in the extracts was calculated by determining the decrease in the absorbance at 290 nm for 2 min due to the oxidation of ascorbic acid using an extinction coefficient of 2.8 mM^{-1} cm^{-1} (Boominathan & Doran, 2002). The activity of CAT in the extracts was estimated by monitoring the decreases in the absorbance of H_2O_2 at 240 nm for 2 min. One unit of activity was defined as the quantity of enzyme needed to decompose 1 μmol H_2O_2 per min, using the extinction coefficient of 0.28 mM^{-1} cm^{-1} (Chance & Maehly, 1954). The activity of peroxidase (POD, EC 1.11.1.7) was assessed in a reaction mixture using guaiacol as substrate, and the enzyme activity was measured at 470 nm using extinction coefficient (26.6 mM^{-1} cm^{-1}) of tetraguaiacol (Ranieri et al., 2001).

The concentration of total soluble proteins was determined using the Bradford assay (Bradford, 1976) and bovine serum albumin (BSA, Merck) as the standard.

2.2.3 H_2O_2, malondialdehyde (MDA), and proline concentrations

The concentration of H_2O_2 in the extracts was determined by recording the absorbance at 390 nm and using the plotted calibration curve in the range of 0-120 μM H_2O_2 (Harinasut et al., 2003). The concentration of malondialdehyde (MDA) as a marker of lipid peroxidation in the leaf extracts was determined by the absorbance at 532 nm due to its reaction with thiobarbituric acid and using a plotted standard curve with 1,1,3,3 - tetraethoxypropane (Hodges et al., 1999). For the estimation of proline, leaf samples were extracted in sulfosalicylic acid, and the supernatants were used for the determination of proline according to the method of Bates et al. (1973) and with ninhydrin as a reagent.

2.2.4 Total phenolics concentration and lignin quantification

Phenolic compounds were extracted in 70 % aqueous methanol three times, and after centrifugation, the supernatant was used for the determination of soluble phenolics, while cell wall-bound phenolics and lignin were quantified in the pellet. For the release of the cell wall (CW)-bound phenolics, the pellet was washed consecutively with water and Triton X-100, then after incubation with 20 mM NH_4-oxalate (70 °C) followed by 100 mM NaOH for 24 h, the phenolics were released from the CW. The concentrations of soluble and CW-bound phenolics were determined using Folin–Ciocalteu reagent at 765 nm and gallic acid as standard (Swain & Hillis, 1959). After air-drying, the residual CW fraction was used for lignin extraction and determination using the acetyl bromide method by recording the absorbance at 280 nm and a specific absorption coefficient value of 8.4 l g^{-1} cm^{-1} (Morrison, 1972).

2.2.5 Phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) activity

Phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) activity in the leaf and root samples were evaluated as the amount of the formed trans-cinnamic acid at 290 nm and was calculated using its extinction coefficient of 9630 mM^{-1} cm^{-1} (Dickerson et al., 1984). Polyphenol oxidase (PPO, EC 1.14.18.1) activity was determined according to the method described by Casado-Vela et al. (2005). The changes in the absorbance at 334 nm due to the oxidation of pyrogallol were used for the calculation of the PPO activity in the extracts.

2.2.6 Mineral analyses

The oven-dried leaf and root samples were used for mineral analyses. The samples were ashed in a muffle furnace at 550 °C for 8 h, and after dissolving in 10 % HCl and filtration, were made to volume with distilled water. The concentrations of Na, K, and Ca were determined by flame photometry (PFP7, Jenway, UK), and the standard solutions of the examined elements were used for the construction of the calibration curves. The concentration of Zn in the samples was analyzed by atomic absorption spectrophotometry (AA-6300, Shimadzu, Japan).

2.2.7 Experimental design and statistical analyses

This experiment was performed using a complete randomized block design with four independent replicates per treatment. Data were presented as mean ± standard deviation (SD). The comparison of means was carried out using SigmaStat 3.5 (Systat Software Inc., USA) with Tukey's test at $p < 0.05$.

3 RESULTS

According to the results of the preliminary experiment, the growth of *L. latifolium* plants was decreased under both salinity and Zn toxicity treatments. Reduction of growth upon exposure to 400 mM salt was 49 % and 52 % for the fresh mass (FM) and dry mass (DM) of the shoots, respectively. The reduction of FM and DM of aerial parts under the effect of 400 μM Zn was 50 % and 43 %, respectively (Table 1).

Table 1: Decline in biomass production (% over control) in *L. latifolium* grown with salt (400 mM NaCl) or toxic Zn concentration (400 µM $ZnSO_4$) for three weeks in a hydroponic medium

	Reduction of FM	Reduction of DM
Salinity	49 ± 6	52 ± 8
Zn toxicity	50 ± 13	43 ± 18

The main experiment was conducted to study salt, Zn, and their interaction effects on the growth, biochemical and mineral attributes of the plants as described below.

3.1 THE EFFECT OF SALT, ZN, AND THEIR COMBINATION ON PLANTS BIOMASS AND CONCENTRATION OF PIGMENTS

Due to a strong reduction of growth in the preliminary experiment, lower concentrations of salt and Zn were applied in the main experiment. According to the obtained results, the shoot DM was decreased by salt, Zn toxicity, and especially under a combination of both treatments (Figure 1A). Root DM decreased significantly under Zn toxicity alone or in combination with salt, while it was not significantly affected by salinity as a single treatment (Figure 1B).

The leaf concentration of Chl was decreased by all applied treatments. The highest reduction (90 %) was observed under Zn toxicity without salt and the lowest decline (17 %) was found under salt as a single treatment (Table 2). Unlike Chl, the leaf concentration of carotenoids increased due to salinity treatment but significantly decreased by Zn toxicity alone or in combination with salt. The leaf concentration of flavonoids decreased by both salt and Zn toxicity as single stresses, while it was significantly higher than the control plant in the combinative treatment (Table 2).

3.2 THE EFFECT OF SALT, ZN, AND THEIR COMBINATION ON THE ACTIVITY OF ANTIOXIDANT ENZYMES AND CONCENTRATION OF RELATED METABOLITES

The activity of SOD decreased by salt treatment alone or in combination with Zn toxicity while remaining unaffected under Zn toxicity as a single treatment (Figure 2A). The activity of CAT showed a significant reduction under the combined treatment but remained unchanged under the individual treatments of either salt or Zn toxicity (Figure 2B). The activity of APX, in contrast, increased by salt treatment alone or in combination with Zn toxicity. The effect of the latter treatment as single stress on the APX activity was not significant (Figure 2C). The activity of POD was significantly increased by salinity and Zn toxicity; the highest enzyme activity was observed under a combination of these treatments (Figure 2D).

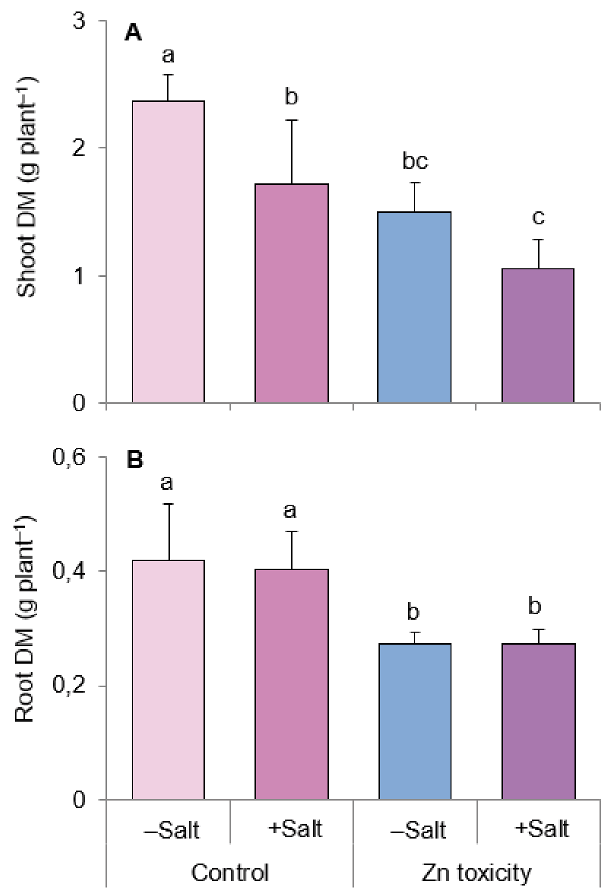

Figure 1: The biomass of shoots (A) and roots (B) in *L. latifolium* grown for three weeks in the absence or presence of salt (100 mM NaCl) and Zn (200 µM as $ZnSO_4$). Bars indicated by different letters are significantly different ($p < 0.05$)

The concentration of H_2O_2 was increased by all applied treatments. The effect of salinity alone or in combination with Zn toxicity was significantly higher than that of Zn toxicity as single stress (Figure 3A). The concentration of MDA increased under salinity and Zn toxicity as single stress. Under the combination of both treatments, however, this parameter did not differ from that in the control plants (Figure 3B). The concentration of proline showed a significant increase under the influence of all applied treatments. The effect of Zn toxicity as a single treatment, however, was higher than that of its combination with salinity; the lowest effect was observed upon salt as single stress (Figure 3C).

Table 2: The leaf concentrations of chlorophylls (Chl a+b), carotenoids (mg g^{-1} FM), and flavonoids (µg g^{-1} FM) in *L. latifolium* grown for three weeks in the absence or presence of salt (100 mM NaCl) and Zn (200 µM as $ZnSO_4$). Data of each column indicated by the different letters are significantly different ($p < 0.05$)

Treatment		Chl (a+b)	Carotenoids	Flavonoids
Control	–Salt	2.06 ± 0.10 [a]	0.16 ± 0.01 [b]	88 ± 5.8 [b]
	+Salt	1.70 ± 0.19 [b]	0.32 ± 0.01 [a]	66 ± 2.6 [c]
Zn toxicity	–Salt	0.19 ± 0.06 [d]	0.08 ± 0.02 [d]	78 ± 5.7 [bc]
	+Salt	1.32 ± 0.02 [c]	0.12 ± 0.01 [c]	147 ± 14.0 [a]

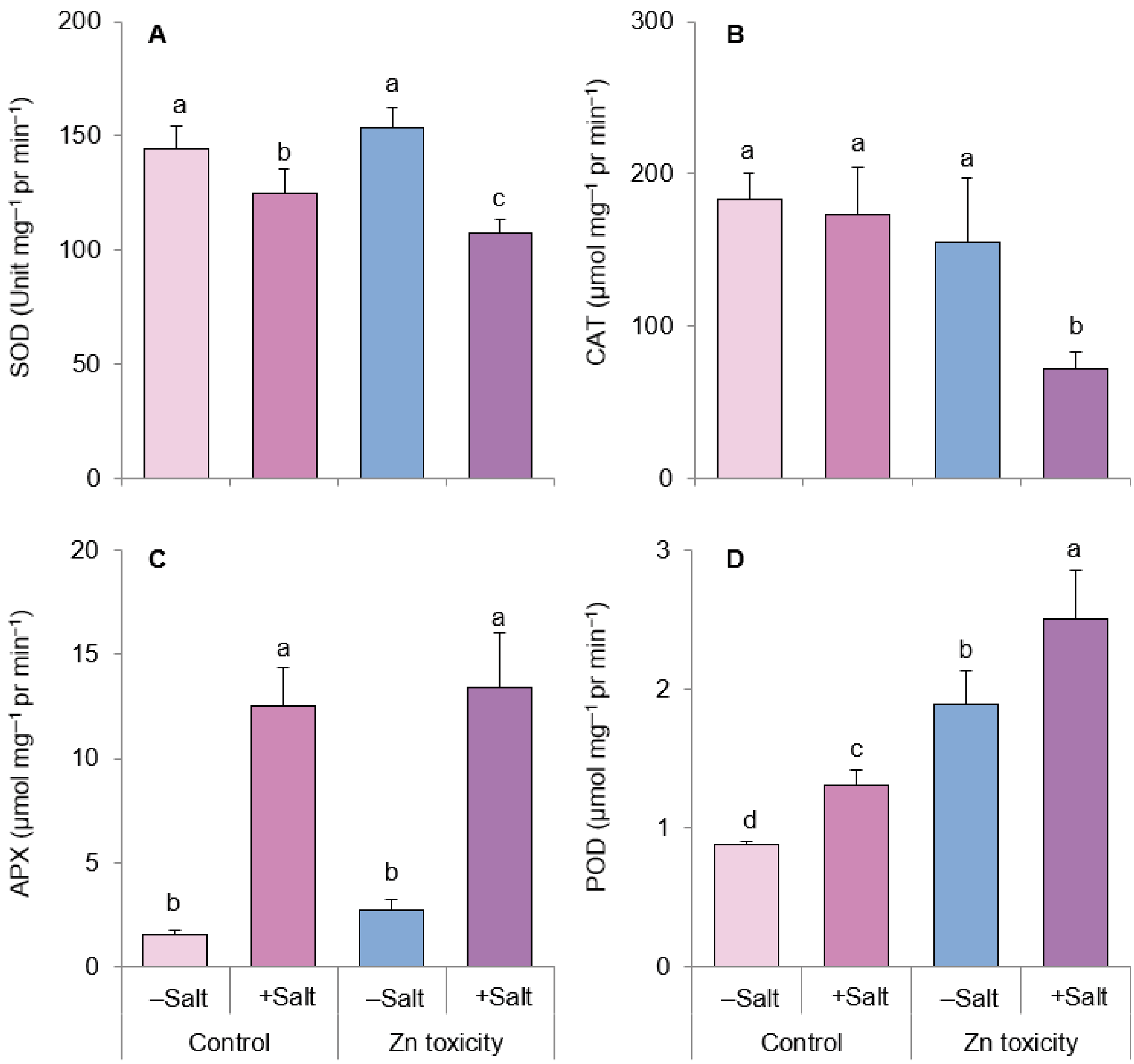

Figure 2: The leaf activity of superoxide dismutase (SOD) (A), catalase (CAT) (B), ascorbate peroxidase (APX) (C), and peroxidase (POD) (D) in *L. latifolium* grown for three weeks in the absence or presence of salt (100 mM NaCl) and Zn (200 µM as $ZnSO_4$). Bars indicated by different letters are significantly different ($p < 0.05$)

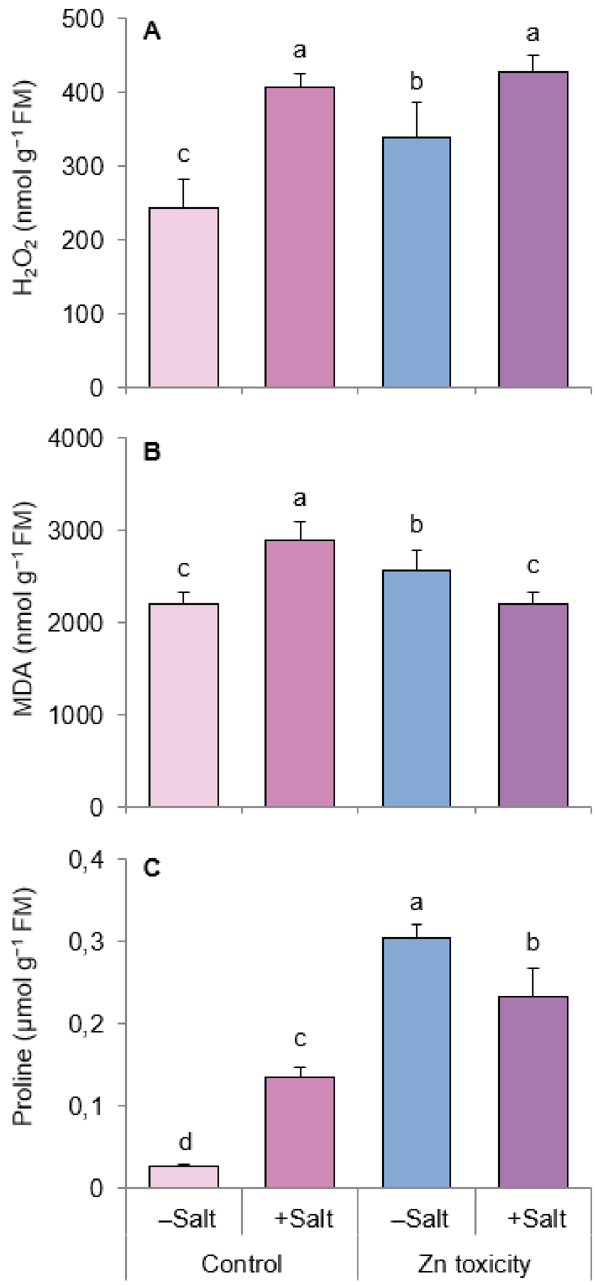

Figure 3: The leaf concentration of hydrogen peroxide (H_2O_2) (A), malondialdehyde (MDA) (B), and proline (C) in *L. latifolium* grown for three weeks in the absence or presence of salt (100 mM NaCl) and Zn (200 µM as $ZnSO_4$). Bars indicated by different letters are significantly different ($p < 0.05$)

3.3 THE EFFECT OF SALT, ZN, AND THEIR COMBINATION ON THE ACTIVITY OF PAL, PPO, AND THE CONCENTRATIONS OF PHENOLICS AND LIGNIN

The leaf activity of PAL was decreased by all applied treatments without difference among the three treatments. The root activity of this enzyme, in contrast, remained unchanged or rather increased. The latter effect was observed under Zn toxicity as a single treatment (Figures 4A and 4B). The leaf activity of PPO increased under Zn toxicity alone or in combination with salinity, while salt treatment as a single stress did not affect this parameter (Figure 4C). In the roots, however, all applied treatments increased the PPO activity; the effect of Zn toxicity alone was significantly higher than that of the combinative treatment (Figure 4D).

The concentration of free phenolics decreased under salt stress, while increased by Zn toxicity treatment, and remained unaffected under the combination of both treatments (Figure 5A). The concentration of CW-bound phenolics, in contrast, increased by salt stress either alone or in combination with high Zn concentration while was not affected by Zn toxicity as a single treatment (Figure 5B). The lignin concentration increased by salt, Zn toxicity, and their combination without difference among the three treatments (Figure 5C).

3.4 THE EFFECT OF SALT, ZN, AND THEIR COMBINATION ON THE CONCENTRATION OF ELEMENTS IN THE LEAVES AND ROOTS

As expected, Zn was accumulated in the leaves and roots of plants treated with a high Zn concentration. In the young leaves, the concentration of Zn was significantly lower under the combinative treatment than that under Zn as a single treatment. The effect of salt on the reduction of Zn concentration, however, was not observed in the old leaves and roots (Figure 6).

Also, as anticipated, Na was accumulated in the young and old leaves and roots upon exposure to salt in the medium. Leaf accumulation of Na in plants treated with a combination of salt and Zn toxicity was significantly higher than that in plants grown with salt as a single treatment. The opposite was observed in the roots; Na concentration in this organ was lower under combinative treatment compared to that under single salt treatment (Table 3).

The concentration of K decreased significantly by salt treatment in the old and young leaves and in the roots. Treatment of plants with toxic Zn concentration caused also a decrease in the K concentration of old leaves and roots but did not affect this parameter in the young leaves. Reduction of K concentration was also observed under the combination of Zn stress and salt treatments. However, the effect of combinative treatment on

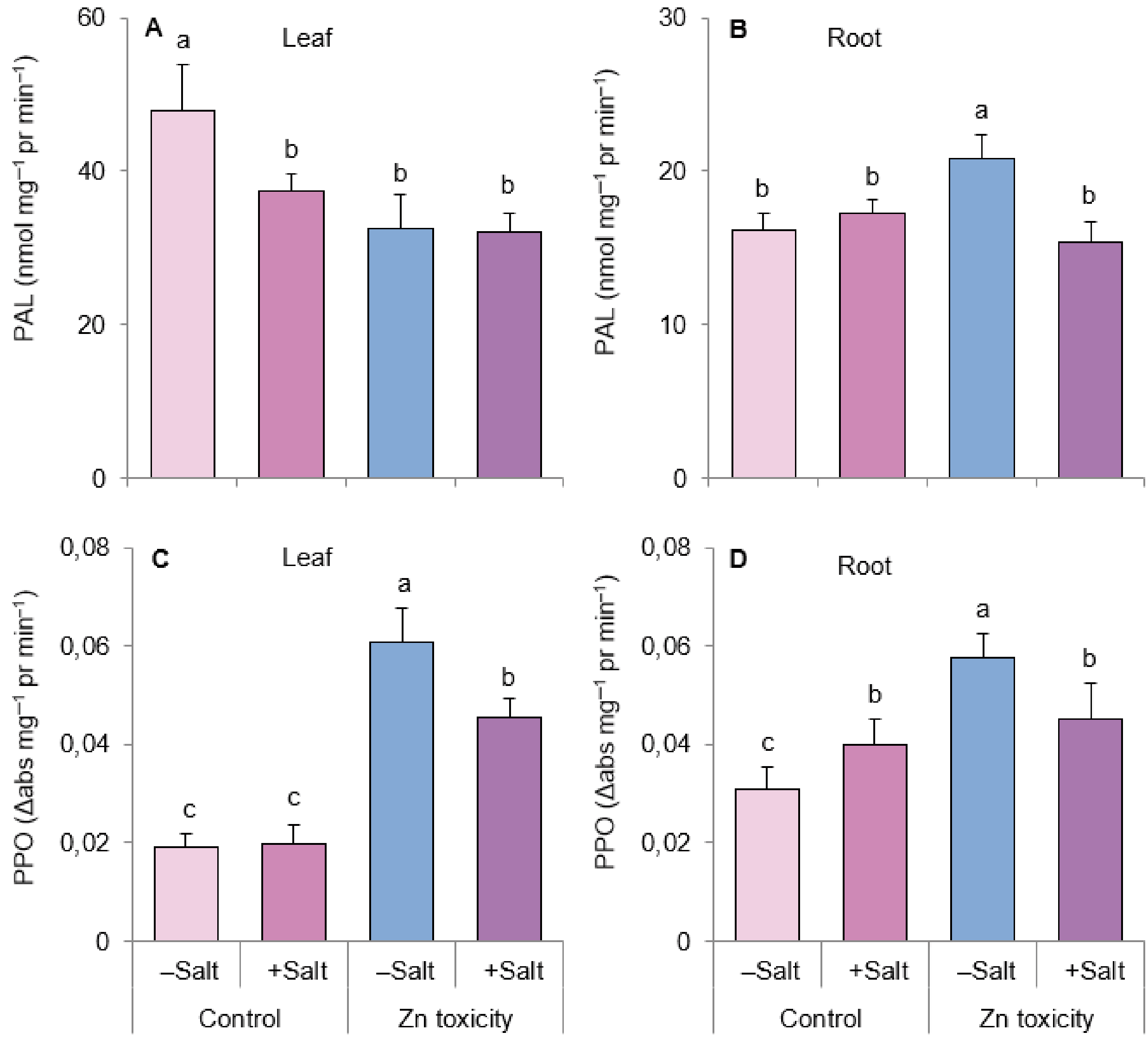

Figure 4: The activity of phenylalanine ammonia-lyase (PAL) (A and B) and polyphenol oxidase (PPO) (C and D) in the leaves and roots of *L. latifolium* grown for three weeks in the absence or presence of salt (100 mM NaCl) and Zn (200 µM as $ZnSO_4$). Bars indicated by different letters are significantly different ($p < 0.05$)

the leaf K concentration was less than the effect of single salt stress (Table 3).

The effect of salt and Zn toxicity treatments on the Ca concentration was dependent on the plant organ. The presence of salt in the medium increased Ca concentration in the roots and did not influence it in the young leaves while decreasing it in the old leaves. Treatment with toxic Zn concentration led to an increase in the Ca concentration of the young leaves but resulted in its reduction in the old leaves and roots. The effect of combinative treatment in the reduction of Ca concentration was observed only in the old leaves and roots (Table 3).

4 DISCUSSION

Studies on the stress tolerance in Brassicaceae species, particularly its halophyte members, attract the attention of plant scientists because of the possibility for comparison of these species with the model plant, *Arabidopsis thaliana* (L.) Heynh.. Based on these studies, *Schrenkiella parvula* (Schrenk) D.A.German & Al-Shehbaz (Hajiboland et al., 2018), *Cakile maritima* Scop. (Debez et al., 2004), and *Thellungiella salsuginea* (Pall.) O.E.Schulz (syn. *Eutrema salsugineum* (Pall.) Al-Shehbaz & Warwick) (Gao et al., 2008) are the most salt-tolerant Bras-

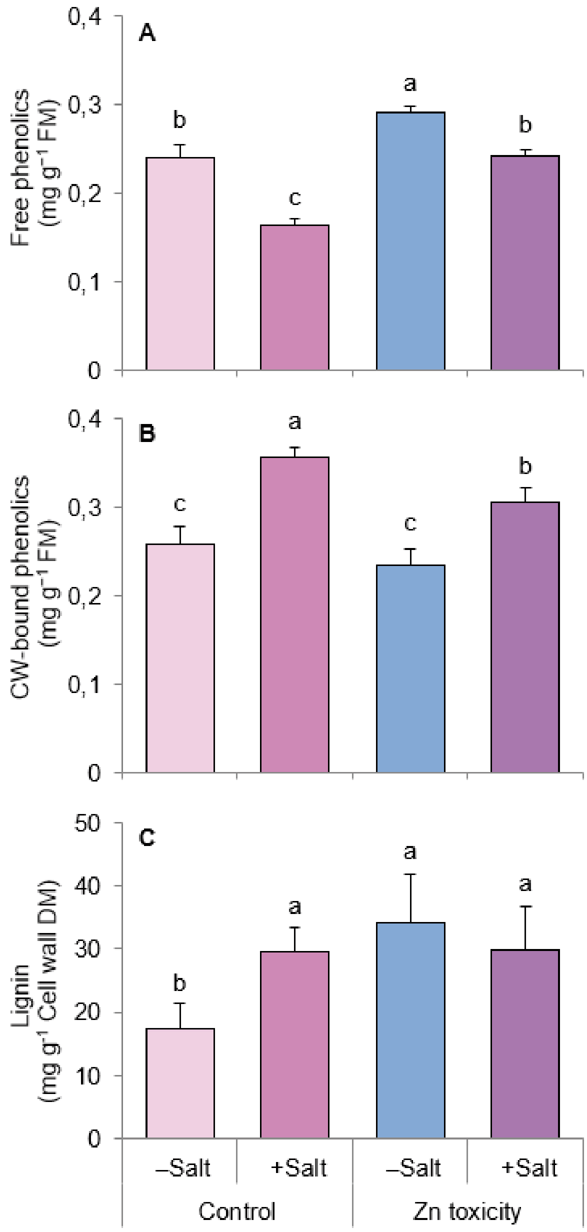

Figure 5: The leaf concentration of free phenolics (A), cell wall (CW)-bound phenolics (B), and lignin (C) in *L. latifolium* grown for three weeks in the absence or presence of salt (100 mM NaCl) and Zn (200 µM as $ZnSO_4$). Bars indicated by different letters are significantly different ($p < 0.05$)

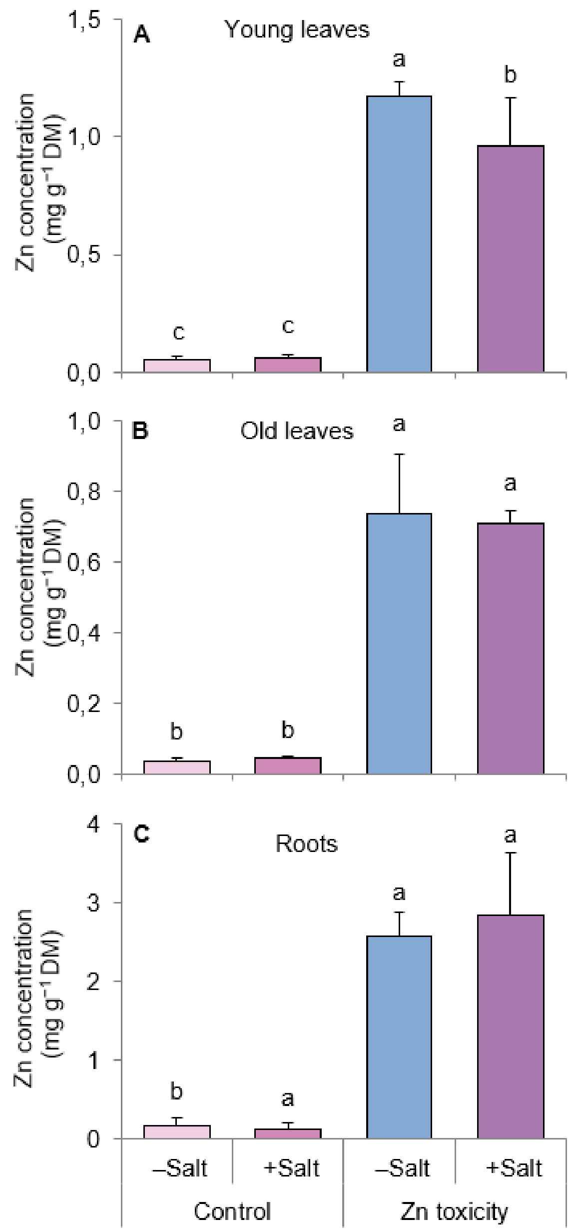

Figure 6: Zn concentration in the young leaves (A), old leaves (B), and roots (C) of *L. latifolium* grown for three weeks in the absence or presence of salt (100 mM NaCl) and Zn (200 µM as $ZnSO_4$). Bars indicated by different letters are significantly different ($p < 0.05$)

sicaceae species, which are able to grow even under salt concentrations of about 400 mM. The tolerance mechanisms in halophytes of this family include avoidance of excessive uptake of Na and maintenance of a proper ratio of K/Na in the cytosol, accumulation of organic osmolytes (including proline), activation of defense against ROS as well as changes in the levels of plant hormones (Van Zelm et al., 2020). To expand our knowledge of the mechanisms of salt tolerance and to find other model plants within Brassicaceae, i.e. *Arabidopsis*-related model species (*ARMS*, Arbelet-Bonnin et al., 2019), more investigations are necessary particularly on the halophytes of this family. In this study, *L. latifolium*, a facultative halophyte species was investigated for salt tolerance and its interaction with plant response to Zn toxicity.

Table 3: Concentrations (mg g^{-1} DM) of Na, K, and Ca in the young leaves, old leaves, and roots of *L. latifolium* grown for three weeks in the absence or presence of salt (100 mM NaCl) and Zn (200 µM as $ZnSO_4$). Data of each column within each organ indicated by different letters are significantly different ($p < 0.05$)

Treatment		Na	K	Ca
		Young leaf		
Control	–Salt	0.6 ± 0.2 ^{c}	43 ± 0.5 ^{a}	35 ± 3 ^{b}
	+Salt	6.5 ± 1.6 ^{b}	32 ± 2.4 ^{c}	38 ± 7 ^{b}
Zn toxicity	–Salt	1.5 ± 0.7 ^{c}	41 ± 2.8 ^{a}	49 ± 2 ^{a}
	+Salt	15 ± 2.8 ^{a}	35 ± 1.6 ^{b}	37 ± 9 ^{b}
		Old leaf		
Control	–Salt	2.3 ± 1.0 ^{c}	68 ± 2.0 ^{a}	66 ± 7 ^{a}
	+Salt	18 ± 3.0 ^{b}	34 ± 1.0 ^{d}	44 ± 9 ^{c}
Zn toxicity	–Salt	2.4 ± 0.6 ^{c}	53 ± 5.0 ^{b}	58 ± 6 ^{b}
	+Salt	42 ± 6.0 ^{a}	41 ± 3.6 ^{c}	45 ± 3 ^{c}
		Root		
Control	–Salt	1.1 ± 0.3 ^{c}	91 ± 8.7 ^{a}	62 ± 11 ^{b}
	+Salt	14 ± 2.0 ^{a}	44 ± 7.9 ^{c}	154 ± 13 ^{a}
Zn toxicity	–Salt	1.2 ± 0.1 ^{c}	78 ± 2.6 ^{b}	44 ± 9 ^{c}
	+Salt	6.5 ± 1.2 ^{b}	38 ± 1.7 ^{c}	23 ± 5 ^{d}

4.1 THE EFFECT OF SINGLE AND COMBINATIVE TREATMENTS OF SALT AND ZN TOXICITY ON PLANT BIOMASS

In comparison to glycophytes, halophytes have generally higher resistance against not only salt but also other soil-derived abiotic stressors, such as drought and heavy metal toxicity (Lokhande & Suprasanna, 2012). Mechanisms such as heavy metal exclusion, reduction of their mobility in soil (MacFarlane & Burchett, 2002), restriction of shoot-root translocation (Mejías et al., 2013), internal detoxification and sequestration, activation of the antioxidant system (Sharma et al., 2016), and even excretion of heavy metals through salt glands and trichomes (Lokhande & Suprasanna, 2012) all contribute to a higher heavy metal tolerance in halophytes. In addition, salt treatment mitigates heavy metal toxicity effects in some halophytes. The biomass of *Sesuvium portulacastrum* (L.) L. under Cd toxicity (Ghnaya et al., 2007) and growth of *Mesembryanthemum crystallinum* L. (Kholodova et al., 2005) and *Spartina densiflora* Brongn. (Mahon & Carman, 2008) under Zn toxicity were higher under simultaneous application of salt and heavy metal toxicity compared to single heavy metal stress. In our study, however, the effect of Zn toxicity was not mitigated by simultaneous treatment with salt suggesting that the ameliorative effect of salt on heavy metal toxicity in the halophytes is not common and is likely dependent on the species and the heavy metal. To the best of our knowledge, the effect of salt on heavy metal toxicity has not been investigated in the Brassicaceae halophytes, but in the glycophytes of this family including *Brassica napus* L. and *Brassica juncea* (L.) Czem. the combined treatment of salt and Cd had a higher inhibitory effect on the growth and photosynthesis of these species compared to single treatments (Shah et al., 2011).

4.2 THE EFFECT OF SINGLE AND COMBINATIVE TREATMENT OF SALT AND ZN TOXICITY ON THE CONCENTRATIONS OF LEAF PIGMENTS

An antagonistic effect of toxic heavy metal concentrations on the uptake and utilization of Fe, the main element for Chl biosynthesis, has been well documented (Leiková et al., 2017). In addition, impairment of biochemical reactions under heavy metal toxicity leads to the formation of excess excitation energy and generation of ROS that in turn cause instability of the thylakoid membranes and destruction of Chl (Riyazuddin et al., 2021). In *L. latifolium*, the leaf concentration of Chl was decreased upon exposure to excess Zn by up to 90 %. This was probably the consequence of both reductions in biosynthesis and the rise of its destruction.

Carotenoids play important roles in the stability of chloroplast membranes and protect photosynthetic apparatus against damages caused by excess excitation energy (Uarrota et al., 2018). A significant increase in the leaf carotenoids observed in salt-treated *L. latifolium* indicates that some protection mechanisms are triggered under these conditions leading to a reduction of injury to the chloroplasts similar to that reported for other halophytes, *Arthrocnemum macrostachyum* (Moric.) Piirainen & G.Kadereit, *Sarcocornia fruticosa* (L.) A.J.Scott (Ghanem et al., 2021), and *Nitraria retusa* (Forssk.) Asch. (Boughalleb & Denden, 2011). However, a salt-mediated increase in the leaf carotenoids has not been observed in all halophytes, as in *Salicornia europaea* L. salt treatment reduced this parameter (Aghaleh et al., 2009). Contrary to salt treatment, leaf carotenoids significantly decreased under Zn toxicity in our study, which may increase the vulnerability of photosynthetic apparatus to ROS-induced damages under these conditions. In *Halimione portulacoides* (L.) Aellen the amounts of carotenoids increased under Zn toxicity (400 mM) (Cambrollé et al., 2012) while in another halophyte, *Avicennia marina*

(Forssk.) Vierh. the amount of this pigment decreased under excess Cu and Zn, but remained unchanged under the Pb toxicity (MacFarlan & Burchett, 2002).

The leaf concentrations of Chl, carotenoids, and flavonoids were significantly higher under the combination of salt with Zn toxicity compared to the single Zn treatment in *L. latifolium*, likely as the consequence of the effect of salt treatment on the stimulation of protection mechanisms in the chloroplasts. Application of low concentrations of salt protects the structure and function of chloroplasts; ascorbate plays a central role in this priming effect because *Arabidopsis* mutants lacking ascorbate show a considerable disruption of photosynthesis under salt stress (Acosta-Motos et al., 2017). Similarly, salt priming decreased high temperature-induced damage to the photosystem II in *Atriplex centralasiatica* Iljin (Qiu & Lu, 2003). In *Suaeda salsa* (L.) Pall. salt priming increased the quantum yield of photosystem II and increased the amount of unsaturated fatty acids (Cheng et al., 2014). Flavonoids are low-molecular-weight polyphenolic metabolites not only involved in ROS scavenging, but also as chelating molecules, bind to heavy metals and thus, play a role in internal detoxification (Keilig & Ludwig-Müller, 2009; Samanta et al., 2011). Under the combination of salt and Zn treatments, the leaf concentration of flavonoids was two-fold higher than that found under control and single-stress conditions in *L. latifolium*. Enhanced flavonoids level under combinative treatment was associated with significantly lower MDA concentration suggesting their contribution to the prevention of membrane damage under these conditions.

4.3 THE EFFECT OF SINGLE AND COMBINATIVE TREATMENTS OF SALT AND ZN TOXICITY ON THE FUNCTION OF THE ANTIOXIDANT DEFENSE SYSTEM

Higher constitutive or stress-induced antioxidant defense is one of the most important mechanisms in halophytes to cope with various environmental stresses (Sruthi et al., 2017). SOD is involved in scavenging superoxide radicals as one of the most damaging ROS in cells (Sruthi et al., 2017). In this study, however, the activity of SOD decreased under both single and combinative salt and Zn stresses. Reduction in the activity of SOD under salinity has also been reported in other halophytes such as *Gypsophila oblanceolate* Barkoudah (Sekmen et al., 2012) and *Salvadora persica* L. (Rangani et al., 2016).

Unlike SOD, the activity of APX was significantly increased by salt treatment alone or in combination with Zn toxicity, which is in agreement with many reports on the effect of salinity on the APX activity in the glycophytes, e.g. *Arabidopsis thaliana* and halophytes, e.g. *Cakile maritima* (Ellouzi et al., 2011) and *Sesuvium portulacastrum* (Ben Amor et al., 2020). The activity of CAT was not affected by the single salt and Zn toxicity stress and significantly decreased under combinative treatments. The lack of any response to low concentrations of salt (100 mM) was similar to another report in the euhalophyte, *Salvadora persica*, where the activity of CAT did not change under 250 mM salt, but increased under 500 mM salt treatment (Rngani et al., 2016). Unlike CAT and APX, the activity of POD increased by all three applied treatments and could probably be considered as an indicator of the effect of treatments. Peroxidases are a large group of enzymes oxidizing a wide array of substrates using H_2O_2 (Veitch, 2004). Several reports showed a consistent increase in the activity of POD by salt stress both in the glycophytes and in halophytes (Yang et al., 2010; Ellouzi et al., 2011). The highest activity of POD was observed under combinative treatment associated with a reduction of MDA to the levels observed in control plants. Overall, our results demonstrated that antioxidant enzymes respond differently to the applied treatments depending on enzyme and stress factors, and suggest the different contributions of each enzyme in the defense of plants against salt, heavy metals, and their combinations.

H_2O_2 plays a dual role in plant stress response: it acts as a signaling molecule at the nanomolar or low micromolar range of concentration (per g FM) while at the millimolar level damages the molecular structure of proteins, lipids, and nucleic acids (Černý et al., 2018). The range of cytosolic H_2O_2 concentration in our plants (200-400 nmol g^{-1} FM, Figure 3A) was highly relevant to its signaling function. Furthermore, the ability of salt treatment in the induction of H_2O_2 signal was higher than that of Zn toxicity, leading in turn to a higher H_2O_2 level in the combinative treatment (Figure 3A). This may be one of the mechanisms for the ameliorative effect of salt on Zn stress in this work, as was also reflected in the levels of biochemical stress markers under combinative treatment. The effect of salt on the induction of H_2O_2 signaling and stress tolerance has been observed in other halophytes (Ellouzi et al., 2011). Interestingly, H_2O_2 signaling in a halophyte (*L. latifolium*) is characteristically different from its glycophyte relative (*Lepidium sativum* L.) in the timing and magnitude of induction (Hajiboland et al., 2020).

Accumulation of MDA, as the final product of peroxidation of poly-unsaturated fatty acids in the membranes, is one of the most common effects of heavy metal toxicity in plants (Manara, 2012). An increase in the MDA content has been reported in *Atriplex rosea* L. and *Arabidopsis hortensis* L. grown in soils contaminated with Ni and Zn (Kumari et al., 2019) or in *Acanthus ilici-*

folius L. under Cd stress (Shakira & Puter, 2019). In our work, the MDA concentration increased by Zn toxicity as a single treatment, while was reduced to the level of that in control plants under the combination of Zn with salt treatment. This may indicate higher protection of cell and plastid membranes in the combinative treatment, as was also reflected in the higher concentration of leaf pigments under these conditions compared with Zn toxicity as a single treatment (Table 2). The protective role of salt under heavy metal stress has also been observed in the halophyte *Atriplex halimus* L. against Cd and Cu toxicities as a reduction of MDA accumulation in the roots under the combination of heavy metals with salt treatments (Bankaji et al., 2016).

Accumulation of proline driven by different environmental stresses is a well-documented response in plants (Hayat et al., 2012). The major function of proline under salt stress is osmotic regulation, which along with other compatible organic osmolytes, e.g. polyols and glycine betaine, confronts the osmotic component of salt stress (Siddique et al., 2018). The function of proline, however, is not limited to an osmotic role but it contributes to a wide range of protective functions, including stimulation of antioxidant defense enzymes, role in the stability of protein structures, and redox homeostasis (Hayat et al., 2012). Under heavy metal stress, proline protects cells against toxicity damage (Siddique et al., 2018). In the halophyte *Acanthus ilicifolius,* proline concentration increased in response to Cd toxicity (Shackira & Puthur, 2019) and in *Mesembryanthemum crystallinum* under excess Zn concentration (Kholodova et al., 2005). The expression of the proline biosynthetic gene (P5CS) was increased under Cr toxicity and its combination with salt in *Chenopodium quinoa* Willd. that was associated with proline accumulation in this species (Guarino et al., 2020). In our work, the leaf concentration of proline increased under salt stress, particularly by Zn toxicity treatment. Although the proline level was lower under combinative treatment compared to single Zn stress, it remained still higher than that found under salt stress and was about 9-fold higher than the proline concentration of control plants. As an indicator of stress (Ashraf & Harris, 2004), lower proline concentration in the combinative treatment could be likely the result of mitigation of the Zn toxicity stress, as was also reflected in the lower MDA content and higher amounts of leaf pigments. In agreement with our findings, the proline concentration in the halophyte *Kosteletzkya pentacarpos* (L.) Ledeb. increased under Cd toxicity, while decreased under the combination of salt and Cd stress (Zhou et al., 2019).

4.4 THE EFFECT OF SINGLE AND COMBINATIVE TREATMENTS OF SALT AND ZN TOXICITY ON THE CONCENTRATIONS OF PHENOLICS, LIGNIN, AND THE ACTIVITY OF RELATED ENZYMES

Phenolic compounds possessing an aromatic ring with one or more hydroxyl substituents are contributed to ROS scavenging and stabilization of membranes and other cell structures (Moura et al., 2010). An enhanced synthesis and accumulation of phenolic compounds under the toxicity of heavy metals has been extensively reported (Ghori et al., 2019). In *Kandelia obovata* Sheue, Liu & Yong, Cd toxicity caused phenolics accumulation accompanied by a significant increase in PPO activity (Chen et al., 2019). Similarly, in *Matricaria chamomilla* L., the concentration of phenolic compounds increased under Ni toxicity associated with an increase in the activity of PAL but a decrease in the activity of PPO (Kováčik et al., 2009). In our work here, the concentration of free phenolics increased under Zn toxicity treatment (Figure 5A), indicating a probable role for phenolics in the increasing plants' resistance against Zn toxicity. In addition to their antioxidant and protective function, a metal chelating capacity of phenolics as a mechanism for the internal detoxification of heavy metals has been documented (Michalak, 2006). The capacity of particular phenolics such as cinnamic acid, ferulic acid, gallic acid, and naringenin for Zn chelation has been demonstrated both *in vivo* and *in vitro* (Fedenko et al., 2022).

In addition to free phenolics, low molecular weight phenolic acids that are bound to various CW components have several important roles including responses to stresses (Wallace & Fry, 1994). The concentration of CW-bound phenolics was higher in the salt-treated plants either as single stress or in combination with Zn toxicity (Figure 5B). The carboxylic groups of CW-bound phenolics have a high affinity for heavy metals and the formed complexes show high stability constants (McDonald et al., 1996). This mechanism may contribute to Zn detoxification in our work, particularly in the combinative treatment with an enhanced concentration of CW-bound phenolics.

The composition of plant CWs is modified under biotic and abiotic stresses (Gall et al., 2015). The biosynthesis of lignin, as one of the major components of the CW, increases under various stresses, including salinity and heavy metal toxicity (Moura et al., 2010). ROS produced under stress in the apoplasts participate in lignin synthesis via various enzymes including POD and PPO (Ali et al., 2006). In *Arabidopsis thaliana*, salt treatment upregulates laccase- and POD-encoding genes leading to lignification in salt-stressed plants (Chun et al., 2019). In *Tamarix hispida* Willd., the expression of genes involved in the lignin biosynthesis, i.e. S-adenosyl methionine synthase (SAM synthase) and catechol-O-methyltransferase (COMT) were increased under salt stress (Han et al., 2022). Interestingly, there is a relationship between the extent of lignification and salt tolerance, so that leaf lignification under salt stress was higher in the halophyte *L. latifolium* than that was found in its glycophyte relative, *L. sativum* (Hajiboland et al., 2020). In this work, the lignin concentration was increased by all applied treatments, especially by Zn toxicity indicating its contribution to the adaptation of plants to stress. An increase in the lignin content mediated by Zn toxicity has also been observed in *Thlaspi caerulescens* J.Presl & C.Presl, following the upregulation of the related biosynthetic genes (Van De Mortel et al., 2006). Interestingly, the lignin deposition under Zn toxicity was higher in *Thlaspi caerulescens* compared with its glycophyte relative, *Arabidopsis thaliana* with higher sensitivity to Zn toxicity, indicating again a relationship between the higher ability to lignin synthesis and tolerance to not only salt (Hajiboland et al., 2020) but also to Zn toxicity (Van De Mortel et al., 2006).

4.5 THE EFFECT OF SINGLE AND COMBINATIVE TREATMENTS OF SALT AND ZN TOXICITY ON THE ACCUMULATION OF ELEMENTS

It has been observed that salt treatment results in higher accumulation of Zn in *B. juncea* (Novo et al., 2014) and *Brassica rapa* L. (Zeiner et al., 2022) because of salt-mediated increase in the mobility of Zn in the soil and within plants and enhancement of the root-shoot translocation of Zn in these glycophyte species (Novo et al., 2014). In the present work, the application of salt reduced Zn accumulation in the aerial parts of *L. latifolium*. Although there is no information about other halophytes of this family, similar results have been obtained for Cd in the halophytes from Aizoaceae (*Carpobrotus rossii* (Haw.) Schwantes) in that application of salt decreased Cd concentration in plant's aerial parts (Cheng et al., 2018).

A relatively low concentration of Na in the leaves (6-18 mg g^{-1} DM) was in agreement with the halophytic behavior of *L. latifolium*. It has been reported that under relatively low salt concentrations (50-150 mM), halophytes are more successful in controlling the Na uptake than glycophytes, and thus, do not accumulate much Na under these conditions (Munns, 2005). Salt treatment significantly reduced the concentration of K and Ca similar to that observed in other halophytes, e.g. *Plantago coronopus* L. (Koyro, 2006).

Zn toxicity treatment increased the leaf Na concentration in salt-treated plants by about 2.3 fold. A similar increase was observed in K concentration however, it was a 'concentration effect' because the K content (mg $plant^{-1}$) was rather decreased by Zn treatment (data not shown). In *Kosteletzkya virginica* (L.) C.Presl ex A.Gray Zn toxicity increased Na and decreased K concentration in the aerial parts (Han et al., 2012). Damages to membranes under Zn toxicity are likely the mechanism for an increase in the Na concentration in Zn-stressed plants that may be in turn prevented the mitigating effect of the combinative treatment on the biomass in our plants.

5 CONCLUSIONS

In contrast to available reports on the ameliorative effects of salt on heavy metal tolerance in the halophytes, in this study, a growth improvement was not observed under the combination of salt and Zn toxicity compared with excess Zn as single stress. This may suggest that the mitigating effect of salt on heavy metal stress is related to the heavy metal and/or the halophyte species. Nevertheless, the improvement of leaf pigments and, reduction of MDA and Zn accumulation in the leaves under the combination of both treatments showed that salt treatment is still able to stimulate the defense mechanisms in plants for protecting membranes and photosynthetic pigments against excess Zn concentrations.

Lepidium latifolium plants grow under diverse environmental conditions, have a high growth rate, and produce considerable biomass with perennial habit. These properties make this species a suitable candidate for revegetation and rehabilitation of saline soils contaminated with heavy metals.

6 ACKNOWLEDGMENTS

The authors would like to express their gratitude to the Council of Shahed University for financial support during the course of this research for the plant physiology Ph.D. thesis. In addition, the authors thank the Ministry of Science, Research, and Technology (Iran) for a short-term research grant to B.N. and the University of Tabriz for providing the facilities for this research and related analyses.

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doi:10.14720/aas.2023.119.1.2254 Original research article / izvirni znanstveni članek

Evaluation of herbicide options for control of invasive annual ground cherry (*Physalis divaricat*a D.Don.) in corn

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Received June 19, 2021; accepted January 28, 2023.
Delo je prispelo 19. junija 2021, sprejeto 28. januarja 2023

Evaluation of herbicide options for control of invasive annual ground cherry (*Physalis divaricata* D.Don.) in corn

Abstract: Annual ground cherry (*Physalis divaricata*) is an invasive weed in farmlands located in western Iran, but there is little information on effective options for its management, particularly in corn. Greenhouse and field studies were conducted to evaluate the performance of herbicide options consisting of mesotrione + s-metolacholor + terbuthylazine, bromoxynil + MCPA, foramsulfuron + iodosulfuron, 2,4-D + MCPA, rimsulfuron and nicosulfuron + rimsulfuron alone and in combination with ammonium sulphate (AMS) and citogate for *Ph. divaricata* control in corn. In the greenhouse study, only Mesotrione + s-metolacholor + terbuthylazine and bromoxynil + MCPA mixture provided satisfactory control of *Ph. divaricata* among the evaluated herbicides. In field study, each of mesotrione + s-metolacholor + terbuthylazine or bromoxynil + MCPA efficiently suppressed *Ph. divaricata*. Nevertheless, bromoxynil + MCPA had higher efficacy so that applying 75% of its recommended dose resulted in more than 80 % control of *Ph. divaricata*. Considering both greenhouse and field studies, mesotrione + s-metolacholor + terbuthylazine as well as bromoxynil + MCPA were found to be suitable options for *Ph. divaricata* in corn. Results also suggest the need of adding of AMS to improving weed control by these herbicides, particularly in bromoxynil + MCPA.

Key words: ammonium sulphate; annual ground cherry; weed control

Ovrednotenje herbicidov primernih za nadzor invazivnega himalajskega volčjega jabolka (*Physalis divaricata* D.Don.) v posevku koruze

Izvleček: Enoletnica, himalajsko volčje jabolko (*Physalis divaricate* D. Don.) je invaziven plevel na kmetijskih zemljiščih zahodnega Irana, a le malo je znanega o možnostih njegovega zatiranja, še posebej v koruzi. Za ovrednotenje učinka herbicidov za zatiranje tega plevela v koruzi so bili v rastlinjaku in v poljskem poskusu uporabljeni herbicidi v naslednjih kombinacijah: mezotrion + s-metolaklor + terbutilazin, bromoksinil + MCPA, foramsulfuron + iodosulfuron, 2, 4-D + MCPA, rimsulfuron in nikosulfuron + rimsulfuron posamično ali v kombinaciji z amonijevim sulfatom (AMS) in citogatom. V rastlinjaku je bila za nadzor plevela izmed preiskušenih herbicidov zadostna samo mešanica herbicidov kot so mezotrion, s-metolaklor, terbutilazin in bromoksil, MCPA. V poljskem poskusu je uporaba mešanic herbicidov kot so mezotrion, s-metolaklor, terbutilazin ali bromoksil in MCPA učinkovito zatrla plevel. Mešanica bromoksila in MCPA je imela večjo učinkovitost in je pri odmerku 75 % od priporočene doze zavrla rast plevela za 80 % v primerjavi s kontrolo. Glede na poskusa v rastlinjaku in na polju je mešanica herbicidov kot so mezotrion, s-metolaklor, terbutilazin, bromoksinilin MCPA primerna za nadzor tega plevela v koruzi. Rezultati nakazujejo tudi potrebo po dodatku amonijevega sulfata za izboljšanje nadzora plevelov s temi herbicidi, še posebej pri mešanici bromoksinila in MCPA.

Ključne besede: amonijev sulfat; himalajsko volčje jabolko; uravnavanje plevelov

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1 INTRODUCTION

Over the last decade, annual ground cherry (*Physalis divaricata* L.) has increasingly become one of the noxious and invasive annual weed of summer crops in Iran (Nosratti et al., 2017). Originating from India, *Ph. divaricate* D. Don. has been adapted to diverse agro-ecological conditions in Iran (Nosratti et al., 2016). It is now a weed of national significance in Iran, with highly infestations found in corn. Despite applied control measures, average yield loss due to weeds in corn farms of Kermanshah province has been reported to be higher than 17 % (Sabeti et al., 2013). Sugar beet, potato, tomato, and other summer crops, are grown especially in western parts of Iran (Alam et al., 2011).. *Physalis divaricata* is very competitive because of its fast growth and large canopy. For instance, Alam et al. (2013) found that presence of only one individual plant of *Ph. divaricata* in square meter resulted in 34 % reduction in root yield of sugar beet. In Iranian farmlands, particularly those of Kermanshah and Lorestan provinces, *Ph. divaricata* is now one of the five most troublesome corn weed. It is especially compatible to corn, where there is free gape between corn rows during early growth stages greatly favor its growth (Murphy et al., 1996). This weed species has the potential to reduce the yield of several main crops and interfere with their harvest. For example, reductions of 10 and 14 % in corn yield have been reported with *Ph. divaricata* at densities of 8 and 16 plants m^{-2}, respectively (Sabeti et al., 2019). In addition, the presence of sticky ingredients in its berries causes harvest problems. Applying herbicide is the predominant weed management option in Iranian fields, particularly in corn. So far, 11 herbicides belonging to different groups of modes of action are available for corn growers in Iran (Zand et al., 2009a). When compared with other developed countries, the number of herbicides for use in Iranian corn fields are lower than those are in other countries (Zand et al., 2009b). The lack of herbicide variation by narrowing spectrum of target weed species may has resulted in increasing the population of *Ph. divaricata* (Alam et al., 2011). In addition, almost all of the commercially available herbicides for use in corn are applied as POST (Zand et al., 2006), which may let some weed species escaping the common herbicide application (Mortimer, 1997). In addition, the efficacy of commonly used corn herbicides on *Ph. divaricata* is not known because little effort has been devoted to investigating chemical control of this weed in corn. Therefore, the main aim of this research was to characterize the reaction of *Ph. divaricata* to applying registered herbicides in Iran.

2 MATERIALS AND METHODS

2.1 GREENHOUSE EXPERIMENT

The Research was conducted at Greenhouse of Kermanshah Agricultural and Natural Resources Research and Education Center during March and April of 2017. This trial was conducted to evaluate the response of *Ph. divaricata* to herbicides listed as follows: mesotrione + s-metolacholor + terbuthylazine, bromoxynil + MCPA, foramsulfuron + iodosulfuron, 2, 4-D+MCPA, rimsulfuron and nicosulfuron + rimsulfuron, which applied alone or in combination with either AMS (2 % w/v) or citogate (1 % v/v). *Ph. divaricata* berries were collected from corn fields of Kermanshah province in the western Iran during October and November of 2016. Seeds were kept in paper bags at room temperature until the beginning of the greenhouse experiment. Seeds of annual ground cherry were sowed in l5-cm-diam pots filled with a silty clay loam soil (an average mixture of 42 % sand, 34 % silt, and 24 % clay). The organic matter content was 11 % and the pH 6.1. Plants were grown by sowing five seeds in each 4.5 l plastic pot. Pots watered as needed during the experiment. After emergence, seedlings were thinned to three uniform Annual ground cherry plants per pot. Greenhouse was operated at 30 °C day and 16 °C night temperatures and at l6-h photoperiod consisting of daylight supplemented with dummy light. Experiment was a randomized complete block (CRD) with four replicates and the whole of experiment was repeated twice. Herbicides were used with a backpack mechanical plot sprayer in 200 liters of water per hectare at 3 bar, using a single Teejet® XR8003 flat fan nozzle. Herbicides were applied at the 3-5 leaf growth stage (10 to 12 cm in height) of annual ground cherry with 25, 50, 75, 100 and 125 % of the recommended dose of six evaluated herbicides (Table 1).Forty-five DAT, *Ph. divaricata* plants were cut at the soil surface, placed in paper bags, and dried in an oven at 72 °C for 48 h, and the dry biomass of each individual pot was calculated as percentage of untreated control.

2.2 FIELD EXPERIMENT

In 2017, two separate field trials were conducted in Research Centers Mahidasht (34°26'N, 46°83'E; elevation: 1366 m a.s.l.; annual average temperature: 14.7 °C; annual average rainfall: 439.2 mm) and Eslamabad–e-Gharb (34°8'N, 47°26'E; elevation: 1346 m a .s. l.; annual

Table 1: Herbicide product, active ingredients, and application rates for herbicide treatments used for *Physalis divaricata* control in greenhouse and field studies

Trade name	Active ingredients	Rates kg ai or ae* ha^{-1}
Bromicide	Bromoxynil + MCPA	300 g a. i. ha^{-1} Bromoxynil + 300 g a. i. ha^{-1} MCPA
Lumax	Mesotrione + s-metolacholor + terbuthylazine	1500 g a.i. ha^{-1} s-metolachlor + 150 g a.i. ha^{-1} Terbuthylazine + 500 g a.i. ha^{-1} Mesotrione
Maister	Foramsulfuron + iodosulfuron	45 g a. i. ha^{-1} foram + 1.5 g a. i. ha^{-1} iod
U 46 Combi fluid	2,4-D + MCPA	540 g a. i. ha^{-1} 2,4-D + 472.5 g a. i. ha^{-1} MCPA
Titus	Rimsulfuron	10 g a. i. ha^{-1}
Utima	Nicosulfuron +rimsulfuron	93.75 g a. i. ha^{-1}

* ai: active ingredients, ae: acid equivalent which is applied for those pesticides that are acids

average temperature: 15 °C; annual average rainfall: 498.4 mm) to further investigation the response of *Ph. divaricata* to mesotrione + s-metolacholor + terbuthylazine and bromoxynil + MCPA. Because satisfactory control of *Ph. divaricata* was achieved only by using these two herbicides under greenhouse conditions, we selected them for further study in field conditions. The field soil in both locations was uniformly infested with high densities of *Ph. divaricata*. The soil type, physical and chemical characteristics in the experimental sites are shown in Table 2.

The experimental procedure for both locations was the same and corn (SC 703) seeds were sowed in mid-May at 75000 seeds ha^{-1} in 75-cm rows. Plot size for each treatment was 3 m wide by 10 m long and arranged in a randomized complete block design with four replications. Each test plot was divided into two parts in length. The upper part of each plot was not sprayed and was considered as a control for each plot separately and the lower part of the plot treatment was applied. When *Ph. divaricata* was between 10 and 12 cm height, 50, 75, and 100 % of the recommended dose of mesotrione + s-metolacholor + terbuthylazine and bromoxynil + MCPA (Table 1) with and without ammonium sulfate (AMS) (2 %) were applied using the described equipment in greenhouse study. A nontreated control was also included for comparison. In both experimental sites, arrangement of treatments was as factorial in a completely randomized block design with four replications. Visual weed control was recorded 15 and 30 DAT on a scale of 0 % to 100 %, with 0 % representing no control compared to nontreated plots and 100 % indicating plant death. At corn maturity, all aerial parts of living *Ph. divaricata* plants were harvested from 50 by 50 cm quadrate placed in each plot, then oven dried and above dry biomass of weeds was recorded.

2.3 DATA ANALYSIS

For both greenhouse and field studies, homogeneity of variance also was analyzed. However, no transformation of the data was needed; therefore, analyses were conducted on the untransformed data. For the greenhouse study, the data were pooled across runs because of lacking a significant run by treatment interaction and then subjected to ANOVA using SAS software (SAS Institute, Cary, NC, USA). Because field study was done at two locations, data analysis was conducted using the PROC MIXED procedure in SAS. The variance was partitioned into fixed effects of treatment and random effects of block, location, and location by treatment interaction. Fisher's Protected LSD (p = 0.05) was used for means separation.

Regression analysis was conducted on data obtained from greenhouse experiment using SigmaPlot software (version 12.0, SyStat Software, Inc., Point Richmond, CA,

Table 2: Physical and chemical properties of soil (0-30 cm depth) in the experimental sites

Site	O.C (%)	N (%)	P (ppm)	K (ppm)	Soil texture	PH	EC (ds m^{-1})
Mahidasht	0.77	0.08	7.4	280	Silty clay loam	7.8	0.84
Eslamabad–e-Gharb	0.63	0.06	8	240	Silty Clay Loam	7.7	0.55

USA). Above dry biomass of weeds reduction (%) resulting from different doses of herbicides in greenhouse studies were fitted to a functional three-parameter exponential model (Chauhan et al., 2006). The fitted model was as follows:

$$G\ (\%) = {G_{max}}\Big/\left[1 + (X/ED_{50})^{Grate}\right]$$

G represents the weed dry biomass reduction (%) at herbicide concentration *X*, G_{max} is the maximum germination (%), ED_{50} is the herbicide concentration for 50 % reduction in above dry biomass of weeds, and G_{rate} indicates the slope.

3 RESULTS AND DISCUSSION

3.1 GREENHOUSE STUDY

Bromoxynil + MCPA and mesotrione + s-metolacholor + terbuthylazine applied at 25 % of the recommended herbicide dose provided 100 and 93.4 % control of annual ground cherry, respectively (Fig. 1). Based on the three-parameter logistic model results, the required concentration of different herbicides to reduce 50 % of the dry biomass of annual ground cherry varied greatly among herbicides when used with AMS, citogate and alone are presented in Table 3. AMS was more effective than citogate in enhancing performance of applied herbicides (Fig. 1).

The ED_{50} (the herbicide concentration for 50 % reduction in dry biomass) of all herbicide was significantly lower when compared with herbicide lonely (Table 3). The control of *Ph. divaricata* improved with increasing dosage of foramsulfuron + iodosulfuron and 2, 4-D (Fig. 1). However, only applying foramsulfuron + iodosulfuron at recommended dose resulted in significant control (84.6 %) of *Ph. divaricata* (Fig. 1). Similar to our results, Kudsk, (1989, 2002) found that very susceptible species can be controlled with less than half of recommended herbicide dose. Rimmsulfuron did not provide control percentages beyond 20 % even at recommended doses (Fig. 1). Mesotrione + s-metolacholor + terbuthylazine is a combination of three highly effective active, which recently has been registered for the control of weeds in Iranian corn fields (Zand et al., 2009a). Tański and Idziak (2009) reported that mesotrione + s-metolacholor + terbuthylazine was able to effectively control *Chenopodium album* L., *Echinochloa crus-galli* (L.) P.Beauv., *Amaranthus retroflexus* L., and *Solanum nigrum* L. in corn. In addition, Chikoye et al. (2009) showed that mesotrione + s-metolacholor + terbuthylazine provides satisfactory control of all main broad-leaved and grass weeds in corn. It has been well demonstrated that bromoxynil + MCPA controls a wide spectrum of broadleaved weeds in corn (Baghestani et al., 2014). Likewise, our results showed that either mesotrione + s-metolacholor + terbuthylazine or bromoxynil + MCPA were highly effective in controlling annual ground cherry. Although foramsulfuron + iodosulfuron, rimsulfuron, 2,4-D + MCPA, nicosulfuron, and foramsulfuron are known to control broadleaved weeds in corn (Vencill, 2002), but they had no or little effect on control of annual ground cherry in this study. Little weed control with mesotrione (30 %) and atrazine (8 %) was consistent with formerly reported results (Vencill, 2002). Generally, weed species responded to various herbicides inconsistently (Khan et al., 2003).

3.2 FIELD STUDIES

Based on the results of greenhouse study, commonly used herbicides are the most important factor in reducing the population of *Ph. divaricata* in corn field. Bromoxynil + MCPA and mesotrione + s-metolacholor + terbuthylazine, which provided optimum control of *Ph. Divaricata*, are not common herbicides often being used by corn growers across Iran, particularly in western provinces. Instead, 2, 4-D + MCPA, nicosulfuron, and foramsulfuron are more popular herbicide option for chemical weed control in corn (Baghestani et al., 2007). There are scarce reports on the response of *Ph. divaricata* to even the main generally used herbicides although such information would be very important for control this newly introduced weed in corn. There was an interaction between herbicide rate by location interaction for *Ph. divaricata* control 15 and 30 DAT. Therefore, the current control data are presented by separate locations.

Symptoms of mesotrione + s-metolacholor + terbuthylazine and bromoxynil + MCPA injury in *Ph. divaricata* were described by chlorosis and general growth reduction causing to final death. Herbicide symptoms were not visible until 15 DAT, the first ranking date on which control was recorded. Research conducted in Eslamabad-e-Gharb resulted in higher control of *Ph. divaricata* by either mesotrione + s-metolacholor + terbuthylazine or bromoxynil + MCPA at 15 and 30 DAT in comparison with Mahidasht observations (Table 4). Differences detected between two sites are likely due to the difference in climate conditions. Furthermore, results clearly

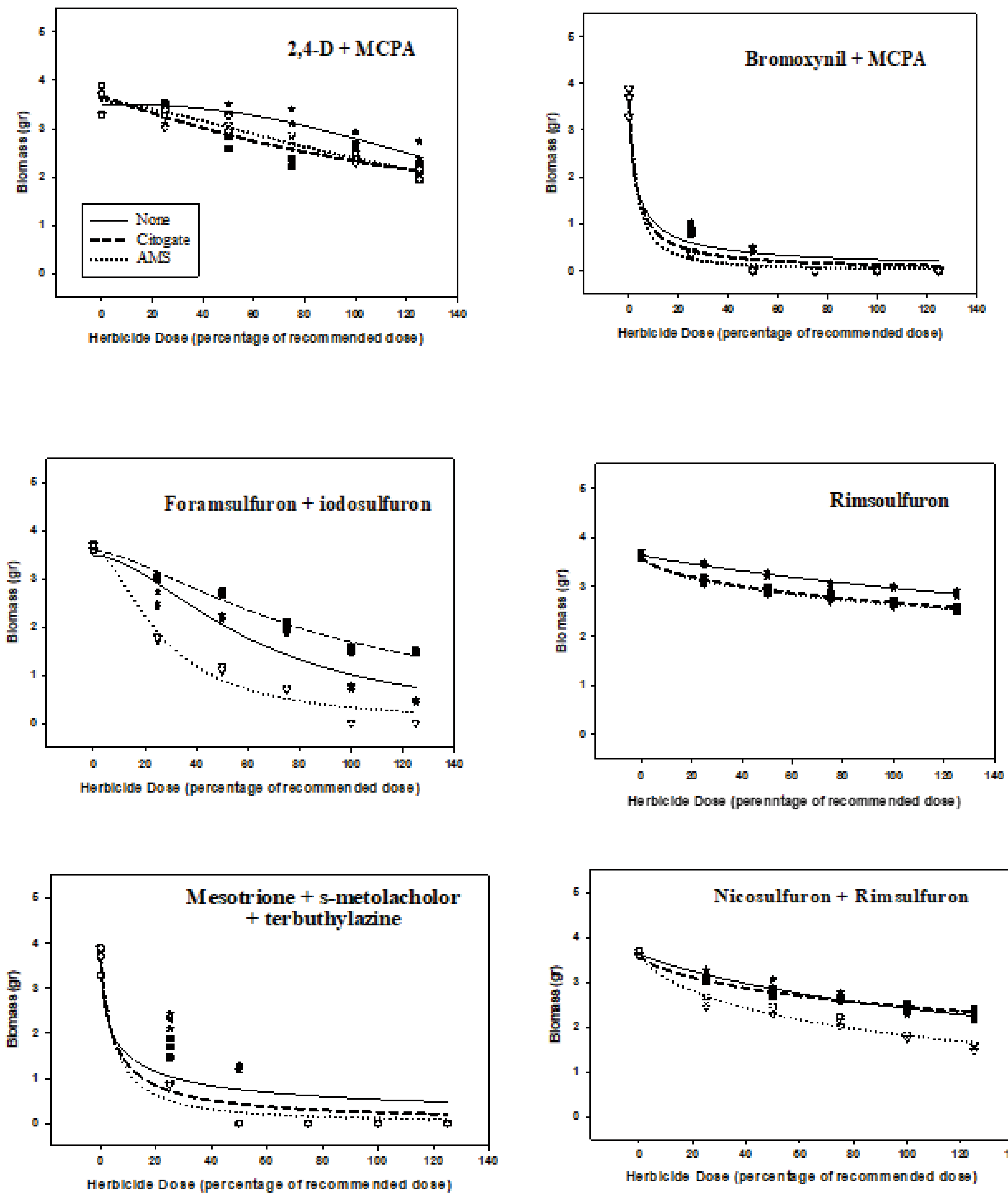

Figure 1: Response of *Physalis divaricate* following foliar applications of various doses of mesotrione + s-metolacholor + terbuthylazine, bromoxynil + MCPA, foramsulfuron + iodosulfuron, 2,4-D + MCPA, rimsulfuron and nicosulfuron + rimsulfuron applied alone and in combination with AMS and citogait. Data are replication of dry biomass reduction of weed, which are fitted to a 3-paramere logistic model

Table 3: Parameter estimates of the three-parameter sigmoid model fitted to dry biomass reduction of *Physalis divaricata* following foliar applications of various doses of different herbicides applied alone and in combination with AMS and citogate

Treatment	G_{max}*	* ED_{50}	G_{rate}*	RMSE	R^2
2,4-D+MCPA	3.51 (0.10)	173.13 (24.75)	2.47 (0.83)	0.24	0.76
2,4-D+MCPA +Citogate	3.68 (0.14)	167.21 (28.38)	1.05 (0.24)	0.25	0.83
2,4-D +MCPA +AMS	3.57 (0.09)	161.29 (15.20)	1.45 (0.26)	0.17	0.90
Bromoxynil + MCPA	3.66 (0.17)	3.00 (1.48)	0.76 (0.22)	0.30	0.95
Bromoxynil + MCPA +Citogate	3.64 (0.15)	3.00 (1.67)	0.94 (0.37)	0.26	0.96
Bromoxynil + MCPA +AMS	3.63 (0.08)	3.00 (1.48)	1.22 (0.67)	0.14	0.99
Foramsulfuron + iodosulfuron	3.48 (0.19)	61.00 (6.43)	1.80 (0.31)	0.34	0.91
Foramsulfuron + iodosulfuron + Citogate	3.60 (0.07)	91.74 (3.80)	1.47 (0.11)	0.12	0.97
Foramsulfuron + iodosulfuron + AMS	3.62 (0.13)	26.25 (2.36)	1.73 (0.21)	0.23	0.97
Rimsulfuron	3.64 (0.06)	508.82 (181.24)	0.92 (0.21)	0.12	0.85
Rimsulfuron + Citogate	3.63 (0.02)	534.32 (74.20)	0.60 (0.04)	0.05	0.98
Rimsulfuron + AMS	3.63 (0.03)	508.22 (70.41)	0.59 (0.04)	0.05	0.98
Mesotrione + s-metolacholor + terbuthylazine	3.83 (0.43)	5.00 (2.29)	0.61 (0.17)	0.74	0.75
Mesotrione + s-metolacholor + terbuthylazine + Citogate	3.71 (0.30)	5.00 (2.32)	0.88 (0.27)	0.52	0.87
Mesotrione + s-metolacholor + terbuthylazine + AMS	3.65 (0.14)	5.00 (2.85)	1.13 (0.45)	0.25	0.97
Nicosulfuron + rimsulfuron	3.63 (0.12)	215.50 (46.62)	0.89 (0.20)	0.21	0.84
Nicosulfuron + rimsulfuron + Citogate	3.63 (0.04)	306.51 (38.11)	0.64 (0.06)	0.07	0.97
Nicosulfuron + rimsulfuron + AMS	3.62 (0.07)	100.46 (8.14)	0.76 (0.08)	0.12	0.97

*G_{max} is the maximum germination (%), ED_{50} is the herbicide concentration for 50 % reduction in above dry biomass of weed, and G_{rate} indicates the slope

showed that as herbicide rate increased, *Ph. divaricata* control also increased. For both locations and regardless of adjuvant (AMS), bromoxynil + MCPA was more effective than mesotrione + s-metolacholor + terbuthylazine in the control of *Ph. divaricata* (Table 4).

There was a significant AMS by herbicide rate interaction on biomass reduction of annual ground cherry. However, treatment by location interaction was not significant. Hence, the biomass reduction data were pooled across the locations. Similar to the control data, *Ph. divaricata* control increased as herbicide rate increased with and without AMS (Table 5). Likewise, bromoxynil + MCPA reduced the biomass of *Ph. divaricata* more efficiently when compared to mesotrione + s-metolacholor + terbuthylazine (Table 5). Applying 75 % of the recommended dose of bromoxynil + MCPA provided more than 80 % control of *Ph. divaricata* regardless of AMS, but even the highest rate of mesotrione + s-metolacholor + terbuthylazine resulted in lower than 90 % control at 15 and 30 DAT. However, by utilizing complete dose of bromoxynil + MCPA plus AMS, *Ph. divaricata* control was 100 % at 30 DAT. Bromoxynil + MCPA is premixed and formulated herbicide that controls broadleaved weeds (Vencill 2002). Bromoxynil + MCPA can provide excellent suppression of some problematic weeds such as *Ambrosia artemisiifolia* L., *Abutilon theophrasti* Medik., *Capsella bursa-pastoris* (L.) Medik., *Polygonum convovulus* L., *Polygonum persicaria* L., *Sinapis arvensis* L., *Solanum* spp., *Thlaspi arvense* L., and *Xanthium strumarium* L. (Soltani et al., 2006); Sheibany et al., 2009). Their research found that using bromoxynil + MCPA as a POST-treatment can effectively control *Portulaca oleracea* L., *Convolvulus arvensis* L., *Amaranthus retroflexus, Chenopodium album* in corn. However, control of *Amaranthus blitoides* S.Wats. was not sufficient.

Zand et al. (2009b) showed that mesotrione + s-metolacholor + terbuthylazine is able to effectively control *P. oleracea, Ch. album, Setaria* spp.,_and *Hibiscus trionum* L.. However, the control of *Sorghum halepense* (L.) Pers. by this herbicide was poor (Zand et al., 2009b). Be-

Table 4: Effect of various doses of mesotrione + s-metolacholor + terbuthylazine and bromoxynil + MCPA herbicides on *Physalis divaricata* control 15 and 30 days after treatment (DAT) at Eslamabad and Mahidasht Research Centers

				%Control	
Location	Herbicide	Rate	Adjuvant	15 DAT	30 DAT
		% of Recommended herbicide dose			
Eslamabad-e-Gharb	Bromoxynil + MCPA	50	-	16.67 p	21.67 k
			AMS	36.67 m	40.67 i
		75	-	58.33 jk	71.67 e
			AMS	69.33 h	71.67 e
		100	-	79.33 de	94.00 b
			AMS	89.00 b	100.00 a
	Mesotrione + s-metolacholor + terbuthylazine	50	-	21.67 o	41.67 i
			AMS	36.00 m	48.33 h
		75	-	51.67 l	55.00 g
			AMS4	56.67 k	66.67 f
		100	-	61.00 ij	83.33 cd
			AMS	71.67 gh	100.00 a
Mahidasht	Bromoxynil + MCPA	50	-	22.00 o	35.00 j
			AMS	34.00 n	50.00 h
		75	-	73.00 gh	85.00 c
			AMS	78.00 ef	100.00 a
		100	-	87.00 bc	100.00 a
			AMS	100.00 a	100.00 a
	Mesotrione + s-metolacholor + terbuthylazine	50	-	33.00 n	40.00 i
			AMS	52.00 l	50.00 h
		75	-	70.00 h	70.00 ef
			AMS	75.00 fg	70.00 ef
		100	-	63.00 i	80.00 d
			AMS	83.00 cd	90.00 b

Means in a column with the same letter are not significantly different

tween the locations, bromoxynil + MCPA was found to be more effective in managing *Ph. divaricata* compared to mesotrione + s-metolacholor + terbuthylazine (Table 4 and 5). Based on the controlled weed by bromoxynil + MCPA, the results of current study are expected. The results of field surveys showed that the addition of AMS improve *Ph. divaricata* control by either mesotrione + s-metolacholor + terbuthylazine or bromoxynil + MCPA, especially at lower herbicide doses (Table 4 and 5). Collectively, AMS had higher ability in increasing the efficacy of bromoxynil + MCPA compared to mesotrione + s-metolacholor + terbuthylazine. For instance, addition of AMS to 50 % of label dose of bromoxynil + MCPA improved *Ph. divaricata* control by 10 % in comparison to herbicide alone while this value was negligible in the respect dose of mesotrione + s-metolacholor + terbuthylazine (Table 5). Adding AMS to 75 % recommended dose of bromoxynil + MCPA resulted in complete reduction of biomass of *Ph. divaricata* (Table 5).

The present results are in agreement with those of similar studies in which control of weeds was improved by adding AMS to various herbicides (Zollinger et al.,

Table 5. Effect of various doses of mesotrione + s-metolacholor + terbuthylazine and bromoxynil + MCPA herbicides on biomass of *Physalis divaricata* when data pooled over location

Herbicide	Rate	Adjuvant	Biomass reduction
	% of Recommended herbicide dose		% of untreated
Bromoxynil + MCPA	50	-	27.49 c
		AMS	38.39 c
	75	-	84.69 ab
		AMS	100.00 a
	100	-	100.00 a
		AMS	100.00 a
Mesotrione + s-metolacholor + terbuthylazine	50	-	35.18 c
		AMS	36.26 c
	75	-	67.99 b
		AMS	68.50 b
	100	-	72.99 b
		AMS	82.84 ab

Means with the same letter are not significantly different

2011; Tanveer et al., 2015). It has been well confirmed that the addition of AMS to herbicide tank mixture could improve the control of *A. theophrasti* by herbicides (Maschhoff et al., 2000; Young et al., 2003; Guza et al., 2002; Krausz et al., 1996). In addition, dust storm is a predominant phenomenon in western parts of Iran. Therefore, most likely the increasing of herbicide efficacy could be attributed to overcoming the adverse impact of dust particles on herbicide performance (Mathiassen et al., 1999; Zhou et al., 2006).

4 CONCLUSION

Results clearly showed that 2, 4-D + MCPA, rimsulfuron and nicosulfuron + rimsulfuron are not suitable options to control *Ph. divaricate* in corn. In conclusion, current study indicated that either mesotrione + s-metolacholor + terbuthylazine or bromoxynil + MCPA, particularly bromoxynil + MCPA can be used for successfully control of *Ph. divaricata* in corn. Furthermore, AMS was very efficient adjuvant in increasing the performance of mesotrione + s-metolacholor + terbuthylazine or bromoxynil + MCPA, against *Ph. divaricata.* These herbicides provide corn growers an herbicide options that can control *Ph. divaricata* and probably other weed species which are not affected by other regularly used herbicide options.

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doi:10.14720/aas.2023.119.1.2679 Original research article / izvirni znanstveni članek

Invertebrate's diversity on persimmon crop (*Diospyros kaki* Thunb.) at low altitude in Mechtras region, North Algeria

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Received May 08, 2022; accepted February 07, 2023.
Delo je prispelo 8. maja 2022, sprejeto 7. februarja 2023

Invertebrate's diversity on persimmon crop (*Diospyros kaki* Thunb.) at low altitude in Mechtras region, North Algeria

Abstract: The inventory of invertebrates on persimmon cultivation using two methods of sampling, Barber traps and colored traps in Mechtras region (Tizi-Ouzou) Algeria, allowed us to collect 115 species divided into 58 families, belonging to 13 orders and 6 classes. The values of the centesimal frequencies applied to invertebrates orders identified in the studied plot vary from one type of trapping to another, each sampling method relates to a representative order group. The diets of insects are extremely diverse, due to the structures and function of the mouthparts, the structural and functional division of the digestive tract; we have established 8 trophic classes. Shannon-Weaver diversity index values are quite high in the study plot. The fairness obtained for each type tend towards 1, which reflects a balance between the species.

Key words: inventory; invertebrates; persimmon; Mechtras; Algeria

Raznolikost nevretenčarjev v nasadih kakija (*Diospyros kaki* Thunb.) v nižinskih predelih območja Mechtras, severna Alžirija

Izvleček: S popisom nevretenčarjev v nasadih kakija z uporabo dveh metod vzorčenja, Barberjevih talnih in barvnih zračnih pasti, na območju Mechtras (Tizi-Ouzou) v Alžiriji, je bilo ugotovljenih 115 vrst iz 58 družin, ki pripadajo 13 redom in 6 razredom. Vrednosti centezimalnih frekvenc, ki so se uporabljale za identifikacijo vrst nevretenčarjev na preučevanem območju, se razlikujejo od ene vrste pasti do druge, vsaka metoda vzorčenja pa se nanaša na reprezentativno redovno skupino. Prehrana žuželk je izjemno raznolika, kot posledica zgradbe in delovanja ustnega aparata in prebavnega trakta. V tej zvezi smo vzpostavili 8 trofičnih razredov. Vrednosti Shannon-Weaverjevega indeksa pestrosti na preučevani ploskvi so bile precej velike. Vrednost indeksa je za vsako ploskev blizu 1, kar odraža ravnotežje med vrstami.

Ključne besede: popis; nevretenčarji; kaki; Mechtras; Alžirija

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1 INTRODUCTION

Fruit arboriculture is an integral part of the economic and social life of Algeria. This large country, due to its geographical position and its various pedoclimatic conditions, indeed has the privilege of cultivating several fruit species and to produce fresh fruit all year round.

The persimmon, like any fruit tree, is attacked by several insects which occupy a very special place in the ecosystem; in addition, they are good biological indicators, in addition, they are good biological indicators, because they are the main food of many vertebrates and are essential pollinators for the farmer (Clere et Bretagnolle, 2001).

According to Bouktir (2003), insects by their diversity abundance, but also their occupation of very diverse ecological niches, they can be useful such as parasitoids and predators. However, insects can be harmful and play various epidemiological roles, which make them a major public health problem (Jolivet, 1980).

The preservation of biodiversity represents an indisputable ecological stake in the functioning of agroecosystems, but also economical for society (Tscharntke and *al*., 2015). In this context, we carried out an inventory of the invertebrates fauna associated with persimmon tree cultivation in Tizi-Ouzou area (Kabylie), with the aim of improving our knowledge of biodiversity invertebrates and their classification according to the different trophic regimes.

2 MATERIALS AND METHODS

The study was realized in a *Diospyros kaki* 'Tipo' ochard which is located in Mechtras area (36° 32′ 41″ Nord, 4° 0′ 18″ East) situated at an altitude of 389 meters, with sub-humid climate and temperate winter (Fig. 1).

The orchard represents an appropriate environment and an extraordinary ecosystem whose biological functions bring together ecological conditions conducive to installation and the multiplication of various invertebrates. So, various sampling methods have been addressed in Mechtras region from December 2019 until November 2020.

2.1 IN THE FIELD

We used two trapping methods (Fig. 2), namely Barber pots or terrestrial traps as well as colored aerial traps, at the rate of one outing per month, from December 2019 until November 2020.

We installed Barber pots with 9 traps 7 cm deep and 15 cm in diameter, filled to 2/3 of their content with soapy water; which are visited once per week. The content was collected and put in jars with labels on which were indicated the date of collection and the trap concerned.

We installed colored pots with 9 traps, placed on tree branches at a height of one meter exceeds the natural vegetation. The pots are filled to 2/3 of their volume with water added with a few drops of detergent. This reduces the surface tension of water and promotes the drowning of species that come into contact with the liquid.

2.2 LABORATORY WORKING METHODS

After each trip, after a week, and according to the different capture methods used, the samples obtained are placed in Petri dishes with indicating the date and the trap.

2.2.1 Identification

The identification of individuals of listed invertebrates is carried out using the different determination keys treating on morphology and chetotaxis (Perrier, 1961; Piham, 1986; Delvare and Aberlenic, 1989; Chinery, 1988 and Seguy, 1651).

2.2.2 Trophic diet

After identification of the invertebrate species captured by the different sampling methods, their trophic regimes are determined after bibliographic research.

2.3 RESULTS TREATMENT

The results obtained are evaluated by several ecological indices.

The total wealth represents the total number of species that includes the population considered in an ecosystem (Ramade, 2003).

The Relative abundance (centesimal frequency) Fc (%) was also evaluated; it gives the percentage of individuals of a species Ni relative to the total number of individuals N (Dajoz, 1971).

Fc = Ni x 100/ N

We have also used Shannon-Weaver index which is calculated by the following formula:

H' = - qi log2 qi

*H'***:** diversity index expressed in bits units

qi: the probability of encountering the species i

The evenness index is the ratio of observed diversity H' to the maximum diversity' max**:** E = H'/H' max (Blondel, 1979). Knowing that H' max is calculated using the following formula:

H' max = Log 2 S

S: total wealth

H'max: is expressed in bits

3 RESULTS

In our study about invertebrates fauna associated to persimmon trees, we have caught 115 species, distributed in 57 families belonging to 13 orders and 6 classes.

Figure 1: Situation of the study region in Algeria (Google maps, 2021)

3.1 TOTAL WEALTH AND CENTESIMAL FREQUENCY

We were able to identify 115 species of captured invertebrates on persimmon plot using colored traps and Barber pots. Table 1 represents total wealth of invertebrate, which were 63 species for colored traps and 66 species for Barber pots.

Invertebrates orders collected on persimmon plot according to their centesimal frequency are shown in Figure 3 for colored traps and Figure 4 for Barber pots. Table 2 represent relative abundance of species identified according to the order, and family.

We have noticed that Hymenoptera is the most dominant order recorded for colored traps is with centesimal frequency equal to 29.8 %, and Coleoptera is the most dominant order recorded for Barber pots with centesimal frequency equal to 30.98 %.

Table 1: Total wealth of species caught

	Colored traps	Barber Pots
Total wealth	63	66

Figure 2: Different sampling methods used: a: Yellow plastic bins serving as an aerial trap, b: Barber pots buried in the ground (Original, 2020)

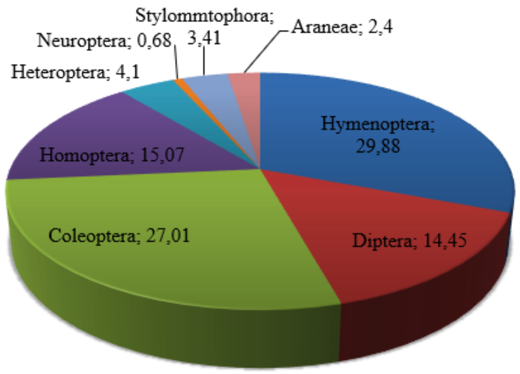

Figure 3: Relative abundance of invertebrate species caught using colored traps

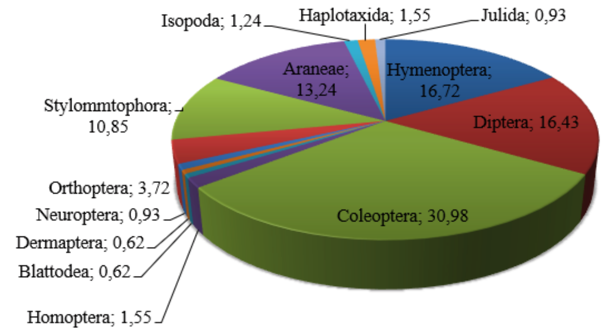

Figure 4: Relative abundance of invertebrate species caught using Barber pots

Table 2: Relative abundance of invertebrates' species caught on persimmon crop

Classes	Orders	Famillies	Species	Colored Trap	Barber Trap
–Insecta	Hymenoptera	Apidae	Apis m–ellifera (Linnée, 1758)	13,7	0,93
		Formicidae	Messor barbarus (Linnée, 1767)	-	5,88
			Messor structor (Latreille, 1798)	-	1,24
			Pheidol pallidula (Nylander, 1849)	1,71	0,31
			Cataglyphis cursor (Fonscolombe, 1846)	-	0,93
			Cataglyphis bicolor (Fabricius, 1793)	-	0,62
			Cataglyphis viatica (Fabricius, 1787)	0,68	4,33
		Braconidae	Aphidius colemani (Viereck, 1912)	0,68	-
		Ichneumonidae	Netelia testacea (Gravenhorst, 1829)	1,13	-
			Ichneumonidae (Latreille, 1802)	1,71	-
		Halictidae	Halictus quadricinctus (Fabricius, 1776)	3,08	-
			Lasioglossum calceatum (Scopoli, 1763)	1,71	-
		Vespidae	Polistes nimpha (Christ, 1791)	1,03	-
			Vespula germanica (Fabricius, 1793)	1,37	0,62
		Pompilidae	Priocnemis confusor (Wahis, 2006)	-	0,93
		Pteromalidae	Systasis angustula (Graham, 1969)	0,68	-
			Coruna clavata (Walker, 1833)	1,37	-
		Megachilidae	Megachile centuncularis (Linnée, 1758)	-	0,93
			Megachile fertoni (Pérez, 1895)	1,03	-
	Diptera	Tephritidae	Xyphosia miliaria (Schrank, 1781)	-	2,17
			Tephritidae (Schrank, 1781)	1,03	-
			Ceratitis capitata (Wiedemann, 1826)	7,53	-
		Muscidae	Graphomya maculata (Scopoli, 1763)	-	0,62
			Musca sp. (Linnée, 1758)	-	0,31
			Musca domestica (Linnée, 1758)	-	1,55
		Sepsidae	Sepsis fulgens (Linnée, 1758)	-	0,62
		Syrphidae	Episyrphus balteatus (De Geer, 1776)	1,71	-
		Stratiomyidae	Chorisops tibialis (Meigen, 1820)	1,71	-

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	Lauxaniidae	Lauxaniidae (Meigen, 1820)	0,68	-
	Sciaridae	Zygoneura sp. (Billberg, 1820)	0,34	-
	Psychodidae	Phlebotomus sp. (Loew, 1845)	-	0,62
	Agromyzidae	Agromyzidae (Fallen, 1823)	0,34	-
	Tipulidae	Tipula oleracea (Linnée, 1758)	-	1,24
	Mydidae	Mydas clavatus (Drury, 1773)	-	1,86
	Calliphoridae	Calliphora vomitoria (Linnée, 1758)	-	2,79
		Calliphora vicina (Robineau-Desvoidy, 1830)	1,03	2,48
		Calliphoridae (Hough, 1899)	-	0,62
		Lucilia caesar (Linnée, 1758)	-	1,55
	Culicidae	Culiseta annulata (Schrank, 1776)	1,03	-
		Aedes albopictus (Skuse, 1894)	0,34	-
		Culex pipiens (Linnée, 1758)	1,71	-
Coleoptera	Staphylinidae	Staphylinus caesareus (Cederhjelm, 1798)	-	0,31
		Creophilus maxillosus (Linnée, 1758)	0,68	0,93
		Philonthus marginatus (O.F. Muller, 1764)	0,68	-
		Ocypus olens (O.F. Muller, 1764)	-	10,84
	Scarabaeidae	Rhizotrogus aestivus (Olivier, 1789)	-	0,62
		Rhizotrogus maculicollis (Villa et Villa, 1833)	-	1,55
		Anisoplia floricola (Fabricius, 1787)	1,71	-
		Oxythyrea funesta (Poda, 1761)	3,42	-
	Apioidae	Apion pomonae (Fabricius, 1798)	1,37	-
	Coccinellidae	Oenopia conglobata (Linnée, 1758)	-	0,93
	Dermestidae	Attagenus fasciatus (Thunberg, 1795)	1,03	-
		Dermestes sp. (Linnée, 1758)	0,68	-
	Curculionidae	Liparus glabrirostris (Kuster, 1849)	1,03	-
		Liparus coronatus (Goeze, 1777)	0,34	-
		Phyllobius oblongus (Linnée, 1758)	-	0,62
		Lixus penctiventris (Boheman, 1835)	2,05	-
		Phyllobius pomaceus (Gyllenhal, 1834)	0,34	-
		Polydrusus sp. (Germar, 1822)	-	1,24
		Polydrusus marginatus (Stephens, 1831)	1,71	-
		Polydrusus impersifron (Gyllenhal, 1834)	0,68	-
		Otiorhynchus sp. (Germar, 1822)	0,68	-
	Elateridae	Elateridae (Leach, 1815)	-	0,31
	Chrysomelidae	Bruchus rufimanus (Boheman, 1833)	7,88	-
	Buprestidae	Anthaxia cadens (Panzer, 1792)	0,68	-
	Cleridae	Trichodes alvearius (Fabricius, 1792)	1,37	-
	Carabidae	Macrothorax morbillosus (Fabricius, 1792)	-	0,93
		Brachinus crepitans (Linnée, 1758)	0,68	-
		Bembidion atripes (Latreilles, 1802)	-	0,93
		Clivina collaris (Herbst, 1784)	-	0,62

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			Carabus auratus (Linnée, 1758)	-	0,62
			Carabus violaceus (O.F. Muller, 1764)	-	0,93
			Harpalus paratus (Casey, 1924)	-	9,6
	Homoptera	Aphididae	Dysaphis plantaginea (Passerini, 1860)	1,03	-
			Aphis nerii (Fonscolombe, 1841)	1,03	-
			Aphis fabae (Scopoli, 1763)	3,08	0,31
		Cicadellidae	Amblysellus curtisii (Fitch, 1851)	5,48	-
			Helochara communis (Fitch, 1851)	1,03	0,62
			Cicadella viridis (Linnée, 1758)	1,03	0,62
			Graphocephala fennahi (Young, 1977)	0,68	-
			Amblysellus sp. (Glover, 1877)	1,71	-
	Heteroptera	Lygaeidae	Lygaeus saxatilis (Scopoli, 1763)	1,37	-
			Nysius helveticus (Herrich-Schaffer, 1850)	1,03	-
			Nysius senecionis (Schilling, 1829)	0,68	-
		Triozidae	Trioza urticae (Linnée, 1758)	0,68	-
		Miridae	Deraeocoris ruber (Linnée, 1758)	0,34	-
	Blattodea	Blattidae	Blatta orientalis (Linnée, 1758)	-	0,62
	Dermapteta	Forficulidae	Forficula auricularia (Linnée, 1758)	-	0,62
	Neuroptera	Myrmeleontidae	Myrmeleontidae (Latreille, 1802)	-	0,93
		Chrysopidae	Chrysoperla carnea (Stephens, 1836)	0,68	-
	Orthoptera	Gryllidae	Gryllus campestris (Linnée, 1758)	-	0,93
			Acheta domestica (Linnée, 1758)	-	0,93
		Tetrigidae	Tetrix undulata (Sowerby, 1806)	-	1,24
			Tetrix subulata (Linnée, 1758)	-	0,62
Gasteropoda	Stylommtophora	Subulinidae	Rumina decollata (Linnée, 1758)	-	1,86
		Hygromiidae	Ganula flava (Tryon, 1866)	-	1,86
			Cernuella virgata (Da Costa, 1778)	0,68	3,41
		Helicidae	Theba pisana (O.F. Muller, 1774)	0,68	-
			Helix aperta (Born, 1778)	0,68	-
			Helix aspersa (O.F. Muller, 1774)	1,37	0,62
			Massylaea vermiculata (OF Muller, 1774)	-	0,62
		Geomitridae	Xerotricha conspurcata (Draparnaud, 1801)	-	0,62
			Cochlicella acuta (O.F. Muller, 1774)	-	0,62
			Cochlicella barbara (Linnée, 1758)	-	1,24
Arachnida	Araneae	Phalangiidae	Phalangium opilio (Linnée, 1758)	-	0,62
		Dysderidae	Dysdera erythrina (Walcknaer, 1802)	-	1,24
		Thomisidae	Thomisus sp. (Walcknaer, 1802)	0,34	2,17
			Synema globosum (Fabricius, 1775)	1,03	0,93
		Salticidae	Heliophans sp. (C.L. Koch, 1833)	1,03	0,31
		Lycosidae	Lycosa narbonensis (Latreille, 1806)	-	5,8
			Lycosidae (Sundevall, 1833)	-	2,79

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Malacostraca	Isopoda	Armadillidiidae	Armadillidium vulgare (Latreille, 1804)	-	0,62
		Oniscidae	Oniscus sp. (Linnée, 1758)	-	0,62
Clitellata	Haplotaxida	Lumbricidae	Eisenia fetida (Savigny, 1826)	-	1,55
Diplopoda	Julida	Julidae	Tachypodoiulus albipes (C.L. Koch, 1838)	-	0,93
6	14	57	115	100	100

Using colored traps we have collected 63 species, represented mainly by *Apis mellifera* with 13.70 %, followed by *Bruchus rufimanus* with centesimal frequency of 7.8 %. The species *Aedes albopictus, Zygoneura* sp., *Liparus coronatus, Phyllobius pomaceux, Thomisus* sp. and *Deraeocoris ruber* presented a low relative abundance equal to 0.34 %.

Using Barber traps, we have caught 66 species, represented mainly by *Ocypus olens* and *Harpalus paratus* with centesimal frequency of 10.84 % and 9.60 % respectively, which are natural predators of various pests. The lowest relative abundance was recorded for the species *Aphis fabae, Pheidol pallidula, Musca* sp., Elateridae, *Staphilinus caesareus,* and Salticidae with 0.31 %.

3.2 RELATIVE ABUNDANCE OF SPECIES DEPENDING ON THEIR TROPHIC RELATIONSHIPS

The centesimal frequency get for species depending on their trophic relationships is shown for colored traps (Fig. 5) and for barber pots (Fig. 6).

Pests represent a large part in invertebrate caught using colored traps and Barber traps with respectively 55.88 % and 37.88 %, whereas the least abundant group for colored traps is necrophagous with only 1.47 % and for Barber traps is omnivorous with only 1.52 %.

Reported pest species attack persimmon, mostly fruit; others attack the trunk by being xylophageous, or to foliage by being phyllophagous. We still find those who suck the sap can seriously affect the proper functioning tree photosynthesis.

3.3 SHANNON DIVERSITY INDEX AND EVENNESS INDEX (E)

Shannon diversity index (H '), maximum diversity (H'max.) and equitability (E) applied to species trapped by the different sampling techniques are presented in Figure 7.

We have registered high value for Shannon diversity index, it is equal to H' = 5.39 bits; with H max = 6.07 bits for colored traps and H' = 5.32 bits; with H max = 6 bits for Barber pots. The evenness values are E = 0.88 for colored traps and Barber pots, this value approaches of 1 which reflects a balance between the middle of species.

4 DISCUSSIONS AND CONCLUSION

In our study about invertebrates fauna associated to persimmon trees, we have caught 115 species, distributed in 57 families belonging to 13 orders and 6 classes.

Chafaa et al. (2019) confirmed in their study on apricot orchards, 125 species belonging to 54 families and 9 orders, in Batna region of North-East Algeria. Vasquez

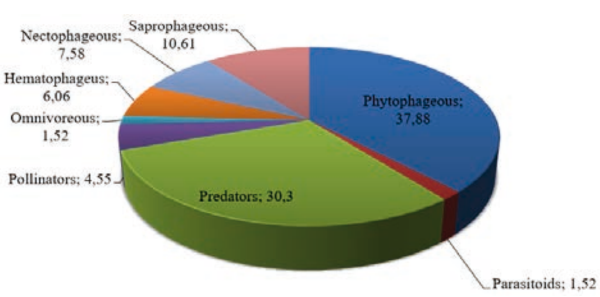

Figure 5: Centesimal frequency of species captured using colored traps according their diet

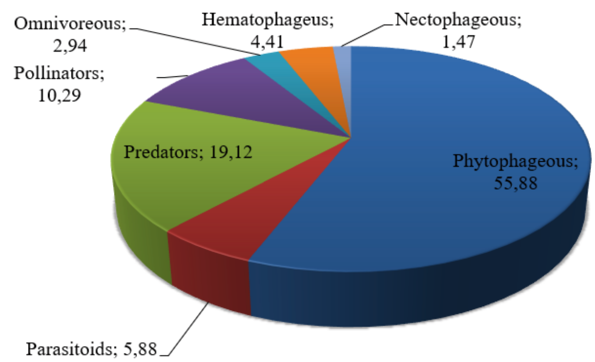

Figure 6: Centesimal frequency of species captured using barber traps according their diet

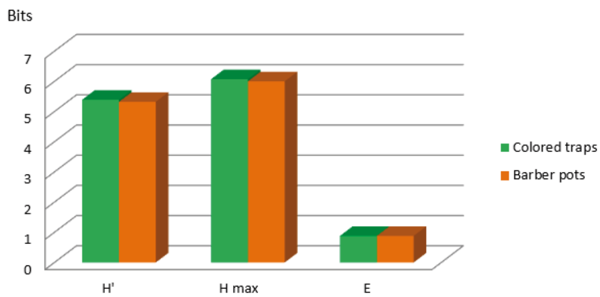

Figure 7: Shannon diversity values H' and evenness of species caught

et al. (2002) in the Peruvian Amazon have counted 36 insect species associated with guava cultivation.

Guermah et al. (2019) reported similar results about 113 species distributed in 64 families belonging to 10 orders in their evaluation of arthropods diversity on apple crop in Tizi-Ouzou. Guermah et al. (2019) collected 42 species divided into 29 families, belonging to 7 orders in their inventory of entomofauna in Tadmait on apple crop.

The total wealth of invertebrate reported were 63 species for colored traps and 66 species for Barber pots.

Chouiet et al. (2012) during a study on the biodiversity of the arthropod fauna of the cultivated areas of the Ghardaia region noted a total richness of 188 species, which is 133 species captured using Barber pots and 124 species using yellow traps. Fritas (2012) estimated total wealth at S = 64 on cereal crops in the Batna region, while Merabet (2014) estimated total wealth at S = 74 by using Barber pots at Agni N Smen.

We have noticed that Hymenoptera is the most dominant order recorded for colored traps is with centesimal frequency equal to 29.8 %, and Coleoptera is the most dominant order recorded for Barber pots with centesimal frequency equal to 30.98 %.

Guermah et al. (2019) registered the most dominant order recorded for sweep net and colored traps which is Hymenoptera with relative abundance of 36.38 % and 37.13 % respectively; for Barber pots, the most dominant order is Coleoptera with relative abundance equal to 50.35 %. Gull et al. (2019) noted that the order of beetles largely dominates with a percentage equal to 89%, followed by Hemiptera with 7% and Lepidoptera with only 3%. Mezani et al. (2016) found dominance about Coleoptera and Hymenoptera with a percentage equal to 23.80% and 23.38%, respectively, by applying the Barber pots. Djetti et al. (2015) in a study on the arthropod fauna of corn cultivation noted that Hymenoptera dominate in the region with a subhumid bioclimatic tier (El Harrach) with a relative abundance equal to 55 %. On the other hand in the region with semi-arid bioclimatic tier, the Coleoptera are best represented with a centesimal frequency equal to 50 %.

Pests represent a large part in invertebrate caught using colored traps and Barber traps with respectively 55.88 % and 37.88 %, whereas the least abundant group for colored traps is necrophagous with only 1.47 % and for Barber traps is omnivorous with only 1.52 %.

Diab and Deghiche (2014) indicated a dominance of phytophages with 53 %, followed by predators with 35 %, then polyphages with 12 % in an olive crop in the Sahara region. According to Beamont and Cassier (1983), in a given area, 40 to 50 % of insect species are phytophagous.

We have registered high value for Shannon diversity index, it is equal to H' = 5.39 bits; with H max = 6.07 bits for colored traps and H' = 5.32 bits; with H max = 6 bits for Barber pots. The evenness values are E = 0.88 for colored traps and Barber pots, this value approaches of 1 which reflects a balance between the middle of species.

Guermah et al. (2019) reported a diversity of Shannon-Weaver values for the various species caught by trapping methods. They are equal to H' = 5.90 bits; H max = 6.40 bits for sweep net; H' = 5.58 bits; H max = 6 bits for colored traps and H' = 5.33 bits; H max = 5.95 bits for Barber pots. Using Barber pot technique for the study of arthropod biodiversity at 3 steppes in the region of Djelfa, Guerzou et al. (2014) reported variations in the diversity values of Shannon between 1.9 and 3.7 bits in Taicha, 3.02 and 3.5 bits in El Khayzar, 3.6 and 4.0 bits in Guayaza.

The variations in the values of the Shannon index are explained by N'zala et al. (1997), who have pointed out that if the living conditions in a given environment are favorable, there are many species and each of them is represented by a small number of individuals. If the conditions are unfavorable, one finds only a small number of species each of them is represented by a large number of individuals. According to Blondel (1979), a community is even more diversified as the index of diversity is higher.

Guermah and Medjdoub-Bensaad (2016) rated fairness at 0.65. In a study on the arthropod fauna of corn cultivation, Djetti et al. (2015) estimated the fairness at E = 0.77 in the region with a subhumid bioclimatic tier (El Harrach) and E = 0.88 in the region with a semi-arid bioclimatic tier. The Pielou's evenness index showed by Gull et al. (2019) varies from 0.62 to 0.87.

In order to consider protection of persimmon crop against possible pests attacks, it is essential to identify the invertebrates cloning this crop and to better understand the interspecific relationship that connects them in this agroecosystem.

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doi:10.14720/aas.2023.119.1.2716 Original research article / izvirni znanstveni članek

Genetic variation and cluster analysis of *Narcissus tazetta* L. genotypes using RAPD and ISSR markers

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Received June 04, 2022; accepted February 10, 2023.
Delo je prispelo 4. junija 2022, sprejeto 10. februarja 2023

Genetic variation and cluster analysis of *Narcissus tazetta* L. genotypes using RAPD and ISSR markers

Abstract: To evaluate the genetic diversity of Iranian *Narcissus* genotypes (e.g., Shomal, Shastpar, Shahla, Yasuj, Shiraz-1, Shiraz-2, Kuchak-e-Atri, Dutch, Khosf-1, Khosf-2, Birjand, and Tabas), different RAPD and ISSR primers were examined at the Plant Breeding Laboratory of the Faculty of Agriculture, University of Birjand, Iran. In sum, RAPD and ISSR primers produced 189 and 80 high-resolution bands. The average values of PIC, Ht, Hs, Nm, DST, FDT, NA, Ne, H, and I indices were 0.287, 0.369, 0.089, 0.486, 0.279, 0.760, 1.952, 1.459, 0.282, 0.437 for RAPD markers and equal to 0.297, 0.380, 0.099, 0.524, 0.278, 0.732, 1.978, 1.495, 0.303, 0.467 for ISSR markers, respectively. The analysis of molecular variance based on the RAPD and ISSR markers showed 23 and 13 % variations for intra-populations and 77 % and 87 % changes for inter-population variabilities, respectively. Cluster analysis based on the RAPD and ISSR markers grouped genotypes into four clusters. Based on RAPD and ISSR markers, the PCoA analysis also showed that the first three components justified equal to 96.9 % and 97.9 % of the total variance, respectively, indicating the dispersion of the primers used. In general, it was concluded that the genetic diversity of narcissus species could be employed for breeding programs.

Key words: cluster analysis; narcissus; genetic parameters; genetic diversity

Genetska raznolikost in klasterska analiza genotipov dvobarvnega narcisa (*Narcissus tazetta* L.) z uporabo RAPD in ISSR markerjev

Izvleček: Za ovrednotenje genetske raznolikosti iranskih genotipov narcisa (e.g., Shomal, Shastpar, Shahla, Yasuj, Shiraz-1, Shiraz-2, Kuchak-e-Atri, Dutch, Khosf-1, Khosf-2, Birjand, in Tabas), so bili preiskušeni različni RAPD in ISSR primerji v Plant Breeding Laboratory, Faculty of Agriculture, University of Birjand, Iran. Sumarno so RAPD in ISSR primerji dali 189 in 80 jasno razvidnih trakov. Poprečne vrednosti PIC, Ht, Hs, Nm, DST, FDT, NA, Ne, H in I indeksov so bile 0,287; 0,369; 0,089; 0,486; 0,279; 0,760; 1,952; 1,459; 0,282; 0;437 za RAPD markerje in 0,297; 0,380; 0,099; 0,524; 0,278; 0,732; 1,978; 1,495; 0,303; 0,467 za ISSR markerje. Analiza molekularne variance na osnovi RAPD in ISSR markerjev je pokazala 23 in 13 % variacij znotraj populacij in 77 % in 87 % sprememb med populacijami. Klasterska analiza na osnovi RAPD in ISSR markerjev je združila genotipe v štiri skupine. Analiza glavnih komponent na osnovi RAPD in ISSR markerjev je pokazala, da so prve tri komponente razložile 96,9 % in 97,9 % celokupne spremenljivosti, kar kaže na razpršenost uporabljenih primerjev. V splošnem lahko zaključimo, da bi se genetska raznolikost vrst narcisa lahko uporabila v žlahtniteljskih programih.

Ključne besede: klasterska analiza; narcis; genetski parametri; genetska raznolikost

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1 INTRODUCTION

Narcissus (*Narcissus tazetta* L.), a member of the family of Amaryllidaceae, has many therapeutic and ornamental properties (Gotti et al., 2006). Narcissus is a beautiful plant of geophytic, monocotyledonous, and perennial plants whose genus has 65 species and 20,000 cultivars and hybrids (Saeedi, 2007). In general, the quality of this flower, especially in terms of color, odor, and characteristics such as the number of sepals and petals, are primarily controlled by genetic factors but environmental factors, such as stress and plant nutrition, have remarkable impacts on these characteristics (Zadebagheri et al., 2011). At present, numerous researches are going on the agronomic characteristics of this plant in Iran, but there are few reports on the plant genetic diversity. Since the study and determination of the genetic diversity of a plant is the first step in the next breeding program, the purpose of this study was to determine the kinship, diversity, and genetic structure of narcissus populations using RAPD and ISSR molecular markers.

Genetic resources can provide desirable genes and supply opportunities for breeders to produce new and desirable cultivars (Clegg, 1997; Bhandari et al., 2017). There are many methods to study the genetic diversity of plants. Among these ways, molecular markers (DNA-based techniques) are the most accurate and reliable method for determining and identifying genetic diversity and (along with multivariate statistical methods) has a significant potential for examining plants phylogenic, evolution, and genetic diversity (Pandey et al., 2008).

Among the molecular markers developed in recent years, RAPD (Random Amplified Polymorphic DNA) marker is a simple PCR-based technique that was developed by Williams et al. (1990) and Welsh & Mc-Clelland in 1990, and is based on the amplification of primer binding regions in DNA genome (Welsh et al., 1991, Ezzat et al., 2016, Shi et al., 2018). In general, this method is widely used due to the lack of basic information needed to design and construct primers, the possibility of simultaneously examining multiple loci in the sample genome, the lack of the need for a special probe in molecular analysis (especially genetic diversity assessment) (Williams et al., 1990). This marker has been used well to study the genetic diversity of narcissus (Jiménez et al., 2017), soybean (Sharma et al., 2018), oil palm (Basyuni et al., 2018), tomato (Maske et al., 2018) genotypes and etc.

ISSRs are semi-optional markers that are amplified by PCR in the presence of a primer that complements the target microsatellite. ISSR markers are useful in studies on genetic diversity, genome mapping, gene tagging, phylogeny and evolution (Pradeep Reddy Reddy et al., 2002). Such multiplication does not require sequential information and leads to the production of multi-position and highly polymorphic patterns. The ISSR fragments are 100- 3,000 bp length of DNA sequences that are located between two microsatellite regions with opposite directions. Microsatellite-based primers are designed and used to amplify the DNA sequence between two ISSRs (Kumar et al., 2009).

ISSR markers are potential markers in identifying genetic diversity compared to other markers and allow the study of the diversity of specific regions of the genome at several locations simultaneously (Mengistu et al., 2004). ISSR markers has been used well for genetic diversity of cuscuta (Tajdoost et al., 2012), Persian violet (Asadi et al., 2016), narcissus (Jiménez et al., 2009), *Solanum nigrum* L. (Suganthi et al., 2018). Also, genetic diversity of different Turkish narcissus has been studied using SRAP markers (Zeybekoğlu et al., 2019). A study using markers (ISSR) investigated the genetic diversity of 31 narcissus species collected from 16 regions of Iran. The average percentage of polymorphic bands was 96.02 %. The highest resolution power (8.32), average polymorphic information content (0.44) and marker index values (5.61) were observed for primers 811, 828 and 811, respectively. They stated that ISSR markers can be used as a diagnostic tool to evaluate genetic diversity in narcissus genotypes and reveal them (Zangeneh and Salehi, 2019).

It should be noted that the Khosf (City of South Khorasan province) has a long historical tradition in narcissus cultivation and its area under cultivation has the highest area in the east of the country. The aim of this study was to investigate the genetic diversity of different narcissus ecotypes that were collected from different regions of Iran. It has also been the determination of the best genotype and its important traits for breeding purposes.

2 MATERIALS AND METHODS

This experiment was carried out in the research farm and laboratory of plant breeding and biotechnology of the Faculty of Agriculture, University of Birjand, Iran during 2017 growing season. In this study, 12 different genotypes of narcissus that been cultivated in Iran (consisted of Shomal, Shastpar, Shahla, Yasuj, Shiraz-1, Shiraz-2, Koochak-e-Atri, Dutch, Birjand, Khosf-1, Khosaf-2, and Tabas) were collected and cultivated both in pots and in the field for relevant experiments.

Leaf samples were taken after 60 days of planting in the four-leaf stage. The youngest leaves were selected for DNA extraction and stored in liquid nitrogen. The required solutions are prepared in stock with higher accuracy. DNA extraction was performed by CTAB method (Doyle and Doyle, 1987). For this purpose, 0.5 g of young leaves was used. The quantity and quality of the extracted DNA were evaluated using two methods of nano drop and 1 % agarose gel electrophoresis. In this experiment, 27 RAPD primers and 11 ISSR primers were used. The primers were purchased in freeze-dried form from Sinaclon Company and diluted 1:10 using double-distilled water. The final concentration of primer was considered to be one µl in each 20 µl PCR reaction (Tables 1 & 2).

The polymerase chain reaction was performed at a final volume of 20 µl (Table 3). 8 µl of the PCR product was stained with 2 µl of loading dye and 10 µl was poured into 1 % agarose gel (with 0.5X TBE buffer) wells. To better examine the bands, 5 µl of DNA size marker was injected into first the gel well. Samples were electrophoresed at voltages 90 for one hour. Amplified fragments were observed and photographed by GelDoc under ultraviolet light. Photos were scored using TL120 Lab Total software; was assigned to the presence of band, number 1and the absence of it, number zero. At first, according to geographical information, genotypes collected from different regions were divided into two groups (South Khorasan in one group and other genotypes in another group) and different statistical analyzes were performed.

In this study, the genetic similarity index was employed to determine genotype grouping, and cluster analysis, similarity coefficient and principal coordinate analysis were used to determine the distribution of primers. These calculations were also performed using NTSYS2.2 software. Molecular indices such as PIC (Sharma & Ghosh, 1955), population genetic index such as number of alleles (Na), number of effective alleles (Ne) (Kimura & Crow, 1973), gene diversity index of Nei (H) (Nei,

Table 1: RAPD and ISSR primers

Primer	Sequence	Primer	Sequence	Primer	Sequence
			RAPD		
R1	5’-TGCCGAGGTG-3’	R10	5’-AGGTGACCGT -3’	R19	5’-ACACCGATGG -3’
R2	5’-AGTCAGCCAC -3’	R11	5’- GTTGCGATCC -3’	R20	5’- CTTCTCGGAC -3’
R3	5’-AGGGGTCTTG -3’	R12	5’- GGAAACCCCT -3’	R21	5’-ACGGGACCTG -3’
R4	5’-GGTCCCTGAC -3’	R13	5’- TGGCGCACAC -3’	R22	5’-CCAGAACGGA -3’
R5	5’-GTGACGTAGG -3’	R14	5’-GGCACGCGTT -3’	R23	5’- GTGGCCGATG -3’
R6	5’-GGGTAACGCC -3’	R15	5’- GTTACGGACC -3’	R24	5’- ACGGAAGTGG -3’
R7	5’-GTGATCGCAG -3’	R16	5’- GGGCGACTAC -3’	R25	5’-GGACCCCTTAC -3’
R8	5’- CAATCGCCGT -3’	R17	5’-TCGCATCCAG -3’	R26	5’-TGAGTGGGTG -3’
R9	5’-TGGGCGATAG -3’	R18	5’-CTGGCGTTC -3’	R27	5’- GTTGCCAGCC -3’
			ISSR		
I1	5’-CTCTCTCTCTCTCTCTRC-3’	I5	5’-GAGAGAGAGAGAGA-GAC-3’	I9	5’-CTCTCTCTCTCTCTCTTG-3’
I2	5’-GAGAGAGAGAGAGAGAYG-3’	I6	5’-GAGAGAGAGAGAGAGA-YA-3’	I10	5’-CACACACACARY-3’
I3	5’-TCTCTCTCTCTCTCTCG-3’	I7	5’-GAGAGAGAGAGAGAGA-YC-3’	I11	5’-GACAGACAGACAGACA-3’
I4	5’-GAGAGAGAGAGAGAGAYT-3’	I8	5’-ATCATCATCATCATC-3’		

Table 2: The PCR reaction temperature program for the ISSR and RAPD markers

	ISSR			RAPD		
Step	Tem.(^{o}C)	Time	Cycle Num.	(^{o}C) Te µLm.	Time	Cycle Num.
Initial denaturation	94	4min	1	95	4min	1
Denaturation	94	45s	40	94	1min	45
Annealing	45	5s	40	35	1min	45
Extension	72	55s	40	72	2min	45
Final extension	72	6min	1	72	6min	1

Table 3: Components of PCR reaction

Materials	Reaction components	The final concentration
Double distilled water	6.5 µl	…….
Master mix (Prepared from Yekta Tajhiz Azma Co.)	10 µl	2X
Primer	1.5 µl	0.67 pm
DNA	2.0 µl	20 ng μl^{-1}

PCR master mix includes the following details:
0.25 U/µl Taq DNA Polymerase; 2x PCR buffer; 0.4 mM dNTPs; 3.2 mM $MgCl_2$; 0.02 % bromophenol blue

1973), Shannon diversity index (I) (Lewontin, 1972), inter-population heterozygosity (Hs), intra-population heterozygosity (Dst), total heterozygosity (Ht), intra-population coefficient of variation (Gst) and fixation index (Fst) (Lynch & Milligan, 1994) was calculated using POPGEN-1.31. Molecular analysis of variance was also performed with Genalex 6.1 software.

3 RESULTS AND DISCUSSION

3.1 DETERMINATION OF DNA QUANTITY AND QUALITY AND ANALYSIS OF MOLECULAR VARIANCE

In this study, the amount of extracted DNA ranged from 83.5 to 565 ng μl^{-1}, which was reduced to less than 12 ng after dilution. Also, in all samples the wavelength ratio of 260:280 was 1.90 to 2.04, which indicated that it was suitable. Results also showed that from 27 RAPD primers produced 189 bands (Figure 1), of which nine bands were single-shaped and 180 had polymorphic bands. An average of seven bands were obtained per primer, so the used primers showed more than 95 % polymorphism. Out of a total of 11 ISSR primers, 80 bands with high-resolution were counted. Of the amplified bands, one band was single-shaped, and 79 bands were polymorphic. An average of 7.27 bands were obtained per primer. Overall, the ISSR primers that were used showed 98 % polymorphism. In general, RAPD and ISSR primers showed more than 95 % and 98 % polymorphism, respectively, and indicated the efficiency of primers in showing polymorphism. Chehrazi (2007) reported that out of 14 RAPD markers used to study the genetic diversity of narcissus genotypes, 105 bands were amplified, of which 92 were polymorphic bands. Consistent with our findings, the average rate was 7.5 bands per primer, and the polymorphism rate was equal to 96 %. Sargazi et al. (2016) studied the genetic diversity of fifteen ajowan populations using 25 RAPD primers and observed that all primers showed good polymorphism. In the study of genetic diversity of fennel using ISSR markers (Taheri et al., 2016), a total of 813 primers were reproduced from six primers, and the number of bands of each primer varied between 7-15, which is the average number of bands per primers consistent with this study.

3.2 GENETIC PARAMETERS

The polymorphism data exhibit notable variations between individuals and populations and are important indicators to compare different primers in terms of their power to differentiate genotypes. According to the presented results, it was found that the primers used had a relatively good PIC index, which indicates their acceptable efficiency in detecting and distinguishing genotypes (Table 4). In RAPD primers, the polymorphic content index differs from 0.15 to 0.47. The highest polymorphic content index (PIC) belonged to R13 (0.47), R1 (0.40), and R27 (0.40) primers, which showed their high efficiency in differentiating these genotypes. The lowest PIC also belonged to R2 (0.16) and R3 (0.15) primers. The polymorphism ratio index differs from 2.25 to 11.00. The lowest value belonged to R19 (2.25) primer, and the highest amount was for the R6 (11.00) primer. Also, the lowest and the highest values of the marker index were obtained for R14 (0.27) and R13 (3.78) primers, respectively. The lowest and the highest values of resolving power belonged to R26 (0.44) and R10 (1.67) primers, respectively. The mean values of total heterozygosity, intra-population heterozygosity, gene flow (Nm), intra-population heterozygosity (Dst), and Fst index were achieved equal to 0.37, 0.09, 0.49, 0.28, and 0.76, respectively.

Overall, the highest and the lowest values of the total heterozygosity were gained equal to R13 (0.46) and R2 (0.20) primers, respectively. The lowest heterozygosity inter-populations were for primers R20 (0) and R10 (0.01), while the highest amount of heterozygosity inter-populations was for R27 (0.22). The highest amount of gene flow belonged to R25 (1.14), and the lowest amount of gene flow was to R20 (0) and R10 (0.02). The highest heterozygosity intra-populations belonged to R10 and R7 (0.38), and the lowest was related to R2 (0.14). Primer R20 (1)

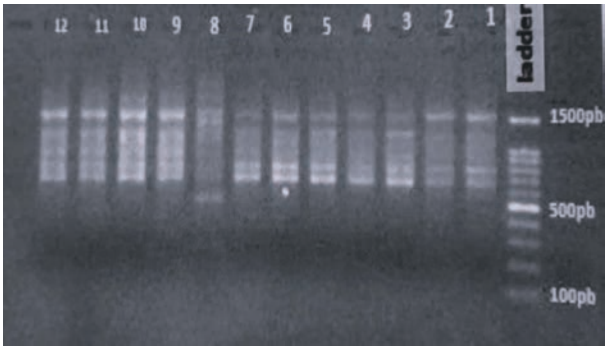

Figure 1: Separation pattern of amplified bands of 12 narcissus genotypes using OPA09 primers

had the highest Fst, and the lowest value with an average value of 0.50 was observed for R27. The maximum number of effective alleles (Ne) calculated for primers R2, R3, R16, R17, R19, and R20 were 1.67, 1.67, 1.89, 1.91, 1.75, and 1.83, respectively, and for other markers, it was equal to 2.0. The lowest number of effective alleles (Ne) related to R19 and R3 (1.21), and the highest these were obtained for primers R27 (1.71), and the average number of effective alleles (Ne) was 1.46. The highest gene diversity index of Nei (H) and Shannon diversity index (I) belonged to R13 primer (0.46 and 0.65), and the lowest was related to R3 primer (0.15 and 0.25), and their average was 0.28 and 0.47, respectively (Table 4).

Table 4: Genetic diversity parameters in narcissus genotypes with RAPD markers

Number	Ht	Hs	Dst	Fst	Na	Ne	H	I	PIC	EMR	MI	RP
R1	0.38	0.16	0.22	0.58	2.00	1.79	0.42	0.60	0.40	8.00	3.19	1.08
R2	0.20	0.07	0.14	0.67	1.67	1.28	0.17	0.28	0.16	2.67	0.42	1.22
R3	0.27	0.03	0.24	0.90	1.67	1.21	0.15	0.25	0.15	4.00	0.62	1.37
R4	0.39	0.10	0.29	0.74	2.00	1.62	0.36	0.53	0.36	8.00	2.89	0.83
R5	0.43	0.08	0.35	0.81	2.00	1.30	0.23	0.37	0.19	6.00	1.67	0.67
R6	0.35	0.06	0.30	0.84	2.00	1.33	0.23	0.38	0.24	11.00	2.68	0.86
R7	0.43	0.04	0.38	0.89	2.00	1.59	0.35	0.52	0.31	5.00	1.55	1.13
R8	0.42	0.16	0.26	0.62	2.00	1.60	0.36	0.54	0.33	6.00	2.01	1.25
R9	0.40	0.11	0.29	0.72	2.00	1.57	0.34	0.51	0.36	6.00	2.24	0.89
R10	0.39	0.01	0.38	0.97	2.00	1.25	0.19	0.33	0.18	8.00	1.44	1.67
R11	0.36	0.14	0.22	0.60	2.00	1.45	0.29	0.45	0.29	5.00	1.43	0.83
R12	0.36	0.12	0.24	0.66	2.00	1.29	0.21	0.37	0.25	5.00	1.28	1.00
R13	0.46	0.14	0.33	0.71	2.00	1.86	0.46	0.65	0.47	8.00	3.78	1.00
R14	0.42	0.14	0.27	0.65	2.00	1.67	0.37	0.55	0.34	8.00	0.27	1.22
R15	0.33	0.06	0.27	0.80	2.00	1.46	0.28	0.44	0.30	6.00	1.80	0.89
R16	0.3	0.08	0.22	0.73	1.89	1.30	0.21	0.35	0.24	8.11	1.70	0.94
R17	0.33	0.04	0.29	0.88	1.91	1.34	0.23	0.37	0.25	9.09	2.31	0.86
R18	0.26	0.07	0.35	0.83	2.00	1.55	0.32	0.49	0.30	7.00	2.11	0.76
R19	0.29	0.06	0.23	0.81	1.75	1.21	0.16	0.27	0.28	2.25	0.41	1.58
R20	0.31	0.00	0.31	1.00	1.83	1.28	0.18	0.30	0.18	4.17	0.76	1.47
R21	0.40	0.06	0.33	0.83	2.00	1.47	0.28	0.44	0.28	7.00	1.97	1.08
R22	0.43	0.06	0.37	0.86	2.00	1.52	0.30	0.47	0.31	7.00	2.17	1.13
R23	0.41	0.10	0.30	0.74	2.00	1.41	0.27	0.43	0.26	8.00	2.11	0.92
R24	0.35	0.14	0.21	0.60	2.00	1.44	0.30	0.42	0.30	7.00	2.72	0.57
R25	0.37	0.10	0.26	0.71	2.00	1.41	0.27	0.42	0.29	9.00	2.62	0.71
R26	0.30	0.05	0.25	0.82	2.00	1.47	0.29	0.45	0.30	3.00	0.92	0.44
R27	0.45	0.22	0.22	0.50	2.00	1.71	0.39	0.57	0.40	6.00	2.40	0.92
Mean	0.37	0.09	0.28	0.76	1.95	1.46	0.28	0.47	0.29	1.90	0.64	1.00

In the ISSR, the polymorphic indices content (PIC) changed between 0.15 and 0.37. The highest PIC belonged to primers I10 and I11 (0.37), which indicates its high efficiency in differentiating used genotypes. The lowest value belonged to the I2 primer (0.15). The polymorphism ratio index was between 3 and 14, with the lowest value belonging to primer I2 and the highest to primer I1. The lowest marker index was related to primer I5 (0.08), and the marker index was related to primer I8 (3.28). The lowest resolution index was related to primer I8 (0.27), and the highest value was related to primer I7 (1.55). The highest total heterozygosity was for primer I6 (0.43), and the lowest was related to primer I9 (0.31). The lowest heterozygosity inter-populations were obtained for primer I7 (0), and the highest value was for primer I10 (0.16). The highest level of gene flow was obtained for primers I1 (0.96) and I11 (0.94), while the lowest level was related to I2 (0). The highest heterozygosity intra-populations belonged to primer I2 (0.37), and the lowest was related to primer I9 (0.17). The highest and the lowest value of Fst were in primer I2 (1) and primer I9 (0.55), respectively. The average number of alleles for 11 primers of ISSR was 1.98. The lowest number of effective alleles (Ne) related to I2 (1.18) and the highest these were obtained for primers I10 (1.81). The means values for Nei's genetic diversity index (H) and Shannon diversity index (I) were 0.30 and 0.47, respectively. The highest value of Nei's genetic diversity index (H) and Shannon diversity index (I) belonged to primer I10 (0.43 and 0.62), and the lowest of these were related to primer I2 (0.15 and 0.29) (Table 5).

Table 5: Genetic diversity parameters in narcissus genotypes with ISSR markers

Number	Ht	Hs	Dst	Fst	Na	Ne	H	I	PIC	EMR	MI	RP
I1	0.37	0.14	0.23	0.62	2.00	1.42	0.28	0.45	0.35	14.00	0.85	0.80
I2	0.37	0.00	0.37	1.00	2.00	1.18	0.15	0.29	0.15	3.00	0.35	1.28
I3	0.35	0.11	0.24	0.70	2.00	1.40	0.27	0.43	0.30	5.00	1.49	0.70
I4	0.42	0.07	0.36	0.84	2.00	1.47	0.29	0.47	0.27	8.00	2.17	0.83
I5	0.41	0.10	0.31	0.75	2.00	1.47	0.30	0.46	0.31	10.00	0.08	1.00
I6	0.43	0.07	0.36	0.83	2.00	1.54	0.33	0.50	0.30	4.00	1.19	1.08
I7	0.33	0.06	0.27	0.81	1.83	1.46	0.28	0.43	0.24	4.16	1.02	1.55
I8	0.37	0.12	0.25	0.68	1.93	1.51	0.30	0.46	0.27	12.07	3.28	0.27
I9	0.31	0.14	0.17	0.55	2.00	1.56	0.33	0.50	0.34	4.00	1.35	0.96
I10	0.41	0.16	0.25	0.61	2.00	1.81	0.43	0.62	0.37	8.00	2.99	1.43
I11	0.37	0.13	0.25	0.66	2.00	1.62	0.36	0.53	0.37	5.00	0.83	1.07
Mean	0.38	0.10	0.28	0.73	1.98	1.49	0.30	0.47	0.30	7.02	2.15	1.09

3.3 DETERMINING SIMILARITIES BETWEEN GENOTYPES

The results showed that in both markers, UN1 similarity coefficient is the best coefficient and UPGMA algorithm is the best clustering pattern due to having the highest correlation coefficient (Table 6). Chehrazi et al. (2007) reported a cophenetic correlation coefficient between native and non-native narcissus genotypes was 0.97. Examination of similarity matrix in RAPD marker showed that the highest similarity was between Khosf-2 and Tabas genotypes (0.95). Genotypes such as Shomal with Shastpar, Shiraz-1 with Shiraz-2 and Birjand with Khosf-1 had high similarity (0.93). Shahla with Yasuj and Shahla with Shiraz-1 also had high similarity (0.92). Shastpar with Shahla and with Shiraz-1 and Yasuj with Shiraz-1 had a similarity in 0.91. Shahla with Shiraz-2, Yasuj with Shiraz-2, Shiraz-2 with Kuchak-e-Atri, Birjand with Khosf-2, and Khosf-1 with Khosf-2 had a relatively high similarity (0.90). The lowest similarity between genotypes was belonged to Dutch genotype with other genotypes, so that the similarity coefficient of this genotype with Birjand (0.43), with Shahla (0.45) and with other genotypes was less than 0.50 (Table 7). In general, the similarity coefficients showed that the similarity between most genotypes except Dutch was more than 0.80 and there was a lot of similarity between genotypes

Examination of similarity matrix of ISSR showed that the highest similarity coefficients were obtained between Shomal and Yasuj genotypes (0.92), Shastopar with Shahla and Yasuj with Shiraz-1 (0.91) and Shomal with Shastopar, Shomal with Shiraz-1 and Yasuj with Shiraz-2 (0.90). The similarity between all genotypes except Dutch was relatively high and averaged above 0.80. The lowest

Table 6: Cophenetic correlation coefficient based on SIM, J, DICE, UNI in RAPD and ISSR

	RAPD			ISSR		
Cophenetic coefficients	UPGMA	Single	Complete	UPGMA	Single	Complete
SM	0.99**	0.98**	0.96**	0.96**	0.96**	0.65**
J	0.99**	0.97**	0.94**	0.95**	0.94**	0.91**
DICE	0.98**	0.98**	0.96**	0.96**	0.95**	0.93**
UN1	0.99**	0.98**	0.97**	0.97**	0.60**	0.96**

Table 7: Similarity matrix based on UN1 similarity coefficient for RAPD marker in different genotypes of narcissus

Genotypes	1	2	3	4	5	6	7	8	9	10	11	12
1- Shomal	1											
2-Shastpar	0.93	1										
3-Shahla	0.88	0.91	1									
4-Yasuj	0.87	0.87	0.92	1								
5-Shiraz-1	0.88	0.91	0.92	0.91	1							
6-Shiraz-2	0.87	0.88	0.90	0.90	0.93	1						
7- Kuchak-e-atri	0.83	0.83	0.86	0.84	0.89	0.90	1					
8-Dutch	0.49	0.49	0.45	0.49	0.48	0.47	0.50	1				
9-Birjand	0.81	0.83	0.83	0.82	0.83	0.82	0.84	0.43	1			
10-Khosf-1	0.81	0.80	0.86	0.83	0.84	0.84	0.82	0.49	0.93	1		
11-Khosf-2	0.80	0.82	0.87	0.88	0.84	0.85	0.85	0.47	0. 90	0.90	1	
12-Tabas	0.78	0.80	0.85	0.87	0.84	0.84	0.86	0.49	0.88	0.89	0.95	1

Table 8: Similarity matrix based on UN1 similarity coefficient for ISSR marker in different genotypes of narcissus

Genotypes	1	2	3	4	5	6	7	8	9	10	11	12
1- Shomal	1.00											
2-Shastpar	0.90	1.00										
3-Shahla	0.86	0.91	1.00									
4-Yasuj	0.92	0.88	0.87	1.00								
5-Shiraz-1	0.90	0.86	0.85	0.91	1.00							
6-Shiraz-2	0.83	0.79	0.82	0.90	0.87	1.00						
7- Kuchak-e-atri	0.84	0.80	0.77	0.86	0.87	0.85	1.00					
8-Dutch	0.51	0.60	0.59	0.51	0.50	0.47	0.48	1.00				
9-Birjand	0.85	0.79	0.78	0.87	0.86	0.79	0.80	0.50	1.00			
10-Khosf-1	0.85	0.79	0.82	0.87	0.84	0.84	0.82	0.44	0.87	1.00		
11-Khosf-2	0.87	0.79	0.67	0.82	0.86	0.86	0.80	0.53	0.83	0.84	1.00	
12-Tabas	0.84	0.83	0.83	0.84	0.87	0.83	0.84	0.46	0.83	0.88	0.88	1.00

similarity coefficient was related to the Dutch with others, so that the similarity coefficient between the Dutch with Yasuj, Shastpar, Shahla, Kouchak-e-Atri, Shiraz-1, Shiraz-2, Birjand, Khosf-1, Khosf-2 and Tabas were estimated as 0.51, 0.60, 0.59, 0.48, 0.50, 0.47, 0.50, 0.44, 0.53 and 0.46; respectively (Table 8). In general, it can be deduced from the similarity coefficients, there is a huge difference similarity between internal genotypes and internal and external genotypes and led to its diversity.

3.4 CLUSTER ANALYSIS AND PRINCIPAL COORDINATE ANALYSIS (PCOA)

Based on the RAPD marker, the genotypes were grouped into four clusters (Figure 2). The first cluster was divided into two sub-clusters. The first sub-cluster included Shomal and Shastpar. The second sub-cluster, which also had two subgroups, included Shahla, Yasuj, Shiraz-1 and Shiraz-2. The Kuchak-e-atri was the only genotype of the second cluster. In the third cluster, there were two sub-clusters that Birjand and Khosf-1 were under the first cluster and Khosf-2 and Tabas were under the second cluster. The Dutch genotype was placed independently in the fourth cluster, which shows a clear difference from others. Based on the ISSR marker (Figure 3), the dendrogram divided the genotypes into four clusters. The first cluster was divided into three sub-clusters. The first sub-cluster included Shomal and Yasuj genotypes, the second sub-cluster included Shiraz-1, and the third sub-cluster included Shastpar and Shahla. The second cluster included two genotypes of Shiraz-2 and Kuchak-e-Atri. The third cluster had three sub-clusters. The first sub-cluster included Birjand genotype, the second sub-cluster included Khosf-2 and the third sub-cluster included Khosf-1 and Tabas genotypes. The Dutch genotype independently formed the fourth cluster. This result is consistent with the results of Zanganeh and Salehi (2019) that a foreign genotype formed a separate group independent of the rest.

In general, several factors such as altering life cycles, environmental changes, climate change, and topography can affect the structure, diversity, and genetic similarity of different populations of a plant species in different ways (Sintayehu, 2018, Zhao et al., 2021). In addition, according to the principle of natural selection, plant populations in similar ecological conditions that grow in different geographical locations have more genetic similarities than plant populations grown in various ecological conditions. Our findings were in sequence with the principle, and the results showed a good relationship between genetic diversity and geographical diversity in most cases, and genetic diversity was followed by geographical diversity. The findings indicate a logical relationship between genetic diversity and geographical location. Similar results related to the relationship between geographical and climatic location with genetic diversity of different plant populations have been reported by other researchers using RAPD and ISSR markers (Gharibi et al., 2011, Abou El-Nasr et al., 2013).

In addition, the obtained results of RAPD marker indicated that the first three components explained 96.9 % of the total variance. In the meantime, the first, second, and third components were justified 87.6, 6.3, and 3.1 % of the total variance. The ISSR data also showed that the first three components explained 97.9 % of the total variance, in which the first component justified 89.3 %, the second component equal to 5.7 % and the third component equal to 2.9 % of the total variance.

In general, molecular markers allow detecting variations and polymorphisms for specific regions of DNA among individuals of a population, and the lower percentage of variance assigned to main factors indicates a more appropriate distribution of markers. It means that molecular markers have examined wider regions of the genome. Thus, they have targeted different parts of the genome that contain gene controlling various traits (Mohammadi & Prasanna, 2003, Marwal & Gaur, 2020). Therefore, it can be concluded that in the present study,

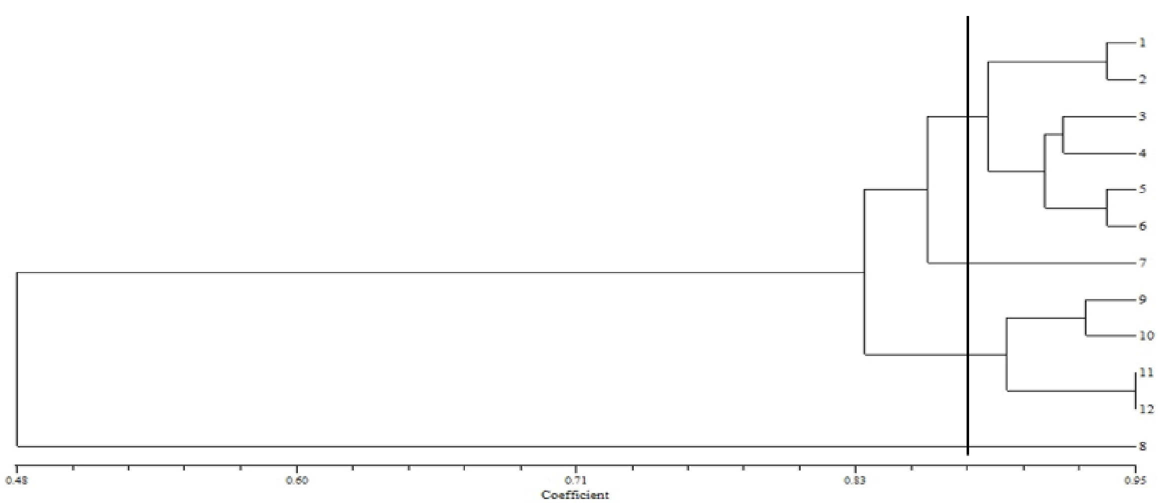

Figure 2: Clustering of Narcissus genotypes based on RAPD markers. 1: Shomal, 2: Shastpar, 3: Shahla, 4: Yasuj, 5: Shiraz-1, 6: Shiraz-2, 7: Kuchak-e-atri, 8: Dutch, 9: Birjand, 10: Khosf-1, 11: Khosf-2, 12: Tabas

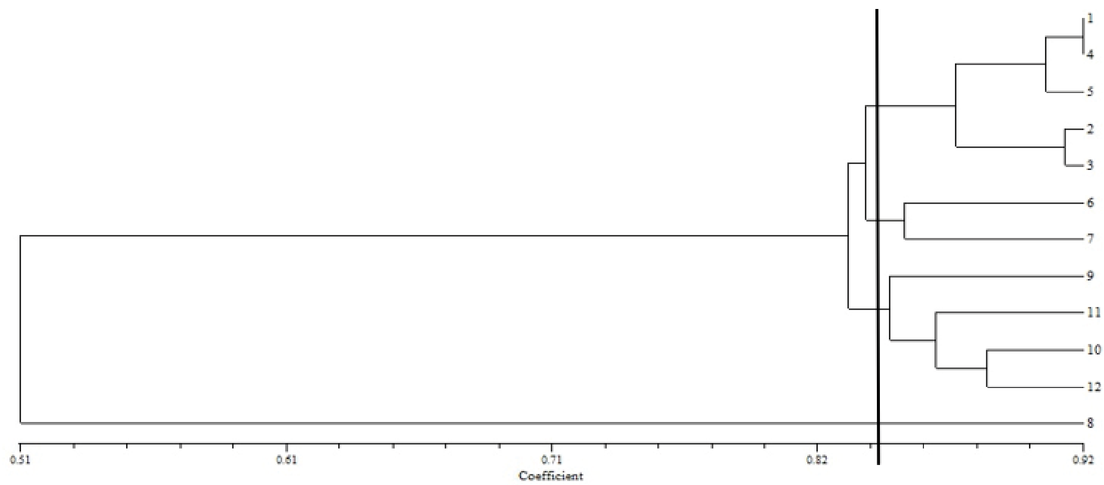

Figure 3: Clustering of Narcissus genotypes based on ISSR markers. 1: Shomal, 2: Shastpar, 3: Shahla, 4: Yasuj, 5: Shiraz-1, 6: Shiraz-2, 7: Kuchak-e-atri, 8: Dutch, 9: Birjand, 10: Khosf-1, 11: Khosf-2, 12: Tabas

RAPD and ISSR markers had good performance and were able to cover a large area of the genome. Hence, it can be concluded that the RAPD and ISSR markers examined in the present study had good performance and were able to cover a large area of the genome. These results are consistent with the results of Zanganeh and Salehi (2019).

4 CONCLUSIONS

In general, based on different coefficients of similarity between genotypes, placement of genotypes in different and distinct clusters and other information, it was been indicated the existence of genetic diversity between the studied genotypes. In most cases, there was a good relationship between genetic diversity and geographical diversity, therefore genetic diversity followed geographical diversity. The study of genetic diversity of narcissus genotypes using 27 RAPD primers and 11 ISSR primers showed that these markers can be useful in identifying polymorphic regions and managing germplasm and can be used to study and determine the genetic distance and differences between narcissus genotypes. The results obtained from RAPD marker were highly consistent with ISSR and conformed the genotypes in clusters according to a distinct pattern. In cluster analysis, placement of genotypes with short geographical distance in close clusters and even one cluster showed that genetic diversity is largely consistent with geographical diversity, and genotypes collected from one geographical area were placed in the same clusters and sub-clusters. Based on principal coordinate analysis, the first three components explained many variances. These results showed that these primers could not evaluate different parts of the sample genome and therefore it is necessary to use more and different primers in future studies.

5 ACKNOWLEDGEMENTS

We thereby appreciate officials of the molecular breeding laboratory of the Collage of Agriculture, University of Birjand, Birjand, Iran.

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doi:10.14720/aas.2023.119.1.2911 Original research article / izvirni znanstveni članek

Correlation and path coefficient analysis of yield and yield components of some Ethiopian faba bean (*Vicia faba* L.) accessions

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Received November 14, 2022; accepted February 16, 2023.
Delo je prispelo 14. novembra 2022, sprejeto 16. februarja 2023

Correlation and path coefficient analysis of yield and yield components of some Ethiopian faba bean (*Vicia faba* L.) accessions

Abstract: The knowledge of correlation and path coefficient analysis allow crop breeders to practice indirect selection to improve traits like grain yield which are complex in nature. The objectives of the present study were to measure association among yield and yield related traits and to identify important traits for indirect selection to improve faba bean grain yield. Eighty-one faba bean accessions were evaluated following 9 × 9 simple lattice design at one of the Bahir Dar University research sites at Mecha district in 2019 rainy cropping season. The result of correlation analysis revealed that grain yield had highly significant ($p < 0.01$) and positive phenotypic and genotypic correlations with plant height, pod length, number of pods per plant, number of branches per plant, biomass yield, 100-seed mass and harvest index indicating the possibility of simultaneous improvement of grain yield with these traits through selection. Path coefficient analysis demonstrated that higher positive direct effects were exerted by biomass yield and harvest index on grain yield both at phenotypic and genotypic levels, as a result, these traits could be used as indirect selection criteria to improve faba bean grain yield.

Key words: correlation; faba bean; indirect selection; path analysis; selection criteria

Korelacijska in usmerjene multipla regresijska analiza pridelka in njegovih komponent nekaterih etiopskih akcesij boba (*Vicia faba* L.)

Izvleček: Poznavanje korelacije in usmerjene multiple regresijske analize omogoča žlahtniteljem posreden izbor za izboljšanje lastnosti kot je pridelek zrnja, na katerega vplivajo v naravi številni dejavniki. Namen te raziskave je bil izmeriti povezave med pridelkom in z njim povezanimi lastnostmi in določiti pomembne lastnosti za posreden izbor izboljšanja pridelka zrnja pri bobu. Enainosemdeset akcesij boba je bilo ovrednotenih v 9 × 9 nepopolnem bločnem poskusu na raziskovalnem polju Bahir Dar University, v območju Mecha, v deževni dobi pridelovalne sezone 2019. Rezultati korelacijske analize so odkrili, da je pridelek zrnja visoko značilno ($p < 0.01$) in pozitivno feno-in genotipsko povezan z višino rastlin, dolžino strokov, številom strokov na rastlino, številom poganjkov na rastlino, biomaso, maso 100-semen. Usmerjena multipla regresijska analiza je pokazala, da so bili večji neposredni pozitivni učinki na pridelek zrnja izraženi s pridelkom biomase in žetvenim indeksom na feno- in genotipski ravni. Na osnovi teh rezultatov bi se te lastnosti lahko uporabile kot posredni selekcijski kriteriji za za izboljšanje pridelka zrnja pri bobu.

Ključne besede: korelacija; bob; posredna selekcija; usmerjena multipla regresijska analiza; selekcijski kriteriji

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1 INTRODUCTION

Faba bean (*Vicia faba* L.), is one of the earliest domesticated food legumes in the world (Singh et al., 2013). Faba bean is one of the most important legume crops and is believed to have originated in the Near East and cultivation started early in Neolithic times, 8000 B.C. (Cubero, 1974; Torres et al., 2006; Karkanis et al., 2018).

Ethiopia is the second largest producer of faba bean in the world after China (Mussa Jarso and Gemechu Keneni, 2006). According to CSA (2016), a faba bean is important pulse covering 3.56 % (about 443,966.09 hectares) of the grain crop area. The report also revealed that the production obtained from faba bean, was 3.18 % (about 848654.57 ton) of the grain production. Between 2007 and 2017 the area under faba bean cultivation was decreased from 520,519 to 437,106 ha (16.02 %) whereas the production and productivity during the corresponding period was increased from 0.69 to 0.92 million ton (25 %) and 1.323 to 2.109 t ha^{-1}(37.27 %), respectively (CSA, 2017). Faba bean has a versatile use and it is an important source of dietary protein to the majority of population in Ethiopia (Mussa Jarso and Fasil Assefa, 2014), while its dry seeds, green haulm and dry straw are used as animal fodder. Faba bean seeds contain proteins, carbohydrates, vitamin B, antioxidants and minerals. Protein content in different varieties varies from 26 % to 41 % (Picard, 1977). Carbohydrate contents varies from 51 % to 68 %, of which major proportion is contributed by starch (41–53 %) (Cerning et al., 1975).

Studies on genotypic and phenotypic correlations among traits of crop plants are useful in planning, evaluating and setting selection criteria for the desired traits in breeding programs (Johanson et al., 1955). Correlations between different traits of crops may arise either from genotypic or environmental factors. Environmental correlations arise from the effect of environmental factors that vary at different environments. Correlations due to genetic causes are mainly pleiotropic effects and linkage between genes affecting different traits (Falconer and Mackay, 1996). When more traits are considered in correlation studies, correlations of traits become more complex; therefore, correlation study followed by path analysis will help to identify yield attributing traits.

Path analysis provides precise picture of character associations for formulating efficient selection strategy. It differs from simple correlation in that it points out the causes and their relative importance, whereas the later measures the mutual association ignoring the causation. The concept of path coefficient was developed by Wright (1921) and it was first used for plant selection by Dewey and Lu (1959). Path coefficient analysis is a standardized partial regression which measures the direct and indirect contribution of independent traits on a dependent trait. Hence, it measures the influence of a trait up on another; and permits the separation of the correlation coefficient into components of direct and indirect effects (Dewey and Lu, 1959). Therefore, the present study was conducted to assess association among yield and yield related traits and to identify important traits for indirect selection to improve faba bean grain yield.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL SITE, MATERIALS AND DESIGN

The experiment was conducted at one of Bahir Dar University research sites in Mecha district of West Gojjam zone of Amhara region, Ethiopia in 2019 main cropping season. Mecha is located about 525 km Northeast of Addis Ababa and 34 km Southwest of Bahir Dar. It is located at latitude of 10°30’ N and longitude of 37°29’ E. It receives annual rainfall of 1572 mm. The mean temperature ranges between 24 °C and 27 °C; and the altitude is 2009 m. a. s. l.

The faba bean genotypes used in the present study include seventy-eight accessions obtained from Ethiopian Biodiversity Institute, two standard checks obtained from Adet Agricultural Research Center, and a local check from local source; 81 in total (Table 1).

The experimental design used was 9 × 9 simple lattice. Each accession was planted on two-row plot. Spacing between rows and between plants were 40 cm and 10 cm, respectively. The plot size was 0.8 m x 1 m (0.8 m^2). Spacing between blocks was 1 meter.

2.2 DATA COLLECTION AND ANALYSES

2.2.1 Data collection

Depending on the nature of traits data were collected on plot and plant basis. Plant height (cm), pod length (cm), number of pods per plant, number of branches were collected on plant basis for which mean of five plants from each plot was used for analysis. Days to 50 % flowering, grain yield (g/plot), 100-seed mass (g), biomass yield (g/plot), harvest index (%), diseases score were gathered on plot basis. Diseases score include the incidence of ascochyta blight, chocolate spot and rust diseases. Each disease was scored using 1-9 score method (where 1 refers highly resistance and 9 indicate highly susceptible) according to Bernier et al. (1993).

Table 1: List of the 81 faba bean accessions used in the study

Number	Accession	Collection Region	District	Number	Accession	Collection Region	District
1	212565	Amhara	Siyadebrina Wayu	42	235709	SNNP	Dirashe Special
2	27279	Amhara	Machakel	43	219089	Oromiya	Amigna
3	25299	SNNP	Angacha	44	25338	SNNP	Meskanena Mareko
4	245140	SNNP	Dila Zuria	45	215748	Amhara	Dessie Zuria
5	212566	Oromiya	Wuchalena Jido	46	213211	Oromiya	Tiro Afeta
6	25006	Amhara	Hulet Ej Enese	47	25336	SNNP	Meskanena Mareko
7	212568	Amhara	Siyadebrina Wayu	48	226125	Amhara	Legambo
8	25018	Oromiya	Wuchalena Jido	49	240497	SNNP	Decha
9	27052	Oromiya	Kofele	50	25346	SNNP	Meskanena Mareko
10	25274	Oromiya	Becho	51	25328	SNNP	Selti
11	229303	Amhara	Lay Betna Tach Bet	52	213214	Oromiya	Limu Seka
12	212567	Amhara	Siyadebrina Wayu	53	25335	SNNP	Meskanena Mareko
13	25298	SNNP	Angacha	54	25340	SNNP	Meskanena Mareko
14	219355	Oromiya	Adolana Wadera	55	25339	SNNP	Meskanena Mareko
15	208085	Amhara	Weremo Wajetuna Mida	56	215129	Amhara	Mama Midrina Lalo
16	212811	Amhara	Wegera	57	215128	Amhara	Mama Midrina Lalo
17	203105	Oromiya	Dedesa	58	228607	Amhara	Goncha Siso Enese
18	235955	Amhara	Debark	59	25337	SNNP	Meskanena Mareko
19	25279	SNNP	Cheha	60	25341	SNNP	Meskanena Mareko
20	208114	Amhara	Weremo Wajetuna Mida	61	25331	SNNP	Selti
21	229310	Amhara	Weremo Wajetuna Mida	62	235433	Tigray	Kola Temben
22	212572	Amhara	Weremo Wajetuna Mida	63	Tumsa		
23	25003	Oromiya	Kuyu	64	25325	SNNP	Selti
24	25323	SNNP	Selti	65	25329	SNNP	Selti
25	25022	Oromiya	Kuyu	66	25309	SNNP	Angacha
26	25280	SNNP	Cheha	67	25304	SNNP	Angacha
27	235956	Amhara	Debark	68	25330	SNNP	Selti
28	220079	Tigray	Adwa	69	25307	SNNP	Angacha
29	212576	Amhara	Lay Betna Tach Bet	70	25334	SNNP	Selti
30	212575	Amhara	Lay Betna Tach Bet	71	25306	SNNP	Angacha
31	25277	SNNP	Cheha	72	25311	SNNP	Angacha
32	220076	Tigray	Adwa	73	25332	SNNP	Sodo
33	27290	Oromiya	Jimma Arjo	74	25310	SNNP	Angacha
34	25264	Oromiya	Becho	75	25333	SNNP	Selti
35	25292	SNNP	Limo	76	25302	SNNP	Angacha
36	25270	Oromiya	Becho	77	25327	SNNP	Selti
37	212580	Amhara	Mama Midrina Lalo	78	Dosha		
38	25017	Amhara	Enarj Enawga	79	25303	SNNP	Angacha
39	212578	Amhara	Geramidirna Keya	80	25301	SNNP	Angacha
40	25010	Oromiya	Gerar Jarso	81	Local		
41	25290	SNNP	Limo				

2.2.2 Correlation analysis

Phenotypic correlation, the observable correlation between variables, which is the sum of genotypic and environmental effects were calculated from variance and covariance components using the formula of Miller et al. (1958) as follows:

Genotypic correlation= $\sigma_g xy / \sqrt{(\sigma^2_g x\, \sigma^2_g y)}$

Phenotypic correlation= $\sigma_p xy / \sqrt{(\sigma^2_p x\, \sigma^2_p y)}$

Where $\sigma_{p\,xy}$ = phenotypic covariance between character x and y, σ^2_p x = phenotypic variance for character x, σ^2_p y = phenotypic variance for character y, $\sigma_{g\,xy}$ = genotypic covariance between characters x and y, σ^2_g x = genotypic variance for character x, and σ^2_g y = genotypic variance for character y

The significance of phenotypic correlation coefficients was tested by the formula of Singh and Chaudhary (1985): $t' = r_p xy \sqrt{(n-2 / 1-r_p^2 xy)}$

The computed t' value was tested against the tabulated t-value at n-2 degree of freedom where n was the number of genotypes studied; whereas the correlation coefficient at genotypic level was tested for significance using the formula proposed by Robertson (1959).

$$t = \frac{rgxy}{SE\ rgxy}$$

The calculated 't' value was compared with 't' tabulated at (n-2) degree of freedom at 1 % and 5 % levels of significances.

Where

$$SE_{r_{gxy}} = \sqrt{\frac{(1-r^2)^2}{2H_x.H_y}}$$

Where SE$r_g$xy is the standard error of genotypic correlation coefficient; Hx and Hy are heritability for traits x and y, respectively.

2.2.3 Path coefficient analysis

Path coefficient analysis is a tool to partition the observed correlation coefficient into direct and indirect effects of yield components on grain yield. Based on correlation, path coefficient which refers to the direct and indirect effects of the yield attributing traits (independent trait) on grain yield (dependent trait) were calculated as per Dewey and Lu (1959) as follows:

$rij = Pij + \Sigma\ rikpkj$

Where rij = mutual association between the independent character (i) and dependent character (j) as measured by the genotypic correlation coefficients. Pij = direct effects of the independent character (i) on the dependent variable (j) as measured by the genotypic path coefficients, and Σrikpkj = Summation of components of indirect effects of a given independent character (i) on a given dependent character (j) via all other independent characters (k).

Residual factor (R^2), the contribution of the remaining unknown factor was estimated using Singh and Chaudhury (1985) method:

$R^2 = \sqrt{(1 - \Sigma Pij\ rij)}$

3 RESULTS AND DISCUSSION

Mean squares of the 14 traits from analysis of variance (ANOVA) as presented below in Table 2 highly significant ($p < 0.01$) differences among accessions were observed for all traits except days to maturity and number of seeds per pod. This highly significant difference indicates the existence of variability among accessions for traits studied. The presence of variability in the accessions offers an opportunity in improvement of yield and its contributing traits through selection in faba bean. Similar results were obtained by Gemechu Keneni et al. (2005) in 160 faba bean accessions.

3.1 CORRELATION AMONG TRAITS

The interrelationship among the 12 traits was estimated through correlation coefficient at genotypic and phenotypic levels are presented in Table 2.

3.2 PHENOTYPIC CORRELATION OF GRAIN YIELD WITH OTHER TRAITS

The result of correlation analysis revealed that grain yield had highly significant ($p < 0.01$) and positive phenotypic correlations with plant height, pod length, number of pods per plant, number of branches per plant, biomass yield, 100-seed mass and harvest index. These results indicate that accessions with high plant height, pod length, number of pods per plant, number of branches per plant, biomass yield, 100-seed mass and harvest index produce high grain yield and vice versa. Therefore, these traits emerged as most important associates of grain yield in faba bean. The results suggested that selection of these traits may have good impact on yield improvement. Similarly, Lal (2019) reported that grain yield had highly significant and positive phenotypic correlations with plant height, number of pods per plant, number of branches per plant, biomass yield, 100-seed mass and harvest index. Kumar et al. (2013) reported highly significant and positive correlation of seed yield with number of branches per plant, number of pods per plant, biomass yield and

Table 2: Analysis of variance for 14 traits of eighty-one faba bean accessions

Traits	Mean square				CV (%)	R^2	R.E. to RCBD
	Rep (DF = 1)	Accessions (DF = 80)	Rep x Block (16)	Error (DF = 64)			
DF	0.10^{ns}	18.57**	3.02^{ns}	2.21	3.30	0.92	101.87
DM	43.56^{ns}	25.29^{ns}	28.53^{ns}	18.28	3.64	0.68	103.76
PH	365.70^{ns}	230.18**	212.36**	109.68	10.75	0.77	108.26
PL	2.57**	0.53**	0.24^{ns}	0.19	11.74	0.80	101.12
NPP	1.03^{ns}	45.44**	7.25^{ns}	6.74	22.36	0.89	100.10
NSP	1.81**	0.17^{ns}	0.09^{ns}	0.12	12.32	0.68	95.91
NBP	0.00^{ns}	0.61**	0.26*	0.17	24.16	0.81	103.24
BY	24966.00^{ns}	9567533.60**	817978.00^{ns}	691793.00	10.23	0.95	100.55
GY	31441.00^{ns}	810072.84**	10553.00^{ns}	14290.00	5.72	0.99	94.77
HSM	6.97^{ns}	173.27**	47.64*	28.96	12.93	0.89	104.69
HI	3.21^{ns}	106.81**	16.36^{ns}	12.71	14.44	0.90	101.23
AB	12.20^{ns}	532.02**	0.04^{ns}	6.19	7.07	0.99	97.23
CS	3.05^{ns}	547.10**	0.33^{ns}	24.49	15.07	0.96	104.92
RT	76.22*	590.28**	0.12^{ns}	14.48	14.30	0.98	100.00

NOTE: ** = Highly significant ($p < 0.01$), * = Significant ($p < 0.05$), ns = Non-significant, CV = Coefficient of variation, DF = days to flowering, DM = days to maturity, PH = plant height(cm), PL = pod length, NPP = number of pods per plant, NSP = number of seeds per pod, NBP = number of branches per plant, BY = biomass yield, GY = grain yield, HSM = 100 seed mass, HI = harvest index, AB = ascochyta blight, CS = chocolate spot, RT = rust disease, R.E to RCBD = relative efficiency to RCBD

harvest index. Verma et al. (2015) also found highly significant and positive correlation of seed yield with harvest index and biomass yield.

Grain yield had highly significant ($p < 0.01$) and negative phenotypic correlations with ascochyta blight, chocolate spot and rust disease scores. These results indicate that as disease severity increase grain yield becomes highly reduced. In addition, grain yield had significant ($p < 0.05$) and negative phenotypic correlations with days to flowering. This result showed that the early flowering accessions have high yield potential than those of late flowering ones, indicating the scope for developing short-cycle varieties. The finding agrees with that of Nchimbi and Mduruma (2007) who reported negative association of days to flowering with grain yield in common bean.

3.3 PHENOTYPIC CORRELATION AMONG OTHER TRAITS

Rust disease score showed highly significant ($p < 0.01$) and positive correlations with ascochyta blight and chocolate spot, indicating that diseases are complementary to each other. Rust disease had highly significant negative correlations with plant height, pod length, number of pods per plant, number of branches per plant, biomass yield and100 seed mass. This indicates that rust is negatively associated with these traits. Chocolate spot had highly significant negative correlations with plant height, pod length, number of pods per plant, biomass yield and100 seed mass. Ascochyta blight had highly significant and negative correlations with plant height, pod length, biomass yield and100 seed mass and significant negative correlations with number of pods per plant. Generally, the results suggested that disease related traits were complementary to each other and negatively affected growth and yield related traits.

Harvest index showed highly significant ($p < 0.01$) positive phenotypic correlations with number of pods per plant. This showed that accessions with high number of pods per plant producing high harvest index. However, harvest index had highly significant negative phenotypic correlations with biomass yield and then significant negative phenotypic correlations with days to flowering. Negative correlations arise due to competition among traits for common precursors which are having restricted supply (Madhur and Jinks, 1994). Hundred-seed mass had highly significant ($p < 0.01$) positive phenotypic correlations with plant height, pod length, biomass yield and significant ($p < 0.05$) positive phenotypic correlations with number of pods per plant. Days to flowering had high significant negative phenotypic correlations with 100-seed mass. Similarly, Alghamdi (2007) and Mulualem et al. (2013) reported a positive and significant phenotypic correlation between pod length and thousand-seed mass. Similar results were also obtained by Lal (2019) that hundred-seed mass had highly significant positive phenotypic correlations with pod length and biomass yield.

Biomass yield had highly significant ($p < 0.01$) positive phenotypic correlations with plant height, pod length, number of pods per plant and number of branches per plant. These traits had positive correlation with biomass yield which augurs well for providing correlated response during selection for improving biomass yield. These observations of positive associations between biomass yield and plant height, pod length, number of pods per plant and number of branches per plant are in agreement with the reports made previously (Kumar et al., 2013; Singh et al., 2015; Verma et al., 2015; Kumar et al., 2017) on faba bean. Similar results were obtained by Lal (2019) that biomass yield had highly significant positive phenotypic correlations with plant height, number of pods per plant and number of branches per plant.

Positive and highly significant ($p < 0.01$) phenotypic correlation was recorded between number of branches per plant with days to flowering, plant height, pod length, number of pods per plant. Similarly, Lal (2019) obtained number of branches per plant had highly significant and positive phenotypic correlations with plant height and number of pods per plant.

Number of pods per plant showed highly significant and positive correlation with plant height and pod length. Azarpour et al. (2012), Sharifi (2014) and Abdalla et al. (2015) reported positive and significant phenotypic correlation of number of pods per plant with plant height. Pod length shows highly significant ($p < 0.01$) positive phenotypic correlations with plant height. Plant height negatively correlated with days to flowering.

3.4 GENOTYPIC CORRELATION OF GRAIN YIELD WITH OTHER TRAITS

In the present study, grain yield had highly significant ($p < 0.01$) and positive genotypic correlations with plant height, pod length, number of pods per plant, number of branches per plant, biomass yield, 100-seed mass and harvest index. These results indicated that accessions with high plant height, pod length, number of pods per plant, number of branches per plant, biomass yield, 100-seed mass and harvest index produce high grain yield. Singh et al. (2017) reported that seed yield had highly significant and positive genotypic correlations with number of pods per plant, 100-seed mass and harvest index. Similar result was reported by Zakira et al. (2010) for harvest index in field pea. Grain yield had highly significant ($p < 0.01$) and negative genotypic correlations with

Table 3: Estimates of phenotypic (above diagonal) and genotypic (below diagonal) correlation coefficients of 12 traits of 81 faba bean accessions

Traits	DF	PH	PL	NPP	NBP	BY	GY	HSM	HI	AB	CS	RT
DF	1	-0.198*	-0.066^{ns}	-0.089^{ns}	0.293**	0.016^{ns}	-0.179*	-0.248**	-0.192*	0.072^{ns}	0.132^{ns}	0.102^{ns}
PH	-0.178^{ns}	1	0.542**	0.588**	0.333**	0.456**	0.576**	0.491**	0.111^{ns}	-0.206**	-0.412**	-0.398**
PL	-0.071^{ns}	0.560**	1	0.287**	0.280**	0.390**	0.360**	0.709**	-0.026^{ns}	-0.239**	-0.289**	-0.263**
NPP	-0.078^{ns}	0.628**	0.271*	1	0.446**	0.518**	0.755**	0.175*	0.228**	-0.186*	-0.348**	-0.394**
NBP	0.381**	0.369**	0.312**	0.454**	1	0.229**	0.336**	0.109^{ns}	0.077^{ns}	-0.028ns	-0.092^{ns}	-0.248**
BY	0.026^{ns}	0.524**	0.438**	0.538**	0.238*	1	0.562**	0.396**	-0.458**	-0.244**	-0.505**	-0.561**
GY	-0.190^{ns}	0.680**	0.407**	0.789**	0.374**	0.579**	1	0.352**	0.418**	-0.281**	-0.483**	-0.413**
HSM	-0.252*	0.510**	0.789**	0.136^{ns}	0.061^{ns}	0.418**	0.362**	1	-0.009^{ns}	-0.301**	-0.411**	-0.263**
HI	-0.227*	0.173^{ns}	-0.007^{ns}	0.272*	0.108^{ns}	-0.427**	0.438**	-0.009^{ns}	1	0.003^{ns}	-0.032^{ns}	0.189*
AB	0.075^{ns}	-0.247*	-0.289**	-0.206^{ns}	-0.041^{ns}	-0.255*	-0.286**	-0.329**	0.004^{ns}	1	0.339**	0.332**
CS	0.142^{ns}	-0.516**	-0.373**	-0.373**	-0.116^{ns}	-0.514**	-0.499**	-0.458**	-0.060^{ns}	0.348**	1	0.413**
RT	0.105^{ns}	-0.495**	-0.332**	-0.429**	-0.294**	-0.578**	-0.422**	-0.283**	0.199^{ns}	0.338**	0.428**	1

** = Highly significant ($p < 0.01$), * = Significant ($p < 0.05$), ns = Non significant, DF = days to flowering, PH = plant height, PL = pod length, NPP = number of pods per plant, NBP = number of branches per plant, BY = biomass yield, GY = grain yield, HSM = 100-seed mass, HI = harvest index, AB = ascochyta blight, CS = chocolate spot, RT = rust disease score ascochyta blight, chocolate spot and rust disease scores. This indicates disease plays a vital role in reduction of grain yield.

3.5 GENOTYPIC CORRELATION AMONG OTHER TRAITS

Days to flowering were positively and highly significantly ($p < 0.01$) correlated with number of branches per plant. The result shows late flowering accessions are expected to produce high number of branches per plant. Days to flowering negatively correlated with 100-seed mass and harvest index. This result showed that the late flowering accessions have less yield potential than those of early flowering ones. These findings are in concurrence with Alghamdi (2007), Tofiq et al. (2016). Kumar et al. (2020) also reported negative correlation of days to flowering with 100-seed mass.

Plant height had positive and highly significant ($p < 0.01$) genotypic correlation with pod length, number pods per plant, number of branches per plant, biomass yield and 100-seed mass. These indicate accessions that have high plant height also had high pod length, number of pods per plant, number of branches per plant, biomass yield and 100-seed mass. Results by Lal (2019) revealed that number of branches per plant and biomass yield had positive and highly significant correlation with plant height in faba bean. Plant height had negative and highly significant ($p < 0.01$) correlation with chocolate spot and rust disease scores, and negatively correlated with ascochyta blight. This indicates disease plays a vital role in reduction of plant height.

Pod length had positive and highly significant ($p < 0.01$) correlation with number of branches per plant, biomass yield and 100-seed mass and positive significant correlation with number of pods per plant. In harmony with the present finding, Lal (2019) reported positive and highly significant correlation of pod length with 100-seed mass. Pod length also had negative and highly significant correlation with ascochyta blight, chocolate spot and rust disease scores. Number of pods per plant had positive and highly significant ($p < 0.01$) correlation with number of branches per plant, biomass yield and positive and significant correlation with harvest index. Improving these traits increases number of pods per plant that support to increases grain yield. This result agrees with Singh et al. (2017) who reported number of pods per plant had positive and highly significant correlation with number of branches per plant and harvest index. Similar result also reported by Fikreselassie and Soboka (2012). Number of pods per plant had negative and highly significant ($p < 0.01$) correlation with chocolate spot and rust disease scores. Number of branches per plant had positive and significant correlation with biomass yield and negative highly significant correlation with rust disease score.

Biomass yield had positive and highly significant ($p < 0.01$) genotypic correlation with 100-seed mass. This indicates that breeding for increased biomass yield might result in bold seeds. This implies that the trait has positive impact on faba bean improvement. Biomass yield had negatively significant ($p < 0.01$) genotypic correlation with harvest index, chocolate spot and rust disease scores and negative significant correlation with ascochyta blight. This implies that these traits inversely correlated with biomass yield. Hundred-seed mass had negative and highly significant correlation with ascochyta blight and chocolate spot diseases but negative significant correlation with rust. These indicate that hundred-seed mass negatively affected by disease and leads to low yield. Ascochyta blight had positive and highly significant correlation with chocolate spot and rust disease scores and also chocolate spot had positive and highly significant correlation with rust disease. This result indicates the occurrence of one disease simply favores another.

3.6 PATH COEFFICIENT ANALYSES

Path coefficient analyses separated the phenotypic and the genotypic correlation coefficients into the corresponding direct and indirect effects. The phenotypic and genotypic direct and indirect effects of different traits on grain yield are presented in Table 3 and 4, respectively.

3.6.1 Phenotypic path coefficients

In the present study, the path coefficient analysis was carried out by using phenotypic correlation coefficients among twelve traits. Plant height, pod length, number of pods per plant, number of branches per plant, biomass yield and harvest index had positive direct effects on grain yield. Higher positive direct effects on grain yield, however, were exerted by biomass yield (0.856) and harvest index (0.778) (Table 3). The results indicate biomass yield and harvest-index are most important traits to which emphasis should be given during simultaneous selection aimed at improving grain yield in faba bean. These results are in close association with previous workers (Bora et al., 1998; Kumar et al., 2013 Lal, 2019) for biomass yield and harvest-index in faba bean.

Biomass yield exerted considerable positive indirect effects on grain yield via number of pods per plant, plant height, pod length and 100-seed mass. Harvest index exhibited high positive indirect effects on grain yield via number of pods per plant and plant height. These results suggested the importance of these traits in selection for the improvement of faba bean grain yield. However, biomass yield exerted considerable negative indirect effects on grain yield via harvest index chocolate spot and rust disease scores and harvest index exhibited high order of negative indirect effects on grain yield via biomass yield. Similarly, Lal (2019) observed biomass yield exerted considerable positive indirect effects on grain yield via number of pods per plant, number of branches per plant and plant height. Harvest index exhibited high order of positive indirect effects on grain yield via number of pods per plant and biomass yield on faba bean. In addition to the important direct contributions, biomass yield and harvest index had considerable positive indirect effects via different traits, implying that biomass yield and harvest index are important traits to be considered during devising selection strategy aimed at developing high yielding varieties in faba bean. Ulukan et al. (2003) and Tadesse et al. (2011) also observed the highest positive direct effect of number of pods per plant together with plant height. Kumar et al. (2017) also reported that the number of branches per plant, number of pods per plant and pod length exhibited positive direct effects on yield. On the contrary, days to flowering, 100-seed mass, ascochyta blight and rust disease scores had negative direct effects on grain yield. Tofiq et al. (2016) also reported negative direct effect of 100-seeds mass on yield.

The magnitude of residual effect (0.297) indicated that the traits included in the study accounted for most of the variability present in grain yield, indicating that the contribution of traits considered was 70.3 % and the rest 29.7 % was the contribution of other traits which were not considered in the present study.

3.6.2 Genotypic path coefficients

The high positive direct effects on grain yield were exerted by biomass yield (0.844) and harvest index (0.756) (Table 4). Thus, biomass yield and harvest-index emerged as most important yield components on which emphasis should be given during simultaneous selection aimed at improving grain yield in faba bean. These results are in close agreement with those of Bora et al. (1998) and Kumar et al. (2013). Similar results also obtained by Lal (2019). The direct effects of the remaining traits were low to be considered important. Biomass yield exerted considerable positive indirect effects on grain yield via number of pods per plant, plant height, pod length and 100-seed mass but biomass yield exerted considerable negative indirect effects on grain yield via harvest index, chocolate spot and rust disease scores. Harvest index exhibited high order of positive indirect effects on grain yield via number of pods per plant and plant height but harvest index exhibited high order of negative indirect effects on grain yield via biomass yield. Similar results were obtained by Lal (2019) for biomass yield and harvest index. In addition to their very high positive direct effects on grain yield, biomass yield and harvest index, having considerable positive indirect effects via different traits, also appeared as most important indirect yield components.

In the present study, path analysis identified bio-

Table 4: Phenotypic direct (bold and diagonal) and indirect effects of 11 traits on grain yield of 81 faba bean accessions

Traits	DF	PH	PL	NPP	NBP	BY	HSM	HI	AB	CS	RT
DF	-0.052	-0.007	-0.001	-0.008	0.013	0.014	0.011	-0.149	-0.005	0.006	-0.002
PH	0.010	0.034	0.006	0.053	0.014	0.390	-0.022	0.087	0.013	-0.019	0.008
PL	0.003	0.019	0.011	0.026	0.012	0.333	-0.032	-0.020	0.016	-0.013	0.005
NPP	0.005	0.020	0.003	0.090	0.019	0.443	-0.008	0.178	0.012	-0.016	0.008
NBP	-0.015	0.011	0.003	0.040	0.043	0.196	-0.005	0.060	0.002	-0.004	0.005
BY	-0.001	0.016	0.004	0.047	0.010	0.856	-0.018	-0.356	0.016	-0.023	0.012
HSM	0.013	0.017	0.008	0.016	0.005	0.339	-0.045	-0.007	0.020	-0.019	0.005
HI	0.010	0.004	0.000	0.021	0.003	-0.392	0.000	0.778	0.000	-0.001	-0.004
AB	-0.004	-0.007	-0.003	-0.017	-0.001	-0.209	0.014	0.003	-0.065	0.015	-0.007
CS	-0.007	-0.014	-0.003	-0.031	-0.004	-0.432	0.018	-0.025	-0.022	0.045	-0.009
RT	-0.005	-0.014	-0.003	-0.035	-0.011	-0.480	0.012	0.147	-0.022	0.019	-0.021

Residual factor = 0.297. DF = days to flowering, PH = plant height, PL = pod length, NPP = number of pods per plant, NBP = number of branches per plant, BY = biomass yield, HSM = 100 seed mass, HI = harvest index, AB = ascochyta blight, CS = chocolate spot, and RT = rust disease score

Table 5: Genotypic direct (bold and diagonal) and indirect effects of 11 traits on grain yield for 81 faba bean accessions

Traits	DF	PH	PL	NPP	NBP	BY	HSM	HI	AB	CS	RT
DF	-0.061	-0.011	0.001	-0.005	0.026	0.022	0.008	-0.172	-0.005	0.009	-0.002
PH	0.011	0.061	-0.006	0.042	0.025	0.442	-0.017	0.131	0.017	-0.034	0.008
PL	0.004	0.034	-0.010	0.018	0.021	0.370	-0.026	-0.005	0.020	-0.024	0.006
NPP	0.005	0.038	-0.003	0.066	0.031	0.454	-0.004	0.206	0.014	-0.024	0.007
NBP	-0.023	0.022	-0.003	0.030	0.067	0.201	-0.002	0.082	0.003	-0.008	0.005
BY	-0.002	0.032	-0.004	0.036	0.016	0.844	-0.014	-0.323	0.017	-0.034	0.010
HSM	0.015	0.031	-0.008	0.009	0.004	0.353	-0.032	-0.007	0.023	-0.030	0.005
HI	0.014	0.010	0.000	0.018	0.007	-0.361	0.000	0.756	0.000	-0.004	-0.003
AB	-0.005	-0.015	0.003	-0.014	-0.003	-0.215	0.011	0.003	-0.068	0.023	-0.006
CS	-0.009	-0.031	0.004	-0.025	-0.008	-0.434	0.015	-0.046	-0.024	0.066	-0.007
RT	-0.006	-0.030	0.003	-0.029	-0.020	-0.488	0.009	0.150	-0.023	0.028	-0.017

Residual factor = 0.297. DF = days to flowering, PH = plant height, PL = pod length, NPP = number of pods per plant, NBP = number of branches per plant, BY = biomass yield, HSM = 100 seed mass, HI = harvest index, AB = ascochyta blight, CS = chocolate spot, and RT = rust disease score

mass yield followed by harvest-index as most important direct as well as indirect yield contributing traits. These indicate that both traits had true association with grain yield and their importance in determining this complex trait. Therefore, important consideration should be given while practicing selection aimed at the improvement of grain yield in faba bean. These results were in accordance with the result of Jivani et al. (2013) who reported the highest direct effects of harvest index and biomass yield on grain yield in chickpea. Plant height, number of pods per plant and number of branches per plant had positive direct effects on grain yield at genotypic level but days to flowering, pod length, 100-seed mass, ascochyta blight and rust disease scores had negative direct effects on grain yield.

The residual effect shows how much the explanatory variables represent the variability of the dependent variable (Singh and Chaudhary, 1985). The residual effect at the genotypic path coefficient analysis was 0.272; as a result, the studied traits explained 72.8 % of the variability in the seed yield and show that few traits were not consider which are related to grain yield.

Residual factor = 0.272. DF = days to flowering, PH = plant height, PL = pod length, NPP = number of pods per plant, NBP = number of branches per plant, BY = biomass yield, HSM = 100 seed mass, HI = harvest index, AB = ascochyta blight, CS = chocolate spot, RT = rust disease.

4 CONCLUSION

Grain yield had highly significant ($p < 0.01$) and positive phenotypic and genotypic correlation with plant height, pod length, number of pods per plant, number of branches per plant, biomass yield, 100-seed mass and harvest index. These results indicated the possibility of simultaneous improvement of grain yield with these traits through selection. Path coefficient analysis showed that harvest index and biomass yield had the highest direct effects on yield both at phenotypic and genotypic levels, indicating the importance of these traits for indirect selection of faba bean accessions for improvement of grain yield.

5 ACKNOWLEDGEMENTS

I would like to thank Bahir Dar University for providing me experimental plot for the field research. I would also like to thank Ethiopian Biodiversity Institute and Adet Agricultural Research Center for the provision of accessions and genotypes for the study.

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doi:10.14720/aas.2023.119.1.2777 Original research article / izvirni znanstveni članek

Agroecological zones influence maize infestation and damage severity by the fall armyworm (*Spodoptera frugiperda* [J. E. Smith, 1797]) in southwestern Nigeria

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Received July 21, 2022; accepted February 20, 2023.
Delo je prispelo 21. julija 2022, sprejeto 20. februarja 2023

Agroecological zones influence maize infestation and damage severity by the fall armyworm (*Spodoptera frugiperda* [J. E. Smith, 1797]) in southwestern Nigeria

Abstract: The fall armyworm (*Spodoptera frugiperda*) is an invasive and highly destructive insect pest that has caused extensive damage to maize in Africa since its first report on the continent in 2016. Information on fall armyworm infestation and damage within African agroecologies is essential for the development of appropriate pest management strategies, but these are scant in Nigeria. Consequently, in this study, fall armyworm infestation levels and severity of damage to maize in the three major maize-growing agro-ecological zones (humid forest, derived savanna, and southern guinea savanna) of southwestern Nigeria was investigated using standard field sampling protocols. Results showed that maize infestation and damage severity varied across agroecological zones, with the humid forest being the most impacted. Information provided will enhance decision-making for effective management of the fall armyworm in southwestern Nigeria.

Key words: agroecology; foliar damage; larval infestation; *Spodoptera frugiperda*; farm sampling; humid forest; derived savanna; guinea savanna

Vpliv agroekoloških območij na okužbo in velikost poškodb koruze zaradi ameriške koruzne sovke (*Spodoptera frugiperda* [J. E. Smith, 1797]) v jugozadni Nigeriji

Izvleček: Ameriška koruzna sovka (*Spodoptera frugiperda*) je invazivna in zelo škodljiva žuželka, ki povzroča obsežne poškodbe koruze v Afriki od njenega prvega pojava na kontinentu leta 2016. Poznavanje okužb in poškodb zaradi te sovke v različnih agroekoloških območjih Afrike je bistveno za razvoj primernih strategij upravljanja, a to vedenje je zelo nezadostno v Nigeriji. V tej raziskavi sta bili s standardnimi metodami vzorčenja preučevani raven okužbe in jakost poškodb zaradi ameriške koruzne sovke v treh glavnih agroekoloških conah jugozahodne Nigerije in sicer v območju vlažnih gozdov, v prehodni savani in južni gvinejski savani. Rezultati so pokazali, da sta se raven okužbe in velikost poškodb koruze razlikovali v teh agroekoloških območjih s tem, da je bila koruza na območju vlažnih gozdov najbolj prizadeta. Pridobljeni podatki bodo pospešili sprejemanje odločitev za učinkovito upravljanje z ameriško koruzno sovko v jugozahodni Nigeriji.

Ključne besede: agroekologija; poškodbe listov; okužba z ličinkami; *Spodoptera frugiperda*; vzorčenje na kmetijah; vlažen gozd; prehodna savana; gvinejska savana

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1 INTRODUCTION

The fall armyworm, (*Spodoptera frugiperda* [J. E. Smith, 1797], Lepidoptera: Noctuidae), **is a**n invasive moth with its origin in the Americas (Sparks, 1979; Liu et al., 2020). It is a highly destructive insect pest of crops (Murúa et al., 2009) that was first reported on the African continent in 2016 (Goergen et al., 2016). The fall armyworm is polyphagous and is known to attack more than 350 plant species spread across 76 plant families (Montezano et al., 2018). In Africa, however, maize (*Zea mays* L.) is its primary host and the most damaged crop on the continent. In addition to causing extensive damage to maize leaves and whorls (CABI, 2020), fall armyworm larvae may feed on reproductive organs like tassels and kernels causing yield losses (Midega et al., 2018; Prasanna et al., 2018). According to ICIPE (2020), maize damage by the fall armyworm has caused yield losses of between 8 – 20 million tonnes in Africa. The fall armyworm thus poses an on-going regional threat to the cultivation of maize – a major staple food to millions of families in sub-Saharan Africa (Prasanna et al., 2018).

Due to their peculiarities, agroecologies in African countries are expected to favour the occurrence, proliferation and development of fall armyworms (Day et al., 2017; Huesing et al., 2018; Chimweta et al., 2019). Consequently, studies that investigate maize infestation and damage by the fall armyworm in different African agroecologies must be undertaken if effective management strategies will be developed for the pest on the continent. The southwestern region of Nigeria, for instance, comprise three major maize-growing agroecological zones namely – humid forest, derived savanna, and southern guinea savanna zones (Onyeka et al., 2008; Olaniyan, 2015). However, information on the similarities or differences in fall armyworm infestations levels and severity of damage to maize in these maize-growing agroecological zones is scarce and remains unclear. This study was therefore carried out to investigate the influence of agrocology on maize infestation and damage severity by fall armyworm larvae in southwestern Nigeria. The specific objective of the study was to compare fall armyworm larval infestation and foliar damage severity on maize plants in the humid forest, derived savanna, and southern guinea agroecological zones of southwestern Nigeria.

2 MATERIALS AND METHODS

2.1 DESCRIPTION OF THE STUDY AREA

On-farm assessment of fall armyworm infestation and severity of damage to maize was conducted in the southwestern region of Nigeria which comprise six

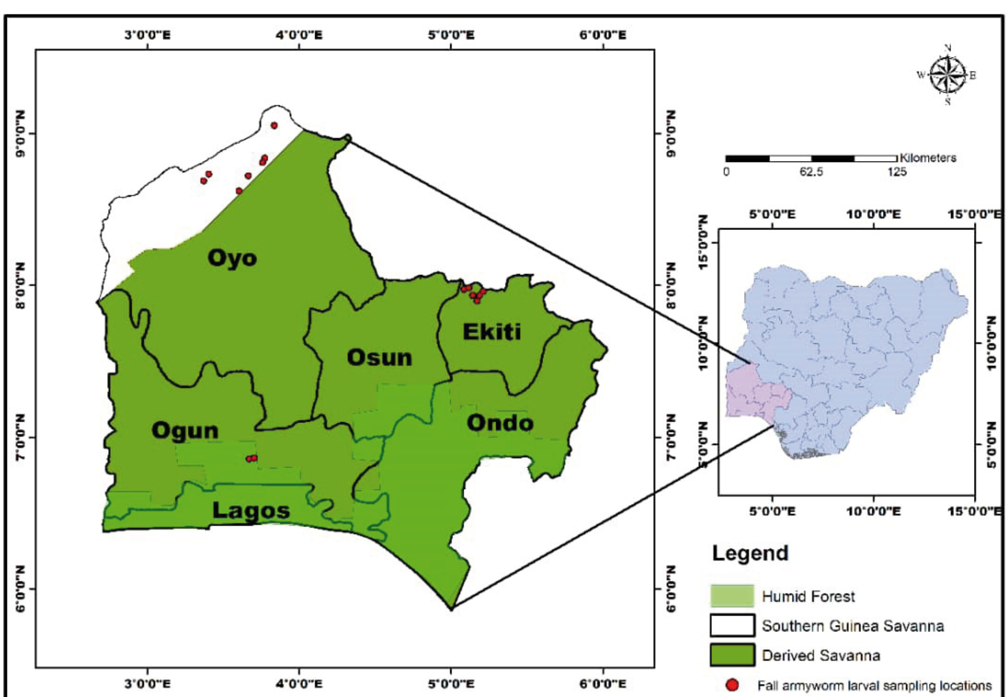

Fig. 1: Map of southwestern Nigeria showing the political states, agroecological zones and sampling location of fall armyworm larvae

geopolitical states – Lagos, Ogun, Ondo, Ekiti, Oyo and Osun states (Fig. 1). The humid forest agroecological zone in the region spreads across Lagos, Ondo, Ogun, and Osun states and is known to experience a relatively longer annual rainfall of at least 2000 mm (Oyenuga, 1967). On the other hand, the southern guinea savanna agroecological zone is characterized by an average annual rainfall of 1051.7 mm (Oyenuga, 1967), and occurs only in the northwestern part of Oyo state. The derived savanna transitional zone is the largest agroecological zone in southwestern Nigeria. It is reported to receive an average annual rainfall of 1314 mm (Sowunmi & Akintola, 2010), and can be found in all the states except in Lagos. All three agroecological zones experience a bimodal pattern of rainfall that peaks yearly in June and September (Aderolu et al., 2013).

2.2 SAMPLING OF MAIZE FARMS

In each of the three major maize-growing agroecological zones, 50 % of existing local government areas (LGA) was purposively sampled (Table 1). Thereafter, two towns were randomly sampled in each LGA. Finally, a maize farm was selected for assessment in each town. Farms selected for fall armyworm infestation and damage assessment were owned by smallholders and typically between 1–5 ha in size; were cultivated solely to maize; had only plants between two and four weeks old; and had not been sprayed with insecticides. Information on plant age and insecticide application was obtained from farm owners through resident agricultural extension officers working in each LGA. In all, a total of 18 farms (four in the humid zone; six in the derived savanna zone and eight in the southern guinea savanna zone) were sampled in the study area in July 2019 (Table 1).

2.3 ASSESSMENT OF FALL ARMYWORM INFESTATION AND DAMAGE TO MAIZE

On each selected maize farm, 20 maize plants were randomly selected for assessment using the ‘W’ sampling method described by McGrath et al. (2018). The method comprise the random sampling of four plants each at five different locations on the farm (away from the border) while following a ‘W’ pattern of movement. All sampled plants were assessed for the presence or absence of fall armyworm larval infestation by gently turning the leaves and carefully unfurling whorls. Plants with one or more

Table 1: Location of farms in southwestern Nigeria sampled for on-farm assessment of maize infestation and damage by the fall armyworm

Agroecological zone	Local Government Area	Town	Geolocation Information
Humid Forest	Sagamu	Sagamu	6°51’16”N 3°40’13”E
		Sagamu	6°51’19”N 3°40’17”E
	Ikenne	Ikenne	6°51’43”N 3°42’10”E
		Ikenne	6°51’46”N 3°42’13”E
Derived Savanna	Ilejemeje	Ewu Ekiti	7°55’49”N 5°11’16”E
		Ijesamodu Ekiti	7°57’39”N 5°12’40”E
	Moba	Osun Ekiti	7°58’15”N 5°05’12”E
		Otun Ekiti	7°58’49”N 5°07’02”E
	Ido-Osi	Aiyetoro Ekiti	7°56’01”N 5°08’32”E
		Usi Ekiti	7°53’55”N 5°10’07”E
Southern Guinea Savanna	Saki West	Saki	8°44’12”N 3°24’11”E
		Saki	8°41’19”N 3°22’11”E
	Saki East	Ago-Amodu	8°38’16”N 3°39’26”E
		Sepeteri	8°37’24”N 3°36’13”E
	Irepo	Igboho	8°50’29”N 3°46’21”E
		Igboho	8°48’37”N 3°45’33”E
	Orelope	Kisi	9°03’12”N 3°50’11”E
		Kisi	9°03’12”N 3°50’03”E

actively feeding larva were taken as infested, and allocated a score of one (1). On the other hand, plants without larval infestation were scored zero (0). Characteristic larval foliar feeding damage symptoms on plants (whether or not infested with larvae) was visually assessed and scored based on severity using the five-point rating scale described by Dal Pogetto et al. (2012) for fall armyworm damage to field maize. Based on the scale, plants without damage were scored 0; plants with erasure leaves were scored 1; plants with pin holes or shot holes due to larval feeding were scored 2; plants with significant number of holes and some whorl damage were scored 3; plants with the whorl completely eaten off or destroyed were scored 4; and a score of 5 was awarded to dead plants**.**

2.4 DATA ANALYSIS

The number of plants infested with fall armyworm on each farm was converted to percentages. Percentage infestation and damage severity data were then summarized with means in Microsoft Excel (Microsoft Office Excel, 2019). Thereafter, data on percentage fall armyworm infestation and foliar damage severity scores recorded in each agroecology and LGA were submitted to a one-way Analysis of Variance (ANOVA) test using a Generalized Linear Model. Where necessary, means were separated using the Tukey's Honestly Significant Difference (HSD) test at 5% level of significance in IBM SPSS statistics software (2011).

3 RESULTS

3.1 FALL ARMYWORM INFESTATION ON MAIZE

Infestation of fall armyworm larvae on maize was highest (86.25 ± 3.88 %) in the humid forest and lowest (56.88 ± 3.93 %) in the southern guinea savanna agroecological zones (Fig. 2). The derived savanna zone, however, had an intermediate level of infestation (71.67 ± 4.13 %). Infestation level of fall armyworm larvae in the humid forest was significantly higher ($p < 0.05$) than in the southern guinea savanna. Similarly, infestation was observed, to varying degrees, in all LGA where maize farms were sampled and assessed (Fig. 3). The top three LGA with high fall armyworm infestation were Ikenne in the humid forest zone (92.5 ± 4.22 %), Ido-Osi in the derived savanna zone (85.0 ± 5.72 %), and Sagamu in the humid forest zone (80.0 ± 6.41 %). In contrast, the lowest infestation levels were recorded in Orelope (47.5 ± 8.00 %); Saki West LGA (50.0 ± 8.01 %), and Irepo (55.0 ± 7.97 %) all in the southern guinea savanna agroecological zone. Significant differences ($p < 0.05$) were observed between the LGA with highest and lowest larval infestation levels.

3.2 FALL ARMYWORM DAMAGE SEVERITY ON MAIZE

Foliar damage ratings were higher (2.63 ± 0.14) in the humid forest agroecological zone, with most maize

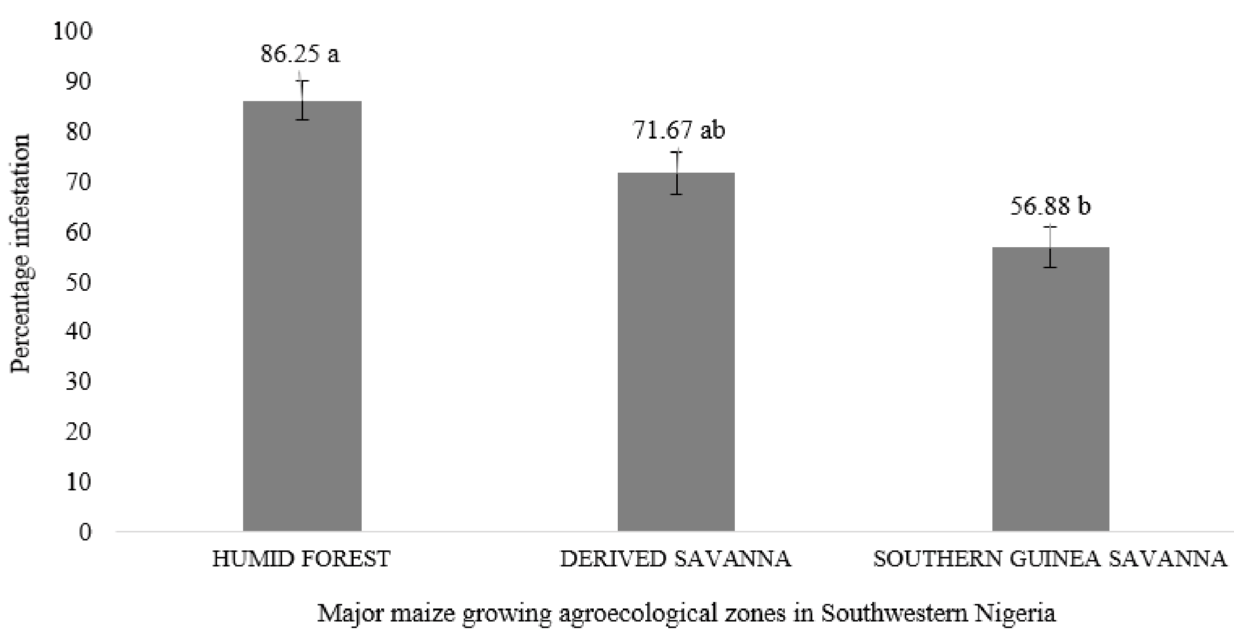

Figure 2: Fall armyworm infestation on maize in the major maize-growing agroecological zones of southwestern Nigeria. Mean values on bars followed by the same letter are not significantly different at $p = 0.05$

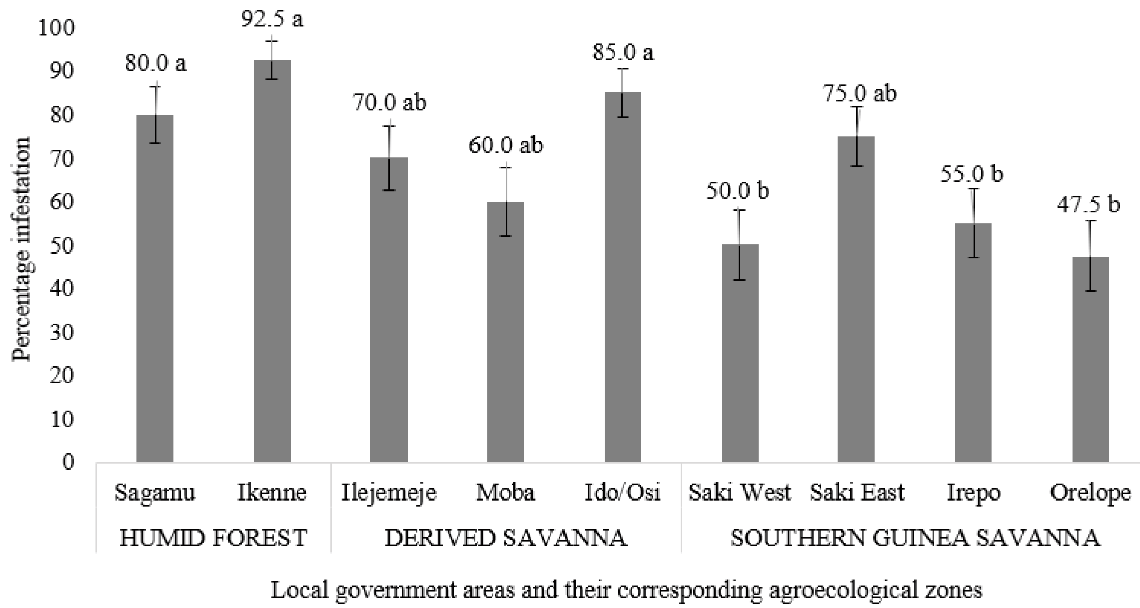

Figure 3: Fall armyworm infestation on maize at representative local government areas in the major maize-growing agroecological zones of southwestern Nigeria. Mean values on bars, in any of the three agroecological zones, followed by the same letter are not significantly different at $p = 0.05$

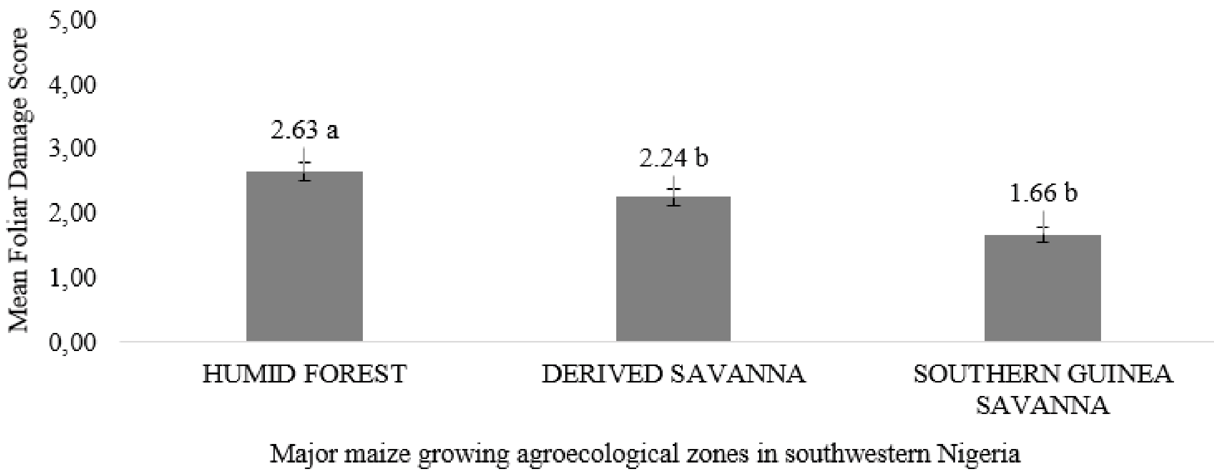

Figure 4: Fall armyworm foliar damage severity on maize in the major maize-growing agroecological zones of southwestern Nigeria. Mean values on bars followed by the same letter are not significantly different at $p = 0.05$

plants having larval feeding holes in leaves and whorls (Fig. 4). On the other hand, foliar damage ratings of 2.24 ± 0.14 and 1.66 ± 0.12 were respectively recorded in the derived savanna and southern guinea savanna zones, indicating the presence of relatively lower fall armyworm damage to plants. Foliar damage was significantly more severe ($p < 0.05$) in the humid forest zone than in the derived savanna or southern guinea savanna zones. In contrast, no significant difference ($p > 0.05$) was observed in the severity of foliar damage recorded between the derived savanna and southern guinea savanna agroecological zones. With respect to foliar damage severity ratings in LGA, maize plants were more severely damaged ($p < 0.05$) at Ikenne (3.05 ± 0.18) and Ido-Osi (2.78 ± 0.21) than in other seven LGA (Fig. 5).

4 DISCUSSION

Many countries in Africa have agroecological conditions that are expected to favour the occurrence, development, and damage severity of fall armyworms (Day

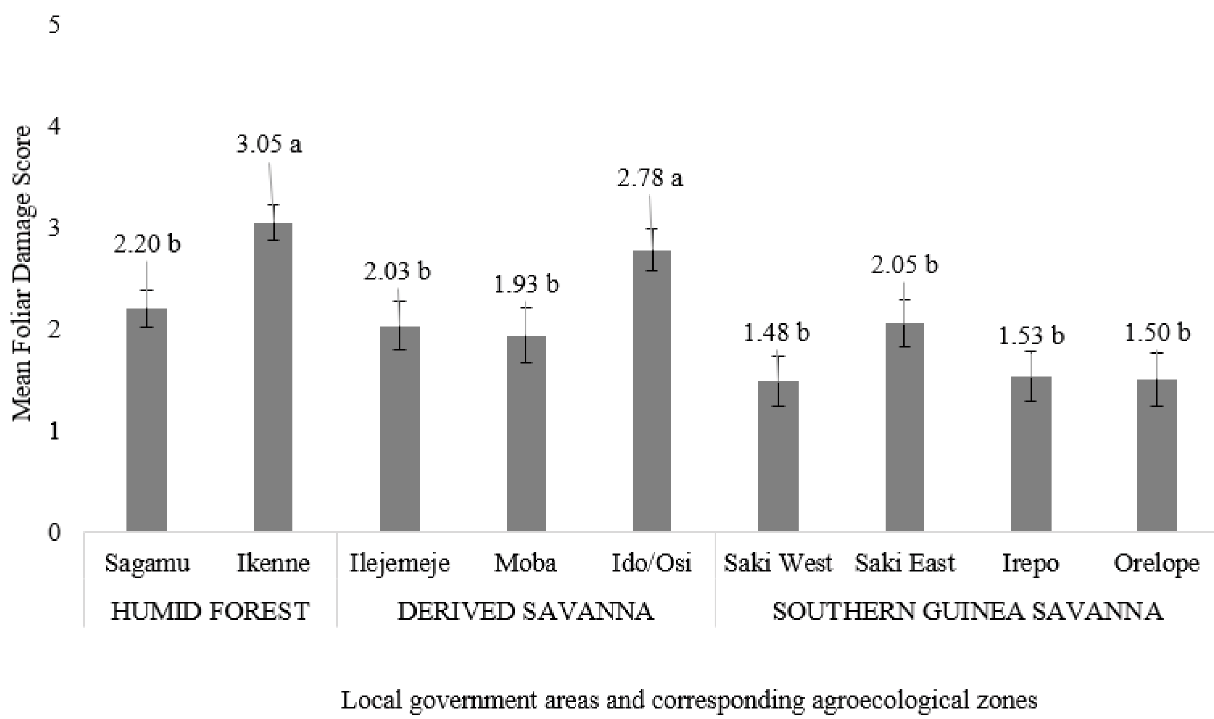

Figure 5: Fall armyworm foliar damage severity on maize at representative local government areas in the major maize-growing agroecological zones of southwestern Nigeria. Mean values on bars, in any of the three agroecological zones, followed by the same letter are not significantly different at $p = 0.05$

et al., 2017; Huesing et al., 2018; Chimweta et al., 2019), necessitating agroecology-based studies on the continent. In the present study, fall armyworm larval infestations increased southwards, that is, from the southern guinea savanna to the humid forest agroecological zone in southwestern Nigeria. In other words, larval infestation was generally higher and foliar damage more severe in the humid forest than in the derived or southern guinea savanna agroecological zones. This is in line with the findings of Odeyemi et al. (2020) who also reported higher fall armyworm damage severity in the humid forest zone than in the derived savanna zone in southwestern Nigeria. The humid forest zone of southwestern Nigeria experiences stable rains as early as March or April, enhancing early and higher maize cultivation. Thus, in a typical year, the humid forest receives up to 1000 mm more rainfall than the southern guinea savanna (Oyenuga, 1967). According to Chimweta et al. (2019), agroecological zones with abundant rainfall and high maize cultivation support multiple and overlapping cropping of maize, which in turn make host plants available all season for fall armyworm. De Groote et al. (2020) and Mutyambai et al. (2022) also reported that hot and wet weather conditions as well as presence of two growing seasons in the coastal lowland agroecological zone of Kenya enhance fall armyworm infestation and damage to on-farm maize compared to the high altitude highland zone of the country. Findings in the present study suggest that the humid forest agroecological zone in southwestern Nigeria favours more maize infestation and damage by the fall armyworm than any of the other two agroecological zones assessed. Nevertheless, it is apparent that all three agroecologies are suitable for fall armyworm reproduction and development.

Apart from weather conditions and number of maize cropping seasons, fall armyworm infestation and severity of damage to on-farm maize in an agroecology may be influenced by other factors like plant growth stage, cropping system, soil type, maize variety type, weeding frequency, and land tillage practice (Koffi et al., 2020; Mutyambai et al., 2022; Ojumoola et al. 2022).

The considerable level of fall armyworm larval infestation and foliar damage observed in the different agroecologies in the present study may be due to the fact that all the maize plants assessed were at the early vegetative growth phase and not more than four weeks old. Fall armyworm larvae are capable of inflicting extensive damage to maize reproductive parts including tassels, silks, and kernels (Midega et al., 2018; Chimweta et al., 2019; Odeyemi et al., 2020). Nevertheless, they are primarily known to be defoliators that tatter and fill leaves, whorls and stems with holes and wet frass, especially between the second and sixth week after planting (Prasanna et al., 2019; CABI, 2020; Odeyemi et al., 2020).

Cropping methods such as intercropping that increase the diversity of plant species on farmer's fields have been posited to be effective and sustainable fall armyworm management tactics in maize systems (FAO, 2018). Intercropping is known to reduce the infestation and damage caused by insects pests like thrips (Trdan et al., 2006) and stem weevils (Cadoux et al., 2015) by disrupting their ability to detect the visual and olfactory cues of host plants (Finch and Collier, 2012), or by increasing the diversity and abundance of natural enemies (FAO, 2018). In Uganda, Hailu et al. (2018) reported significantly lower fall armyworm infestation in maize intercropped with common beans, soybeans, or groundnuts compared with sole maize, especially in the early and late vegetative growth stages. In the present study, maize was planted as a sole crop on all the farms assessed. This might also explain why considerable infestation and damage were observed in all the three maize-growing agroecological zones.

Unlike in more compact soils, loose sandy soils enhance successful soil pupation of fall armyworms, and by extension, higher infestations and damage of the maize crops growing in them (Sims, 2008; Mutyambai et al., 2022). Furthermore, due to differences in morphology and constitutive phytochemical compounds, different maize varieties often have different resistance and tolerance levels to fall armyworm infestation and damage (Morales et al 2021; Ojumoola et al. 2022). In addition, frequent weeding using mechanical methods reduce fall armyworm infestation and damage by destroying the soil dwelling pupa stage and the shelter or food sources provided by reservoir weed hosts (Hay-Roe et al., 2016; Moraes et al., 2020; Mutyambai et al., 2022). Similarly, land tillage practices like conservation tillage or zero tillage, which cause little to no disturbance to the soil, promote higher populations of generalist predators of the immature stages of fall armyworm thus reducing seasonal infestations and damage of maize (Clark et al., 1993; Landis et al., 2000; Rivers et al., 2016; Baudron et al., 2019).

Nevertheless, the potential of the foregoing agronomic factors in reducing or preventing fall armyworm infestation and damage on maize in the humid forest, guinea savanna and southern guinea savanna agroecologies of southwestern Nigeria will require further investigations.

5 CONCLUSIONS

Fall armyworm larval infestation and foliar damage to maize has been shown in this study to be more prevalent in the humid forest agroecological zone than in the guinea savanna or southern guinea savanna agroecological zones of southwestern Nigeria. Notwithstanding, maize plants in all three major maize-growing agroecological zones are susceptible to fall armyworm attack and damage. The study recommends that further studies be conducted to develop suitable agroecology-specific management strategies for fall armyworm in southwestern Nigeria.

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doi:10.14720/aas.2023.119.1.2787 Original research article / izvirni znanstveni članek

Preliminary assessment of genetic diversity between *Glebionis coronaria* and *G. discolor* (Asteraceae) by AFLP markers

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Received July 25, 2022; accepted February 21, 2023.
Delo je prispelo 25. julija 2022, sprejeto 21. februarja 2023

Preliminary assessment of genetic diversity between *Glebionis coronaria* and *G. discolor* (Asteraceae) by AFLP markers

Abstract: *Glebionis coronaria* is a valuable and medicinal herb native of Mediterranean region. Recently, *G. coronaria* var. *discolor* has been elevated to the rank of species as *G. discolor* (d'Urv.) Cano based on morphological characteristics, distinguishing it from *G. coronaria* var. *coronaria* (=*G. coronaria*). To investigate the genetic basis of this diversity, AFLP markers were applied to genotypes of *G. discolor* and *G. coronaria* sampled in three different Mediterranean regions (Italy, Spain, and Portugal). Our results showed that among 1347 fragments identified with five primer combinations 99.55 % were polymorphic. The genetic distance and the Shannon Index values suggested that the two species can be genetically distinguished, but further studies are needed to confirm this hypothesis.

Key words: biodiversity; Compositae; *Chrysanthemum*; daisy; garland chrysanthemum; taxonomy

Preliminarno ovrednotenje genetske raznolikosti med vrstama *Glebionis coronaria* and *G. discolor* (Asteraceae) z AFLP markerji

Izvleček: Vrsta *Glebionis coronaria* je cenjeno zdravilno zelišče, samoniklo na območju Mediterana. V zadjem času je bila različica *G. coronaria* var. *discolor* dvignjena na rang vrste kot vrsta *G. discolor* (d'Urv.) Cano na osnovi morfoloških lastnosti, po katerih se razlikuje od vrste *G. coronaria* var. *coronaria* (=*G. coronaria*). Za preučitev genetske osnove te raznolikosti so bili uporabljeni AFLP markerji za analizo genotipov vrst *G. discolor* in *G. coronaria* vzorčenih na različnih območjih Mediterana (Italija, Španija in Portugalska). Rezultati so pokazali, da je bilo med 1347 fragmenti, identificiranih s kombinacijami petih primerjev 99,55 % polimorfnih. Genetska razdalja in vrednosti Shannonovega indeksa nakazujejo, da sta vrsti genetsko ločeni, a so potrebne nadaljne raziskave za potrditev te hipoteze.

Ključne besede: biodiversiteta; Compositae; *Chrysanthemum*; ivanjščica; užitna ivanjščica; taksonomija

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1 INTRODUCTION

Asteraceae is one of the worlds' richest and the most diverse plant families in biological and ecological terms (Cano et al., 2020). This angiosperm family comprises 1,739 genera including 36,033 species (Hassler, 2021), taxonomically continuously updated. Several of them have various uses around the world, due to their chemical composition for medicinal and food purposes (Mezhoud et al., 2012; Saoud et al., 2019; Pace et al., 2020; Nkuimi Wandjou et al., 2020; Singh et al., 2020; Bhat et al., 2021; Sicari et al., 2021). Among all, *Glebionis coronaria* (L.) Cass. ex Spach (synonym: *Chrysanthemum coronarium* L.) is an annual plant, frequent in ruderal vegetation, in field margins, road verges, and urban wastelands, widely distributed in the Mediterranean basin, Western Africa and Asia (da Silva et al., 2005). In the last few years there has been a growing interest in this species due to its biological activities, such as insecticidal, antifungal, cancer prevention, antioxidant and anti-inflammatory (Yildirim et al., 2018; Khareba et al., 2021). Historically, based on the different colour and morphology of the flowers, d'Urville (1822) distinguished two varieties of this species: *Chrysanthemum coronarium* var. *concolor* d'Urv. (= *G. coronaria* var. *coronaria*) and *Chrysanthemum coronarium* var. *discolor* d'Urv. (= *G. coronaria* var. *discolor* (d'Urv.) Turland). Furthermore, a recent study demonstrated that *G. coronaria* var. *discolor* can be separated and elevated to the rank of species, based on the disposition of the intercostal glands, the size of the disc cypsela wings and bioclimatological traits, as *Glebionis discolor* (d'Urv.) Cano, Musarella, Cano-Ortiz, Piñar Fuentes, Spamp. et Pinto Gomes (Cano et al., 2017). These authors documented that *G. coronaria* has totally yellow ray florets and intercostal glands aligned, while *G. discolor* has white ray florets on a yellow base and intercostal glands arranged randomly. Another feature highlighted by Cano et al. (2017) concerns the distribution of these two species in the Mediterranean basin: indeed, *G. coronaria* is distributed mainly throughout the thermo-Mediterranean bioclimatic belt, while *G. discolor* is spread also in the meso-Mediterranean one. Few other authors phenotypically recognize and/or report *G. discolor*, such as Cueto et al. (2018), Bartolucci et al. (2018), Portal to the Flora of Italy (2022) and POWO (2022). Among them, the last three sources endorse that *G. discolor* is a doubtful taxon or is a synonym of *G. coronaria*, contrary to Cano et al. (2017). In this contest, DNA-based markers (also named molecular markers) represent a powerful tool to fingerprint unequivocally the identity of these species. Indeed, in the last decades, they have been successfully used for investigation of interspecific and intraspecific genetic variability in various plants (Carputo et al., 2013; Villano et al., 2014, 2022, 2023). Among the *plethora* of molecular markers available, the best choice for *Glebionis* spp. can be represented by AFLP (Amplified Fragment Length Polymorphism) markers, due to the absence of a reference genome. Their main advantage is the employment of a standard protocol in combination with different restriction endonucleases to achieve optimal fingerprints without prior knowledge of the organism's genome sequence. These markers have been pioneered by Vos et al. (1995) and have been used in various species, such as *Dioscorea* spp. (Rivera-Jiménez et al., 2011) and *Gynerium sagittatum* (Aubl.) P.Beauv. (Rivera-Jiménez et al., 2008). This paper aims to analyse the genetic variability between *G. coronaria* and *G. discolor* using combinations of AFLP markers.

2 MATERIAL AND METHODS

2.1 PLANT MATERIAL AND DNA EXTRACTION

Three samples of *G. coronaria* and three of *G. discolor* (Figure 1) were collected in Italy, Spain, and Portugal in three biological replicates and stored at the herbarium of the Mediterranean University of Reggio Calabria (REGGIO) (acronym follows Thiers, 2023), as below detailed according to the original labels:

Italy:

1) *G. coronaria* - SIC Fiumara di Melito P.S. (Reggio Calabria). 03/05/2018. Collectors: C.M.Musarella & G.Spampinato.

2) *G. discolor* - Spiaggia di Palizzi Marina, Palizzi (Reggio Calabria). 14/05/2018. Collectors: C.M.Musarella & G.Spampinato.

Spain:

1) *G. coronaria* - Near Urbanización Salobreña (Granada). Alt. 13 m asl. 30S0448140/4064980. 05/05/2018. Collectors: E.Cano, A.Cano-Ortiz & J.C.Piñar Fuentes.

2) *G. discolor* - Near Hotel Soto, Andujar (Jaén). Alt. 220 m asl. 30S0405061/4209759. 06/05/2018. Collectors: E.Cano & A.Cano-Ortiz.

Portugal:

1) *G. coronaria* - Faro. 20/05/2018. Collector: R. Quinto Canas

2) *G. discolor* - Tavira, Pedras del Rey. 15/06/2018. Collector: R. Quinto Canas.

The samples were processed using the DNeasy Plant Mini Kit (Qiagen) previously described by Tengel et al. (2001). The quantity and quality of the isolated gDNA were measured using the NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE) and Qubit 2.0 fluorometer (Life Technologies, Carlsbad, CA).

Figure 1: Capitula of (left) *Glebionis coronaria* (L.) Cass. ex Spach (Pentidattilo, Reggio Calabria, Italy – April 18, 2022) and (right) *G. discolor* (d'Urv.) Cano, Musarella, Cano-Ortiz, Piñar Fuentes, Spamp. et Pinto Gomes (Sevilla, Spain – March 28, 2013). Ph. C.M.Musarella

2.2 AFLP ANALYSIS

The analysis was performed using the method described by Vos et al. (1995) and the commercially available AFLP kit and protocol (Gibco-BRL AFLP analysis System I, Life Technologies, Gaithersburg, MD), which employs EcoRI and MseI as restriction enzymes. For selective amplification, five combinations of primers were used (E-AGG + M-CAG; E-AGC + M-CAC; E-AGG + M-CTT; E-AGC + M-CAC; E-ACT + M-CAG) with the E primer labelled with FAM or HEX. Amplicons were separated with the ABI PRISM® 3130 DNA Analyzer system (Life Technologies, Carlsbad, California, USA). Size calibration was performed with the molecular weight ladder GenScan® 500 ROXTM Size Standard (Life Technologies, Carlsbad, California, USA). AFLP fragments were detected and scored using Peak Scanner® software (Applied Biosystems, Foster) and combined into a binary matrix. Three technical and three biological replicates were considered.

2.3 DATA AND PHYLOGENETIC ANALYSIS

The statistical software Genalex 6.5 (Peakall & Smouse, 2006) was used for data analysis. The input file was created considering each band as one diallelic locus (1 means presence of band, 0 means absence of band). Per each species were calculated the observed number of alleles (Na), the number of total bands and number of bands unique, the effective number of alleles (Ne), the Shannon's information index (I) and the percentage of polymorphic loci (P %). To visualize interspecies and individuals' relationships, a principal coordinates analysis (PCoA) was performed (Nei & Li, 1979).

3 RESULTS AND DISCUSSION

Five selective AFLP primer combinations generated a total of 1347 fragments, distributed between 50 and 500 bp, of which 1341 (99.55 %) were polymorphic (Table 1). The combinations E-AGG/M-CAG and E-AGG/M-CTT were the most informative ones with 248 and 274 polymorphic bands, respectively (Table 1). Two-hundred seventy-two species-specific bands were identified in *G. discolor* and 224 in *G. coronaria* (Table 1).

The genetic variation was measured within the two species considering the number of effective and different alleles (named Ne and Na, respectively), the Shannon's information index (I), the percentage of polymorphic loci (P %) and the number of species-specific bands, called private bands (Table 2). Among all, the Shannon's indexes in *G. coronaria* and *G. discolor* species were 0.369 and 0.353, respectively. The Na values were 1.398 in *G. coronaria* and 1.341 in *G. discolor* while the Ne values were 1.414 in *G. coronaria* and 1.398 in *G. discolor*. The percentage of polymorphic loci showed that the most

Table 1: Results of AFLP analyses used to differentiate *Glebionis coronaria* (L.) Cass. ex Spach and *G. discolor* (d'Urv.) Cano, Musarella, Cano-Ortiz, Piñar Fuentes, Spamp. et Pinto Gomes genotypes

AFLP combinations	Total bands	Polymorphic bands, n°	Polymorphic bands, %	Specie-specific bands, n°	
				G. coronaria	*G. discolor*
E-AGG/M-CAG	249	248	99.60	61	53
E-AGC/M-CAC	310	308	99.35	21	45
E-AGG/M-CTT	275	274	99.64	51	54
E-AGC/M-CAC	327	325	99.39	30	74
E-ACT/M-CAG	186	186	100.00	61	46

Table 2: Statistical analysis of *Glebionis coronaria* (L.) Cass. ex Spach and *G. discolor* (d'Urv.) Cano, Musarella, Cano-Ortiz, Piñar Fuentes, Spamp. et Pinto Gomes. Na = No. of different alleles; Ne = No. of effective alleles; I = Shannon's information index; P % = percentage of polymorphic loci; No. Private Bands = No. of bands unique to a single species

Species	Na	Ne	I	P %	No. Private Bands
G. coronaria	1.398	1.414	0.369	67.12%	359
G. discolor	1.341	1.398	0.353	63.90%	315

Table 3: Nei's Original Measures of genetic distance among samples of *Glebionis coronaria* (L.) Cass. ex Spach and *G. discolor* (d'Urv.) Cano, Musarella, Cano-Ortiz, Piñar Fuentes, Spamp. et Pinto Gomes from: P = Portugal; S = Spain; I = Italy. The highest values are reported in bold

	G. discolor P	*G. discolor* I	*G. coronaria* I	*G. coronaria* S	*G. discolor* S	*G. coronaria* P
G. discolor P	0					
G. discolor I	0.574	0				
G. coronaria I	0.580	0.599	0			
G. coronaria S	**0.642**	0.527	0.556	0		
G. discolor S	0.595	0.546	**0.642**	0.583	0	
G. coronaria P	0.415	0.514	0.576	0.483	0.522	0

polymorphic species was *G. coronaria,* with 67.12 % of polymorphic loci and 359 private bands.

To investigate the genetic distance of the analysed species, the Nei's value was calculated. Our results showed that the highest variation was found between *G. coronaria* from Spain and *G. discolor* from Portugal (0.642), and between *G. coronaria* from Italy and *G. discolor* from Spain (0.642) (Table 3).

In order to obtain further information on the grouping of the two species, we carried out PCoA, using AFLP band pattern as raw data. The PCoA (Figure 2) clearly reflected the relationships among and between the genotypes analysed. The first and second component could explain 22.6 % and 20.7 % of the variation, respectively. *G. discolor* from Portugal have been classified apart from *G. coronaria* genotypes along the two axes. Furthermore, the first axis separated two *G. coronaria* genotypes (Portugal and Italy) from two *G. discolor* ones (Spain and Italy).

The obtained results showed that the *G. coronaria* and *G. discolor* can be distinguished using these markers, but further studies with a higher number of molecular markers are needed to confirm it. The genetic difference between these species has been always investigated using phenotypic attributes; only Ata et al. (2017) investigated the relationships of 12 species belonging to Asteraceae, including *G. coronaria* and *G. discolor*, using ITS sequence barcoding. They affirm that the analysed species could be distinctly separated on the genetic basis. This claim is in line with our results. Indeed, the high number of polymorphic bands, the Shannon index and Nei's gene diversity values suggest that the two groups of genotypes are sharing only part of the analysed fragments, and so can be considered as distinct genotypes. The level of polymorphism obtained here in terms of percentage of polymorphic bands with AFLP markers and the genetic diversity expressed as Nei's gene diversity as well as Shannon's information index values is higher than that

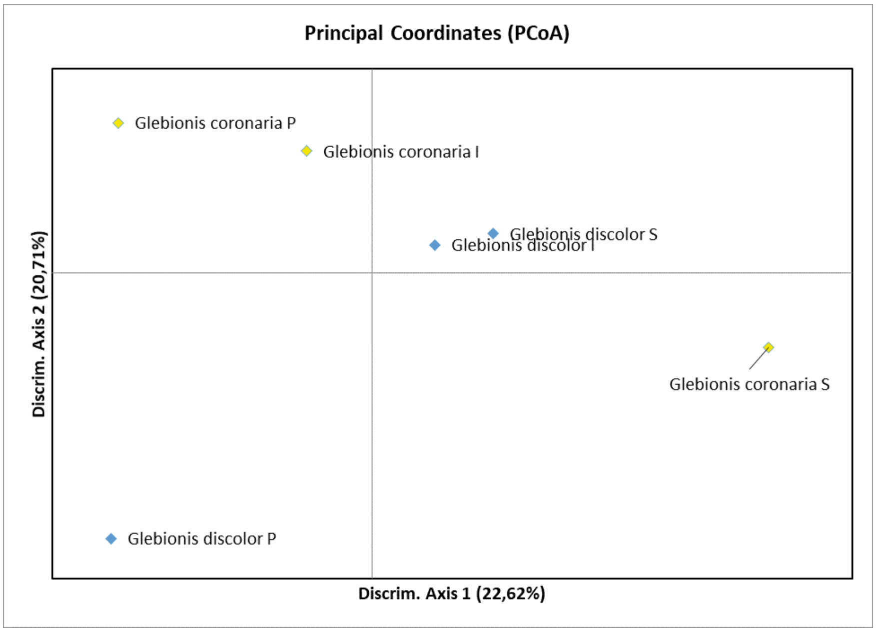

Figure 2: Principal coordinates analysis (PCoA) of three samples of *Glebionis coronaria* (L.) Cass. ex Spach and three of *G. discolor* (d'Urv.) Cano, Musarella, Cano-Ortiz, Piñar Fuentes, Spamp. et Pinto Gomes based on AFLP data (S = Spain; I = Italy; P = Portugal)

reported for other Asteraceae (Nguyen et al., 2013; Kropf et al., 2017; Wu et al., 2019), confirming the effectiveness of the markers used to distinguish the population studied. Furthermore, the PCoA analysis clearly separated *G. coronaria* apart from *G. discolor* along both axes. The different distribution of *G. coronaria* from Portugal and Italy vs genotypes from Spain and of *G. discolor* from Spain and Italy vs genotypes from Portugal could be related to the area of origin.

4 CONCLUSIONS

In the present study, the genetic diversity of two *Glebionis* species has been investigated through an AFLP analysis. The genotypes considered came from *G. coronaria* and *G. discolor* samples collected in three different countries (Spain, Portugal and Italy). Our results, together with the phenotypical studies conducted by Cano and collaborators (2017), allowed the separation of *G. discolor* from *G. coronaria*, confirming that the best way to distinguish some individuals is the combo of molecular markers and phenotypic attributes. We contemplate that this study is showing partial results. Indeed we know that the number of samples used is not fully representative of the *Glebionis* germplasm. However, these preliminary results confirmed the potential resolving power of AFLP analysis for a genome lacking species and could be considered as a starting point for future researches in a larger collection of genotypes.

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doi:10.14720/aas.2023.119.1.1932 Original research article / izvirni znanstveni članek

Strah potrošnikov glede prehranske varnosti v Sloveniji v obdobju prvega vala COVID-19 krize

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Received October 16, 2020; accepted February 27, 2023.
Delo je prispelo 16. oktobra 2020, sprejeto 27. februarja 2023

Consumers' fear regarding food security in Slovenia during the first wave of COVID-19 crisis

Abstract: The various crises are having a significant impact on the entire food sector and are changing the attitudes of Europeans as well as policies on the importance of food security and sustainably produced quality and safe food for consumer health. The paper focuses on the consumer's fear of food security for the time of the first wave of COVID-19 and the associated concern for food security in the future and the changes in consumer behaviour. The online survey in Slovenia was conducted in June 2020 using a "snowball" method. The sample included 490 individuals. The results showed that both measured forms of fear (i) fear over food security during the first wave of COVID-19 crisis, and (ii) fear over food security in the future were statistically significant, moderately strong and positively associated with almost all forms of self-perceived behaviour change caused by the COVID-19 crisis. The respondents focused more on buying locally produced and processed food, food stockpiling and decreasing food waste. Only minor changes were expressed with regards to their food purchasing channels, with the elderly, the highly educated and those who classified themselves in a higher social class buying more often directly from farmers. In the future, the results of this research should be compared with other countries and the impact of an individual's economic situation and the impact of promotional campaigns on agricultural products on changing consumer behaviour should also be analysed in more detail.

Key words: food self-sufficiency; food security; COVID-19; consumer; food chain; statistical analysis; consumer habits

Strah potrošnikov glede prehranske varnosti v Sloveniji v obdobju prvega vala COVID-19 krize

Izvleček: Raznovrstne krize znatno vplivajo na celoten prehranski sektor in spreminjajo stališča Evropejcev in politik o pomenu prehranske varnosti, trajnostno pridelane, kakovostne in varne hrane za zdravje potrošnikov. Prispevek se osredotoča na strah glede prehranske varnosti v času prvega vala COVID-19 krize v Sloveniji, na z njim povezan strah glede prehranske varnosti v prihodnje in na spremembe potrošnih vedenj. Spletno anketiranje je bilo v Sloveniji izvedeno v juniju 2020 na način »snežene kepe« in je zajelo 490 posameznikov. Rezultati so pokazali, da sta obe merjeni obliki strahu (i) strah o prehranski varnosti med prvim valom COVID-19 krize in (ii) strah o prehranski varnosti v prihodnosti statistično značilno, srednje močno in pozitivno povezani s skoraj vsemi samozaznanimi spremembami lastnega vedenja zaradi pojava COVID-19 krize. Anketiranci so se najbolj osredotočili na nakup živil, ki so pridelana in predelana v Sloveniji, na ustvarjanje večjih prehranskih zalog ter na večjo pazljivost pri tem, koliko hrane zavržejo. Zgolj manjše spremembe pa so izrazili glede nakupnih kanalov, pri čemer so neposredno od kmetov pogosteje nakupovali starejši, višje izobraženi in ti, ki se samouvrščajo v višji družbeni razred. V prihodnje bi bilo treba vključiti reprezentativen vzorec in primerjati rezultate z rezultati v drugih državah in podrobneje analizirati vpliv posameznikovega ekonomskega položaja in vpliv promocijskih kampanj na spremembo potrošnih vedenj.

Ključne besede: samooskrba s hrano; prehranska varnost; COVID-19; potrošnik; prehranska veriga; statistična analiza; nakupovalne navade

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1 UVOD

Pandemija COVID-19 ni zgolj zdravstvena kriza, temveč zaobjema tudi ostale sektorje družbe, med drugim vpliva tudi na agro-živilski sektor v Sloveniji. Zaprtje države med prvim valom je bilo nepričakovano in je povzročilo različne posledice. Zadnja desetletja so različne krize (npr. globalna finančna v letu 2008, suše, poplave in druge nepredvidljive vremenske razmere tudi kot posledica podnebnih sprememb) in prehranski škandali (prisotnost ostankov pesticidov, antibiotikov, potvorbe živil) spreminjale stališča prebivalcev Evropske unije (EU) in tudi politike o pomenu prehranske varnosti in trajnostno pridelane kakovostne in varne hrane za zdravje potrošnikov. Tovrstne krize so med drugim imele pomembno vlogo pri spreminjanju miselnosti in sprejetju Zelenega dogovora Evropske unije (Evropska komisija, 2023) ter nove strategije »Farm to Fork« za pravičen, zdrav in okolju prijazen prehranski sistem, kjer so med dolgoročnimi cilji zmanjšanje uporabe pesticidov za 50 %, mineralnih gnojil za 20 %, zmanjšanje porabe antibiotikov v reji živali in povečanje obsega ekološke pridelave v EU na najmanj 25 % kmetijskih obdelovalnih površin do leta 2030 (Evropska komisija, 2020a). Prehranska varnost in večja samooskrba s hrano sta tudi strateški usmeritvi nacionalne kmetijske politike (Resolucija o nacionalnem …, 2020, Strateški načrt …, 2022).

Pandemija COVID-19 je med drugim znatno vplivala na prehranske tokove, tako na lokalni kot tudi na svetovni ravni. Organizacija Združenih narodov za prehrano in kmetijstvo (FAO) opredeljuje štiri stebre zanesljivosti preskrbe s hrano: razpoložljivost hrane (ali preskrba s hrano), dostop do hrane (ekonomski in fizični), uporaba hrane (prehransko stanje posameznikov) in stabilnost ostalih treh dimenzij skozi čas (FAO, 2008). V času pandemije COVID-19 so bili v marsikateri državi ogroženi vsi štirje stebri prehranske varnosti (Barcaccia in sod., 2020, Deaton in Deaton, 2020, Devereux in sod, 2020, Laborde in sod., 2020). Po drugi strani pa moramo upoštevati, da so prehransko industrijo v času pandemije pogosto zaščitili ustrezni vladni ukrepi, kar je prispevalo k zmanjšanju negativnih učinkov, ki bi jih sicer lahko utrpela. Vprašanja razumevanja razsežnosti te krize in zagotavljanje prehranske varnosti prebivalstva so tako postala prioritetna področja vladnih politik in medijskih poročanj. Pomembno vlogo pri oblikovanju tovrstnih politik pa bi moralo imeti podrobno poznavanje vedenja potrošnikov v obdobju krize COVID-19 in faktorjev, ki vplivajo na spremembe tega vedenja.

Raziskava se primarno osredotoča na strah potrošnikov glede prehranske varnosti v obdobju prvega vala krize COVID-19 in z njim povezan strah glede prihodnje prehranske varnosti v prihodnje. Prav tako preverja, ali sta ti dve obliki strahu vplivali na spremembe potrošnih vedenj zaradi epidemije COVID-19. Kriza v obdobju COVID-19 je namreč izredno stanje, v katerem so potrošniki izpostavljeni velikim spremembam, pričujoča raziskava pa dodaja koščšek k mozaiku zbiranja podatkov o psiholoških mehanizmih in demografskih razlikah, ki pojasnjujejo spremembe v vedenju potrošnikov za časa kriz, kot je epidemija COVID-19.

1.1 PREHRANSKA VARNOST IN SAMOOSKRBA S HRANO V SLOVENIJI

V Sloveniji smo v javnem diskurzu pogosto priča mešanju različnih terminov, ki so sorodni, vendar ne enaki konceptu prehranske varnosti. Zato v nadaljevanju podajamo prevode iz angleščine in nemščine ter kratke povzetke definicij ključnih terminov (Preglednica 1).

Prehranska varnost obstaja, kadar imajo vsi ljudje ves čas fizični in ekonomski dostop do zadostne količine varne in kakovostne hrane, ki ustreza njihovim prehranskim potrebam za aktivno in zdravo življenje (FAO, 2008).

Varnost živil FAO (2003) opredeljuje kot odsotnost vseh nevarnosti, kroničnih ali akutnih, zaradi katerih bi živilo lahko bilo nevarno za zdravje ljudi. Do nevarnosti, ki ogrožajo živila lahko pride v katerikoli stopnji živilsko predelovalne verige, t.j. od pridelave oziroma proizvodnje živila do zaužitja in so posledica okoljskih onesnaženj, neprimernih kmetijskih ali proizvodnih praks in skladiščenja ter neznanja in neosveščenosti ljudi pri izbiri in pripravi hrane. Varna živila, ki ne ogrožajo človekovega zdravja so osnova zdrave prehrane in pomemben dejavnik javnega zdravja (NIJZ, 2023).

Ideja prehranske suverenosti se prvič pojavi v javnosti na Svetovnem vrhu hrane leta 1996 s strani mednarodne iniciative La Via Campesina *(*La Via Campesina, 2003*)*, ki prehransko suverenost opredeli kot osnovno človekovo pravico in pravico vsakega naroda, da vzdržuje in razvija lastno kapaciteto pridelave hrane ob hkratnem upoštevanju kulturnih in pridelovalnih razlik. Prehranska suverenost je po tej definicij predpogoj za doseganje prehranske varnosti in predstavlja alternativo neoliberalnim politikam.

Prehranska varnost je povezana s stopnjo samooskrbe s hrano. Stopnja samooskrbe s hrano je obseg, v katerem domača proizvodnja pokriva domačo potrošnjo (potrošnja za prehrano, krmo in potrošnjo v industriji) pri posameznih kmetijskih proizvodih (Hiti Dvoršak in Bele, 2020a). V Sloveniji se stopnja samooskrbe razlikuje za živinorejo in rastlinsko pridelavo. Na slednjo namreč bolj vplivajo vremenske razmere posameznih letin. V povprečju let 2014–2018 je dosežena večja samooskrba

Preglednica 1: Definicije pojmov v zvezi s prehrano

Definicija	Angleški izraz	Nemški izraz	Slovenski prevod
Stanje zanesljivega dostopa do zadostne količine cenovno dostopne in kakovostne hrane (FAO, 2008).	Food security	Ernährungssicherheit	Prehranska varnost
Obseg, v katerem lahko država (regija, gospodinjstvo, posameznik) zadovolji svoje potrebe po hrani iz lastne domače pridelave (FAO, 1999).	Food self-sufficiency	Selbstversorgung mit Lebensmitteln	Samooskrba s hrano
Odsotnost vseh nevarnosti, kroničnih ali akutnih, zaradi katerih bi hrana lahko bila nevarna za zdravje potrošnika (FAO, 2003).	Food safety	Lebensmittelsicherheit	Varnost živil
Pravica ljudi do zdrave in kulturno ustrezne hrane, pridelane po okoljsko sprejemljivih in kulturno ustreznih metodah, in njihova pravica do opredelitve lastnih prehranskih in kmetijskih sistemov (La Via Campesina, 2003).	Food sovereignty	Ernährungssouveränität	Prehranska suverenost

pri koruzi 86 % in ječmenu 72 %, ki sta namenjena krmi, v primerjavi z rastlinami za neposredno prehrano ljudi, kot so krompir 56 %, pšenica 50 %, zelenjava 40 % in sadje 37 %. Zaradi manjše porabe na prebivalca in večje prireje je v 2019 dosežena samooskrba pri mesu večja za 2 % od povprečja petih predhodnih let, ki znaša za mleko 127 %, meso perutnine 108 %, goveje meso 106 %, meso drobnice 87 % in prašičje meso 37 %. Zmanjšala se je samooskrba z jajci in medom (petletno povprečje 93 % oz. 55 %) (Bedrač in sod., 2020). Kljub nekaterim velikim deležem samooskrbe pri živinoreji ostaja dejstvo, da je tudi za to prirejo skoraj vsa beljakovinska krma (prevladujejo gensko spremenjene sojine tropine) iz uvoza (Grobelnik Mlakar in sod., 2017). To je tudi širši evropski problem, saj je npr. le 8 % pokritost potreb po soji s pridelavo v Evropi in ta uvozna odvisnost vpliva na prehransko varnost in predstavlja veliko izpostavljenost evropske živinoreje krizam, ki so se zvrstile v zadnjem obdobju kot npr. 2018 trgovinska vojna s krmo za živali med ZDA in Kitajsko, v 2019 epidemija prašičje kuge, podnebne spremembe s pomanjkanjem padavin, suša in visoke temperature ter požari v Amazoniji, čemur je sledilo zaprtje tovarn in tudi državnih meja ob COVID-19 krizi v letu 2020 (Evropska komisija, 2020b).

Slovenija je del globalnega trga tako kot uvoznik kot izvoznik hrane. Pomemben delež v Sloveniji pridelanih kmetijskih pridelkov in vzrejenih živali ali njihovega mesa in mleka ter tudi živil iz v Sloveniji pridelanih surovin se izvozi – pri nekaterih segmentih je zunanjetrgovinska bilanca celo pozitivna. V letih 2018 in 2019 je bila ta dosežena pri skupinah "razna živila", "izdelki iz mesa", "žive živali" ter "oljna semena" (Bedrač in sod. 2020), kar pomeni, da se npr. ves pridelek semen oljne ogrščice izvozi, surovo olje te in drugih oljnic (sončnice) pa se uvozi in v Sloveniji samo rafinira. Prav tako se v Slovenijo v velikem obsegu uvažajo slabše kategorije kakovosti mesa in užitni klavni odpadki za predelavo v mesne izdelke , medtem ko se veliko živih živali in svežega govejega mesa izvozi. V Sloveniji je več kot 70 % potrošene hrane uvožene, le približno tretjina je slovenskega izvora (Hiti Dvoršak in Bele, 2020b). V zadnjih letih je izvoz hrane pokril približno polovičen delež uvoza, pri čemer se je vrednostni izvoz povečal predvsem na račun nepredelanih kmetijskih proizvodov, uvoz pa na račun predelanih proizvodov (Hiti Dvoršak in Bele, 2020c). Slovenska pridelava pogosto cenovno ni konkurenčna masovni industrijski pridelavi/reji v tujini. Razlogov za to je več; med drugim majhnost kmetij in težjih razmer pridelave na kar 85 % kmetijskih obdelovalnih površin (gorska in hribovska območja ter druge omejitve), zaščitena območja Natura 2000, omejitve pridelave na vodovarstvenih območjih, stroški delovne sile in tudi tehnološka zaostalost nekaterih panog, z izjemo majhnega števila kmetijskih gospodarstev in panog (npr. hmelj) (MKGP, 2020a). V luči teh dejstev je zato izrednega pomena analizirati, kakšen je odziv slovenskih potrošnikov na krizo, kot je epidemija COVID-19.

1.2 ODZIVI POTROŠNIKOV NA OSKRBO S HRANO V ČASU PANDEMIJE COVID-19

Medtem ko se svet bori s pandemijo in so motena tako gospodarstva kot preživetje posameznikov, bodo v razmerju do prehranske varnosti verjetno najbolj trpeli revni in ranljivi (IFPRI, 2020). Laborde in sod. (2020) podajajo pregled tveganj za globalno prehransko varnost in napovedujejo, da bodo pri tem, v finančnem smislu, najbolj prizadeta najrevnejša gospodinjstva, ki za hrano namenijo do 70 % svojih skupnih prihodkov. Prav tako napovedujejo motnje v proizvodnji hrane, prehranskih verigah in trgovinskih omejitvah. To bo po napovedih Laborde in sod. (2020) na odločitve potrošnikov vplivalo tako, da bodo posegali k cenejši hrani slabše kakovosti.

Tudi slovenski potrošniki so pri izbiri živil na ceno hrane zelo občutljivi, saj je dostopna cena živila pomembna za več kot polovico (60,6 %) odraslih in starejših odraslih (Gregorič in sod., 2019). Arndt in sod. (2020) prihajajo do podobnih zaključkov v svoji makroekonomski analizi o izgubi dohodka gospodinjstev v povezavi z njihovimi izdatki v gospodinjstvu in stopnjo izobrazbe. Rezultati njihove študije so pokazali, da bodo najbolj ranljiva gospodinjstva z nizko izobrazbo in veliko odvisnostjo od prihodkov iz dela. Do podobnih ugotovitev prihaja tudi Béné (2020), ki opozarja na to, da je slaba pripravljenost odločevalcev politik za ustrezno krmiljenje med zajezitvijo zdravstvene krize in gospodarstvom, najbolj vplivala na revnejši sloj prebivalstva.

Boumphrey (2020) primerja prehransko potrošnjo z drugimi tipi potrošnje in napoveduje, da bodo zgolj prehranski trgi ostali na približno enakih ravneh, medtem ko bo COVID-19 kriza zelo negativno delovala na skoraj vse ostale tipe potrošnje, saj se potrošniki fokusirajo na varčevanje. Potrošniki bodo v prihodnje bolj osredotočeni nase in družino, na preventivne zdravstvene ukrepe in povečanje odpornosti. Nadalje napoveduje fokus potrošnikov na vprašanja zavržkov hrane in prehranske varnosti, medtem ko bodo ostala vprašanja, vezana na trajnost pridelave in pakiranje, v času krize, izgubila na pomembnosti (Boumphrey, 2020).

Raziskave, ki proučujejo potrebe in vedenje potrošnikov nakazujejo številne preobrate nakupnih navad potrošnikov po COVID-19 krizi, nekateri izmed njih so bili identificirani tudi v Sloveniji. Jribi in sod. (2020) navajajo, da je COVID-19 povečal skrb potrošnikov za zmanjševanje zavržkov hrane. Podobno je konec aprila 2020 Inštitut za nutricionistiko opravil raziskavo na 602 prebivalcih Slovenije. Petina slovenskih anketirancev je poročala o manjši količini zavržene hrane (Kušar, 2020, Janssen in sod., 2021).

Po podatkih Gerholda (2020), je bil primaren odziv potrošnikov na krizo povečevanje prehranskih zalog, kar je potrdila tudi raziskava Inštituta za nutricionistiko v Sloveniji, kjer je kar 43 % gospodinjstev poročalo o povečanih zalogah hrane (Kušar, 2020, Janssen in sod., 2021). Glede na interpretacijo raziskovalcev je to na eni strani posledica izražene skrbi pred nezadostno preskrbo s hrano, na drugi strani pa tudi skrbi, povezane s tveganjem, da bi se pri nakupovanju živil lahko okužili. Posledica povečevanja prehranskih zalog, ki jo napoveduje Boumphrey (2020), naj bi bilo tudi zmanjševanje t.i. impulzivnega nakupovanja, saj bodo potrošniki šli po nakupih manj pogosto, vendar po večje zaloge.

Pomembne spremembe pa se pričakujejo glede nakupnih kanalov potrošnikov. Glede na Boumphrey (2020) je pandemija COVID-19 med številnimi potrošniki, predvsem starejšimi, spodbudila spletno nakupovanje, medtem ko je mlajša generacija potrošnikov kupovala manj pogosto in na zalogo (predvsem osnovna živila). V Sloveniji je bila dve zaporedni leti (februar 2019 in julij 2020) izvedena reprezentativna anketa o odnosu slovenskih potrošnikov do hrane in nakupnih navadah s fokusom na ekološko pridelana živila, ki pa je slučajno sovpadla tudi s COVID-19 krizo (Aragon, 2020). Po teh podatkih se je v prvem valu COVID-19 pogostost nakupa živil in s tem tudi ekoloških živil v letu 2020 v primerjavi z 2019 zmanjšala, kar je potrdila tudi podobna anketa Inštituta za nutricionistiko - pogostost nakupov se je v obdobju prvega vala COVID-19 iz večkrat na teden v povprečju zmanjšala na tedenske nakupe ali še redkeje (Kušar, 2000, Janssen in sod., 2021). Povprečno število prodajnih kanalov, ki jih potrošnik obišče za nakup živil, se je po krizi COVID-19 statistično značilno zmanjšalo iz 3,2 prodajnih mest v letu 2019 na 2,9 prodajnih mest v letu 2020 (Aragon, 2020).

Raziskava Aragon je pokazala, da je v letu 2020 nakup živil v trgovskih centrih in diskontih postal še pomembnejši kraj nakupa kot v enaki raziskavi v letu 2019, ko je več anketirancev izjavilo, da kupujejo neposredno od kmetov bodisi na kmetiji ali na tržnicah, ki so glavni nakupni kanal zlasti za tiste potrošnike, ki zelo pogosto posegajo po ekološko pridelani hrani (Aragon, 2020). Inštitut za nutricionistiko pa nasprotno ugotavlja, da so slovenska gospodinjstva v času pandemije za preskrbo s hrano bistveno manj uporabljala večje trgovine, medtem ko se je znatno povečal obseg naročanja živil na dom. Pri preskrbi s hrano so se anketiranci bolj posluževali živilskih prodajaln, ki so bližje domu in lokalnih ponudnikov, kjer kupuje 19 % gospodinjstev. Pri tem opažajo še posebej značilne razlike med podeželjem v primerjavi z mesti - na podeželju se je skoraj izenačil z mesti delež nakupovanja z dostavo na dom (13 % oz. 15 % gospodinjstev) pri čemer se je nakupovanje z dostavo na dom povečalo za 152 % pri svežem sadju in zelenjavi (Kušar, 2020, Janssen in sod., 2021). Tako glede na neskladnost podatkov iz teh dveh raziskav ostaja odprto vprašanje, ali so potrošniki za časa COVID-19 res v večji meri naročali neposredno od kmetov, ali ne.

Kot zadnje, proučevanje odnosa slovenskih potrošnikov do hrane kaže, da je v zadnjem desetletju pri nakupu slovensko poreklo (pridelano in predelano v Sloveniji) pridobilo na pomenu, čeprav potrošniki v večini primerov ne ločijo te kategorije od v Sloveniji samo predelanih živil iz uvoženih surovin, ki se na trgovskih policah oglašujejo kot slovenski proizvodi večinoma pod različnimi blagovnimi znamkami trgovskih verig, ki so med potrošniki najbolj prepoznavne (Aragon, 2020). Rezultati Nacionalnega inštituta za javno zdravje kažejo, da je najpomembnejša lastnost za nakup hrane sicer okus (78 %), a je na drugem mestu (64,6 %) za odrasle in starejše odrasle lokalna pridelava ali domača hrana (Gregorič in sod., 2019). Manjkajo pa podatki o tem, v kolikšni meri so slovenski potrošniki zaradi COVID-19 krize dali poudarka na pridelanem in predelanem v Sloveniji in ali je na to vplival strah glede prehranske varnosti za časa COVID-19 krize in glede prehranske varnosti v prihodnosti. Glede na Boumphreyeve napovedi (2020) o tem, da bodo v krizi potrošniki dali manj fokusa na vprašanje porekla pridelave hrane, bi namreč po eni strani pričakovali, da bo torej fokus potrošnikov na pridelano in predelano v Sloveniji izgubil na pomenu. Po drugi strani pa je v tem obdobju v Sloveniji bilo dano še več pozornosti različnim promocijskim kampanjam za ozaveščanje potrošnikov o pomenu slovenske hrane (MKGP, 2020 in GZS-ZKŽP, 2020) in je zato zanimivo vprašanje, kakšno je bilo dejansko stanje med slovenskimi potrošniki.

Cilj pričujoče raziskave je nadgraditi dosedanje dostopne raziskave in analizirati globlje psihološke mehanizme, ki povzročajo spremembe nakupnega vedenja zaradi COVID-19 krize. Specifično, raziskovalci Inštituta za nutricionistiko (Kušar, 2020, Jansenn in sod., 2021) interpretirajo rezultate čez prizmo skrbi pred nezadostno preskrbo s hrano, pri čemer nimamo podatkov o tem, koliko so ljudje zares bili zaskrbljeni in kakšna, če sploh je povezava med tovrstnim strahom in nakupnim vedenjem potrošnikov. Tako pričujoča raziskava oblikuje poglobljen merski instrument za merjenje strahu glede prehranske varnosti in preverja v kolikšni meri je strah vzrok za spremembe vedenj, ob standardnih demografskih spremenljivkah starosti, izobrazbe, družbenega razreda, kraja prebivanja in spola.

2 MATERIAL IN METODE

Za izvedbo raziskave je bila uporabljena metoda spletnega anketiranja s pomočjo spletnega orodja 1ka (1ka, 2020). Prednost te metode je relativno hiter proces zbiranja podatkov in enostavnost za udeležence. Omejitev je predvsem v vzorčenju: omejitev na uporabnike interneta in omejitev t. i. vzorca »snežne kepe«, kjer je vzorec omejen na družbene skupine, do katerih segajo prvotni kontakti (če so npr. prvotni kontakti med visoko izobraženimi, bo delež v samem vzorcu večji).

Vprašanja se delijo na stališčna vprašanja in vedenjska vprašanja. Stališčna vprašanja merijo dve obliki strahu: zaznavanje prehranske varnosti v obdobju pandemije COVID-19 ter zaznavanje grožnje prehranski varnosti v prihodnosti. Glede na štiri stebre prehranske varnosti opredeljene s strani FAO sta bili v anketnem vprašalniku zajeti dve dimenziji: zaznavanje razpoložljivosti hrane (angl. food availability) in zaznavanje dostopa do hrane (angl. access to food). Vprašanja so bila deloma oblikovana po zgledu t. i. zaznavanje dostopa do zdrave hrane (Freedman in Bell, 2009), prilagojenega na situacijo COVID-19 v Sloveniji. Zaznavanje grožnje prehranski varnosti v prihodnosti je bila merjena s prilagojenimi vprašanji, po zgledu teorije »apelov strahu« (Witte, 1994). Potrošnikom so bila zastavljena vprašanja glede tega, kako verjetno je, da bo prehranska varnost pomembno tveganje v prihodnosti. V vseh primerih je šlo za tip trditev po zgledu Likertove petstopenjske lestvice od »1: sploh se ne strinjam« do »5: popolnoma se strinjam«. Celoten vprašalnik je bil razdeljen v tri različne sklope: ob demografskih vprašanjih tudi 17 trditev o strahu in vedenjih, razdeljenih v štiri različne sklope (natančne trditve so navedene v Preglednicah 3, 4 in 5).

Pri vedenjskih vprašanjih je bilo merjeno samozaznavanja učinkov COVID-19 krize na potrošnjo in vedenje. Vprašanja, vezana na odziv zmanjševanja prehranskih odpadkov, so bila pripravljena po zgledu Jribi in sod. (2020); vprašanja, vezana na prehranske zaloge, pa po zgledu Gerhold (2020). Dodana so bila še vprašanja o spremembi nakupnih kanalov in vprašanje o večji osredotočenosti na nakup hrane, ki je bila pridelana in predelana v državi prebivanja. Tudi v teh primerih je šlo za 5-stopenjsko Likertovo lestvico. Vprašanja so bila omejena na dejstvena vprašanja, to je na vprašanja o vedenjih za časa COVID-19 krize spomladi 2020, pri čemer je tako omejitev raziskave samozaznavanje in ne dejansko spremljanje vedenj.

Za analizo podatkov je bil uporabljen program SPSS . Deskriptivna analiza je bila omejena na izračun povprečnih vrednosti in standardnih odklonov oz. delež veljavnih odgovorov po skupinah. Trditve, ki so bile vsebinsko »obrnjene«, so bile rekodirane. Za testiranje zanesljivosti vprašalnika na treh ločenih sklopih vprašanj je bila uporabljena metoda analize notranje konsistentnosti s pomočjo Cronbachovega alfa koeficienta. Za preverbo konstruktivne veljavnosti vprašanj je bila za vsa vprašanja, ki merijo isto teoretsko spremenljivko, uporabljena faktorska analiza s pravokotno rotacijo. Faktorska analiza je študij povezav med spremenljivkami, s katerim poizkušamo najti novo množico spremenljivk, ki predstavljajo kar je skupnega opazovanim spremenljivkam (Ferligoj, 2001). Z njo je bilo preverjeno ali lahko za indikatorje ene spremenljivke najdemo en osrednji dejavnik, ki pojasni čim večji delež variance - in s tem potrdimo, da gre za dober merski instrument, ki z različnimi indikatorji meri isto teoretsko spremenljivko. Za analizo korelacij med odvisnimi spremenljivkami (indikatorji samozaznavanja učinkov COVID-19 krize na lastno prehransko potrošnjo) in neodvisnimi razmernostnimi spremenljivkami (oba tipa strahu in starost) je bil uporabljen Spearmanov koeficient. Za analizo razlik samozaznavanja učinkov COVID-19 krize na lastno prehransko potrošnjo glede na neodvisne nominalne spremenljivke pa sta bila uporabljena Kruskal-Wallisov test (izobrazba, razred, kraj) in Mann-Whitneyev U test za neodvisne vzorce (spol).

3 REZULTATI IN DISKUSIJA

3.1 OPIS VZORCA

Vzorčenje je potekalo v juniju 2020, dober mesec po sprostitvi strogih ukrepov samoizolacije in v obdobju, ko je Slovenija razglasila uraden konec prvega vala epidemije v Sloveniji. Vprašanja v raziskavi so bila zastavljena na način, da so povpraševala o obdobju najstrožjih ukrepov v prvem valu epidemije COVID-19 v Sloveniji, torej med februarjem in majem 2020.

Postopek vzorčenja je potekal na način t. i. »snežne kepe«, kjer so bili anketiranci naprošeni, da spletno anketo delijo naprej do svojih znancev. Začetni kontakti so bili osebni kontakti avtoric tega prispevka (elektronski naslovi, Facebook in LinkedIn kontakti) in objava vabil k sodelovanju v raziskavi prek Facebook skupin v slovenščini, ki združujejo ljudi z interesom za hrano ali vrtnarjenje (5 tovrstnih skupin). Prednost tega pristopa je bila relativno hitra odzivnost in doseganje večjega števila udeleženih brez potrebnih dodatnih finančnih sredstev.

Vzorec je zajel 490 posameznikov, nekateri od njih niso želeli odgovoriti na vsa sociodemografska vprašanja. Najmanjše število veljavnih odgovorov je bilo pri vprašanju o samouvrstitvi v družbeni razred (n veljavnih = 428), kjer se preostali anketirani niso želeli opredeliti. V nadaljevanju zato podajamo število veljavnih odgovorov pri vsaki preglednici z rezultati analize posebej.

Pomembna omejitev uporabljenega pristopa vzorčenja je nereprezentativnost vzorca in s tem omejitev rezultatov s pristranostjo k ženskemu spolu, višji izobrazbi in višjemu družbenemu razredu (Preglednica 2). Pri tem je potrebno izpostaviti, da je glede spola vzorec nereprezentativen s stališča prebivalstva Slovenije – da pa je po drugi strani smiselno reprezentativen (podoben namenskemu vzorcu) glede na kulturne razlike spolno določenih družbenih vlog pri skrbi za prehrano v slovenskih gospodinjstvih. Povečini so za nakup hrane in prehranskih izdelkov v gospodinjstvu zadolžene ženske, kar izhaja iz tradicionalne vloge žensk v preteklosti (Crane in sod., 2019).

Po drugi strani je vzorec reprezentativen čez prizmo ruralno proti urbanemu: približno 40 % anketirancev po lastni presoji prebiva na podeželju (v kraju z manj kot 2.000 prebivalci), medtem ko približno 40 % anketiranih prebiva v mestnih naseljih in 20 % v naseljih mestnega območja, kar sovpada tudi z Eurostatovo statistiko po kateri 44 % odstotkov Slovencev prebiva na podeželju, na vmesnih območjih med mestom in podeželjem ter mestnih območjih pa okoli 56 % odstotkov (Eurostat, 2018).

Glede na omejitve vzorčenja je pri interpretaciji rezultatov treba imeti v mislih, da je posploševanje omejeno na tako oblikovan vzorec. Bolj kot v sami analizi deskriptivnih podatkov specifičnih za ta vzorec, je vrednost te raziskave v posploševanju pri korelaciji med stališči in vedenji, vezanimi na prehransko varnost in COVID-19 krizo.

Preglednica 2: Opis vzorca

Spol (n veljavnih = 460)	veljavni delež (%)
Moški	21,5
Ženski	78,5
Starost (n veljavnih = 454) (arit. sredina: 35,6 let, st. odklon: 18,5 let)	
20 let ali manj	1,8
21 do 35 let	33,7
36 do 55 let	48,2
56 let ali več	16,3
Izobrazba (n veljavnih = 463)	
Srednja šola ali manj	21,4
Višja šola	16,6
Univerzitetna izobrazba	44,7
Specialistični študij, magisterij, doktorat	17,3
Velikost kraja prebivanja (n veljavnih = 467)	
Do 2,000 prebivalcev	40,9
Več kot 2,000 do 50,000 prebivalcev	38,5
Več kot 50,000 prebivalcev	20,6
Samouvrstitev v družbeni razred (n veljavnih = 428)	
Nižji srednji razred, nižji razred ali delavski razred	18,9
Srednji razred	54,4
Višji srednji razred ali višji razred	25,5

3.2 STRAH O PREHRANSKI VARNOSTI MED COVID-19 KRIZO

Pri vprašanjih o strahu o prehranski varnosti med COVID-19 krizo (Preglednica 3) je faktorska analiza pokazala en istoimenski faktor, s katerim lahko pojasnimo 54,37 % skupne variance. Test zanesljivosti samo za ta sklop vprašanj pa je pokazal zmerno zanesljivost (Cronbachov alfa: 0,785). Tako lahko sklepamo, da je sestavlje-

Preglednica 3: Strah o prehranski varnosti med COVID-19 krizo (Cronbachov alfa: 0,785; faktorska analiza pojasni 54,37 % skupne variance)

Strah o prehranski varnosti med COVID-19 krizo (Cronbachov alfa: 0,785)	Aritmetična sredina	Standardni odklon	Faktorska analiza (54,37 % variance)
V zadnjih nekaj mesecih sem bil-a zaskrbljen-a o tem ali bom lahko enostavno kupoval-a sveže sadje in zelenjavo.	2,57	1,31	0,68
V času COVID-19 epidemije nisem bil-a v skrbeh glede nakupa svežega sadja in zelenjave. (rekodirano)	2,32	1,27	0,80
Sploh nisem bil-a zaskrbljen-a, kje bom lahko dobil-a/kupil-a sveže sadje ali zelenjavo v času COVID-19 epidemije. Na voljo je bilo in še vedno je dovolj kakovostnih prehranskih pridelkov in izdelkov. (rekodirano)	2,09	1,14	0,84
Prepričan-a sem, da so bile trgovine v moji okolici odlično založene z vsemi potrebnimi prehranskimi pridelki in izdelki, tudi v času COVID-19 epidemije. (rekodirano)	2,09	1,14	0,63
Bil-a sem zaskrbljen-a, da bodo lokalne trgovine v moji neposredni bližini prodajale zgolj staro in gnilo sadje in zelenjavo.	1,90	1,09	0,72
Indeks: povprečje (N = 490)	2,2	0,87	

na lestvica vsebinsko primerna in so vsa vprašanja na istem konstruktu, ki smo ga poimenovali "strah o prehranski varnosti med COVID-19 krizo".

Večina anketirancev v povprečju (2,2) ni bila zelo zaskrbljenih glede dostopnosti svežega sadja in zelenjave v času epidemije COVID-19 (Preglednica 3). Veliko večjo skrb so anketiranci izrazili glede enostavnosti dostopa do svežega sadja in zelenjave v obdobju zaprtja. To gre najverjetneje pripisati vladnim ukrepom za zajezitev epidemije COVID-19, ki so omejevali gibanje prebivalstva v sami občini prebivanja in tudi izven nje ter ukrepu, ki je omejeval časovno obdobje nakupov v trgovinah za določeno skupino prebivalstva (starejši, invalidi, nosečnice).

3.3 STRAH O PREHRANSKI VARNOSTI V PRIHODNOSTI

Pri vprašanjih, ki so merila zaznavanje grožnje prehranski varnosti, je faktorska analiza s pravokotno rotacijo ponovno pokazala samo en faktor, ki smo ga poimenovali dovzetnost grožnje, s katerim lahko pojasnimo 57,47 skupne variance. Cronbachov alfa (0,802) pokaže visoko zanesljivost merskega instrumenta. Tudi v tem primeru tako lahko sklepamo, da so vprašanja v skladu s teorijo Wittove (1994) oblikovana smiselno in skupaj merijo eno dimenzijo strahu o prehranski varnosti v prihodnje.

V povprečju vseh trditev so anketirani srednje zaskrbljeni o prehranski varnosti v prihodnosti (2,46). Da je za prihodnost človeštva prehranska negotovost največja grožnja, je za anketirance visoko ocenjena bojazen (3,49). Prav tako srednje visoko ocenjujejo prehransko negotovost Slovenije v prihodnosti (2,40), medtem ko je na individualni ravni ta nevarnost najmanj izražena (1,94 in 2,13), strah o prehranski varnosti bližnjih v prihodnosti pa je rahlo višji (2,35).

3.4 SAMOZAZNAVANJE SPREMEMB VEDENJ ZARADI COVID-19 KRIZE

Na tem mestu smo povpraševali o vedenjih: bolj specifično, spraševali smo o tem, v kolikšni meri so anketiranci sami pri sebi zaznali spremembe v svojih vedenjih, ki jih pripisujejo COVID-19 krizi. Pri sklopu vprašanj, ki so merila samozaznavanje sprememb vedenj za čas COVID-19 krize je Cronbachov alfa pokazal zmerno, a še vedno sprejemljivo zanesljivost (0,728). Faktorska analiza pa je tokrat pokazala dve komponenti s skupnim deležem pojasnjene variance 54,43 (Preglednica 5), ki smo ju poimenovali Komponenta 1 – Vsi učinki, ter Komponenta 2 – Prehranske zaloge in naročanje prek spleta.

Podrobna analiza komponent pokaže, da je v resnici primaren prvi faktor, saj so vse trditve na tej komponenti večje od 0,4 - tudi pri edinih dveh vprašanjih, ki imata na drugi komponenti vrednost nad 0,4. Pri tem se vsebinsko ne zarisujejo pomembne razlike med tema dvema vprašanjema proti drugim petim vprašanjem. V primerjavi z zgornjimi meritvami strahu, ki so stališčne narave, so različni vedenjski indikatorji dosti bolj pod vplivom zunanjih dejavnikov kot pod vplivom notranjih

Preglednica 4: Strah o prehranski varnosti v prihodnosti (Cronbachov alfa: 0,802)

Dovzetnost grožnje: zaznavanje verjetnosti, da bo prehranska varnost pomembno tveganje v prihodnosti (Cronbachov alfa: 0,802)	Aritmetična sredina	Standardni odklon	Faktorska analiza (57,47 % variance)
Prehranska negotovost predstavlja največjo grožnjo za človeštvo v bližnji prihodnosti.	3,49	1,20	0,52
V državi, v kateri trenutno prebivam, bodo ljudje v kriznih razmerah, podobnim tistim v času epidemije COVID-19 resno prehransko ogroženi.	2,40	1,25	0,72
Zelo verjetno je, da moji bližnji v prihodnjih krizah, podobnim krizi COVID-19, ne bodo imeli dostopa do kakovostne in zdrave hrane.	2,35	1,16	0,85
Zelo verjetno je, da bom tudi sam-a v prihodnjih krizah, podobnim krizi COVID-19, ostal-a brez kakovostne in zdrave hrane.	2,13	1,10	0,86
Sam-a sem v nevarnosti, da v prihodnosti ne bom imel-a dovolj kakovostne in zdrave hrane.	1,94	1,06	0,78
Indeks: povprečje (N = 446)	2,46	0,87	

vzgibov posameznika, zato ne pričakujemo enako velike skladnosti med vedenji, kot bi jo pri stališčih. Tako tega sklopa sedmih vprašanj v nadaljnji analizi povezav med spremenljivkami nismo analizirali s pomočjo indeksa, temveč smo vsako vprašanje obravnavali ločeno kot odvisno spremenljivko.

Analiza nakazuje, da lahko krizne situacije, kot je epidemija COVID-19, v določeni meri spremenijo potrošniške navade (Preglednica 5). Še posebej izstopa, da so se anketiranci zaradi epidemije COVID-19 najbolj osredotočili na nakup živil, ki so pridelana in predelana v Sloveniji (3,52). Osredotočenost na nakup živil lokalnega izvora v času epidemije dokazujejo tudi številne druge raziskave iz tujine (Nemes in sod., 2021, Zhang in sod., 2022).

Drugi najpogostejši odziv anketirancev na CO-

Preglednica 5: Samozaznavanje učinkov COVID-19 krize na lastno prehransko potrošnjo (Cronbachov alfa: 0,728)

Samozaznavanje učinkov COVID-19 krize na lastno prehransko potrošnjo (Cronbachov alfa: 0,728)	Aritmetična sredina	Standardni odklon	Faktorska analiza: Komponenta 1- Vsi učinki (38,28 % variance)	Faktorska analiza: Komponenta 2- Prehranske zaloge in naročanje prek spleta (16,15 % variance)
Zaradi epidemije COVID-19 sem se bolj osredotočil-a na nakup hrane, ki je pridelana in predelana v državi, kjer trenutno živim.	3,52	1,51	0,633	-0,581
Zaradi epidemije COVID-19 sem si naredil-a več prehranskih zalog kot ponavadi.	3,22	1,42	0,558	0,524
Zaradi epidemije COVID-19 sem bil-a bolj pazljiv-a, koliko hrane zavržem.	3,24	1,53	0,674	-0,114
Moje nakupovalne navade se zaradi epidemije COVID-19 niso popolnoma nič spremenile. (rekodirano)	2,87	1,40	0,672	0,238
Zaradi epidemije COVID-19 sem bolj pogosto kupoval-a hrano neposredno od kmeta bodisi prek spleta oziroma na kmetiji.	2,64	1,56	0,679	-0,421
Zaradi epidemije COVID-19 sem začel-a kupovati hrano v trgovinah, kjer je običajno nisem.	1,97	1,28	0,627	0,066
Zaradi epidemije COVID-19 sem hrano naročal-a po spletu bolj pogosto kot ponavadi.	1,73	1,29	0,457	0,518
Povprečje (N = 477)	2,75	0,88		

VID-19 krizo je povečati zalogo hrane (3,22), kar je v skladu s podobnimi raziskavami v tujini (Gerhold 2020, Wang in sod., 2020, Kušar 2020, Janssen in sod., 2021) in v Sloveniji. V določeni meri je to najverjetneje posledica medijskega poročanja v tem obdobju. Številne medijske objave (Delo, 2020, Slovenske novice, 2020, Žurnal24, 2020) so npr. med in tudi še pred uradno razglasitvijo epidemije, poročale o izpraznjenih policah z osnovnimi potrebščinami v trgovinah.

Na tretjem mestu po pogostosti je bila večja pazljivost pri tem, koliko hrane anketiranci zavržejo. Tudi ta rezultat je v skladu z raziskavami tako v Evropi (Kušar, 2020, Janssen in sod., 2021, Vidal-Mones in sod., 2021, Theodoridis in Zacharatos, 2022, Masotti in sod., 2023), kot izven nje (Jribi in sod. 2020, Everitt in sod., 2021, Chang in sod., 2022, Allahyari in sod., 2022, Deliberador in sod., 2023).

V času epidemije je bila deloma opazna tudi sprememba nakupovalnih poti, vendar so podatki le deloma v skladu z napovedmi in s podobnimi raziskavami tako doma (Kušar 2020) kot v ostalih državah po svetu (Boumphrey 2020, Aragon 2020, Janssen in sod., 2021, Masotti in sod., 2023). Na splošno anketiranci nekoliko pogosteje pritrjujejo, da so se nakupovalne navade spremenile zaradi COVID-19 (2,87). Podobno se spremembe v rahli meri kažejo tudi pri nakupu neposredno od kmetov (2,64). Nasprotno pa se pri ostalih vprašanjih anketiranci večinoma ne strinjajo s tem, da so spremenili svoje nakupne kanale. Še posebej preseneča odgovor anketirancev glede pogostejšega nakupa hrane prek spleta. Večina anketirancev se namreč s to trditvijo ni strinjala (1,73) in je bil ta odziv med najredkejšimi odzivi na krizo. Podobno majhen je bil odgovor na trditev, da bi zaradi COVID-19 anketiranci začeli hrano kupovati v trgovinah, kjer je običajno niso (1,97).

3.5 ANALIZA POVEZAV MED SPREMENLJIVKAMI IN RAZLIK MED SKUPINAMI

Za potrebe nadaljnje analize smo izračunali indekse kot povprečja na obeh spremenljivkah strahu. Kot odvisne spremenljivke smo obravnavali vse trditve, ki so merile samozaznavanje vedenjskih sprememb zaradi COVID-19 krize. Shapiro-Wilkov test je pokazal, da nobena izmed spremenljivk ni normalno porazdeljena. V namen

Preglednica 6: Spearmanovi koeficienti korelacije in statistična značilnost povezav

Trditev	Indeks: Strah o prehranski varnosti med COVID-19 krizo - povprečje 5 indikatorjev	Indeks: Strah o prehranski varnosti v prihodnosti - povprečje 5 indikatorjev	Starost
Zaradi epidemije COVID-19 sem si naredil-a več prehranskih zalog kot ponavadi.	,300**	,218**	-0,085
Zaradi epidemije COVID-19 sem hrano naročal-a po spletu bolj pogosto kot ponavadi.	,230**	0,067	-0,047
Zaradi epidemije COVID-19 sem bil-a bolj pazljiv-a, koliko hrane zavržem.	,235**	,224**	0,107*
Zaradi epidemije COVID-19 sem začel-a kupovati hrano v trgovinah, kjer je običajno nisem.	,238**	,212**	-0,006
Zaradi epidemije COVID-19 sem bolj pogosto kupoval-a hrano neposredno od kmeta bodisi prek spleta oziroma na kmetiji.	,220**	,204**	0,130**
Zaradi epidemije COVID-19 sem se bolj osredotočil-a na nakup hrane, ki je pridelana in predelana v državi, kjer trenutno živim.	,116*	,162**	0,283**
Moje nakupovalne navade, se zaradi epidemije COVID-19 niso popolnoma nič spremenile. (rekodirano)	,351**	,166**	0,002

**Korelacija je statistično značilna s < 1 % tveganjem.
*Korelacija je statistično značilna s < 5 % tveganjem.

Preglednica 7: Neparametrični testi razlik po skupinah glede na izobrazbo, samouvrstitev v družbeni razred, velikost kraja bivanja in spol

	Kruskal-Wallisov test, statistična značilnost			Mann-Whitneyev U test za neodvisne vzorce, statistična značilnost
Trditev	Izobrazba	Samouvrstitev v družbeni razred	Velikost kraja prebivanja	Spol
Zaradi epidemije COVID-19 sem si naredil-a več prehranskih zalog kot ponavadi.	0,116	0,065	0,638	0,001**
Zaradi epidemije COVID-19 sem se bolj osredotočil-a na nakup hrane, ki je pridelana in predelana v državi, kjer trenutno živim.	0,000**	0,392	0,057	0,648
Zaradi epidemije COVID-19 sem bil-a bolj pazljiv-a, koliko hrane zavržem.	0,043*	0,066	0,225	0,001**
Moje nakupovalne navade se zaradi epidemije COVID-19 niso popolnoma nič spremenile. (rekodirano)	0,038*	0,146	0,029*	0,029*
Zaradi epidemije COVID-19 sem bolj pogosto kupoval-a hrano neposredno od kmeta bodisi prek spleta oziroma na kmetiji.	0,000**	0,001**	0,705	0,438
Zaradi epidemije COVID-19 sem začel-a kupovati hrano v trgovinah, kjer je običajno nisem.	0,206	0,615	0,054	0,715
Zaradi epidemije COVID-19 sem hrano naročal-a po spletu bolj pogosto kot ponavadi.	0,008**	0,282	0,036	0,772

*** Pozor: ti testi dajejo informacijo o statistični značilnosti razlik med skupinami in ne o moči povezave, kot je to zgoraj pri Spearmanovem koeficientu v Preglednici 6)
**Razlike po skupinah so statistično značilne s < 1 % tveganjem.
*Razlike po skupinah so statistično značilne s < 5 % tveganjem.

preverjanja povezav med samozaznavanjem sprememb vedenja in obeh tipov strahu ter starosti smo analizirali Spearmanove koeficiente korelacije in statistično značilnost povezav (Preglednica 6).

Rezultati so pokazali, da sta obe obliki strahu - tako strah o prehranski varnosti med COVID-19 krizo kot tudi strah o prehranski varnosti v prihodnosti - statistično značilno pozitivno povezani s skoraj vsemi oblikami samozaznavnih sprememb vedenja zaradi COVID-19 krize. Slednje je v skladu s psihološkimi teorijami o učinkih strahu na posameznikovo vedenje (Witte, 1994).

Edina izjema pri tem je trditev o naročanju hrane prek spleta - slednja je pričakovano v korelaciji s strahom o prehranski varnosti med COVID-19 krizo, ni pa povezana s strahom o prehranski varnosti v prihodnosti. Po eni strani je to sicer razumljivo čez prizmo, da strah o prihodnosti ne napove nujno vedenja v sedanjosti. Vendar pri vseh drugih oblikah vedenj najdemo statistično značilno povezanost, predvsem pa jo najdemo pri primerljivih vedenjih, to je spremembah nakupnih kanalov (nakup neposredno od kmeta v živo ali prek spleta in nakup v trgovinah, kjer običajno anketiranci niso prej nakupovali). Tako je vprašanje nakupovanja prek spleta potrebno raziskovati tudi v prihodnje, z namenom, da se ugotovi, zakaj ravno to vedenje odstopa od ostalih.

Analiza glede na starost (Preglednica 6) je pokazala srednje močne pozitivne povezave s starostjo pri treh trditvah od sedmih: pri pazljivosti glede zavržkov hrane, več nakupov neposredno od kmeta in bolj pogostih nakupih hrane, ki je pridelana in predelana v Sloveniji. V vseh treh primerih starejši statistično značilno izkazujejo spremembe vedenj v potrošnji zaradi COVID-19 krize. Pri ostalih oblikah merjenih sprememb potrošnih vedenj pa ni statistično značilne razlike glede starosti.

Kot zadnji korak so bili analizirani neparametrični testi povezanosti med samozaznavanjem sprememb vedenj zaradi COVID-19 in neodvisnimi spremenljivkami izobrazbe, samouvrstitve v družbeni razred, velikosti kraja prebivanja in spola. V preglednici 7 so navedeni rezultati Kruskal-Wallisovega testa in Mann-Whitneyega U testa za neodvisne vzorce. Kot kažejo rezultati o statistični značilnosti razlik rangov med skupinami, je izobrazba najpogosteje statistično značilno povezana s spremembami vedenja, sledi spol, medtem ko sta samouvrstitev v družbeni razred in velikost kraja prebivanja statistično značilno povezani zgolj v enem od primerov

Preglednica 8: Kruskal-Wallisov test, povprečje rangov glede na izobrazbo

Trditev	Izobrazba	N	Povprečje rangov
Zaradi epidemije COVID-19 sem hrano naročal-a po spletu bolj pogosto kot ponavadi.	Srednja šola ali manj	98	195,17
	Višja šola	76	202,16
	Univerzitetna izobrazba	205	247,04
	Specialistični študij, magisterij, doktorat	79	252,88
	Skupaj	458	
Zaradi epidemije COVID-19 sem bil-a bolj pazljiv-a, koliko hrane zavržem.	Srednja šola ali manj	98	228,65
	Višja šola	75	262,79
	Univerzitetna izobrazba	204	226,36
	Specialistični študij, magisterij, doktorat	80	204,48
	Skupaj	457	
Zaradi epidemije COVID-19 sem bolj pogosto kupoval-a hrano neposredno od kmeta bodisi prek spleta oziroma na kmetiji.	Srednja šola ali manj	97	185,28
	Višja šola	76	224,88
	Univerzitetna izobrazba	205	237,94
	Specialistični študij, magisterij, doktorat	80	265,87
	Skupaj	458	
Moje nakupovalne navade, se zaradi epidemije COVID-19 niso popolnoma nič spremenile. (rekodirano)	Srednja šola ali manj	96	187,73
	Višja šola	74	238,50
	Univerzitetna izobrazba	205	239,49
	Specialistični študij, magisterij, doktorat	80	237,16
	Total	455	
Zaradi epidemije COVID-19 sem se bolj osredotočil-a na nakup hrane, ki je pridelana in predelana v državi, kjer trenutno živim.	Srednja šola ali manj	97	226,49
	Višja šola	76	245,07
	Univerzitetna izobrazba	204	233,84
	Specialistični študij, magisterij, doktorat	80	204,44
	Total	457	

sprememb vedenj. V nadaljevanju so podrobneje podani rezultati o razlikah med skupinami glede na neparametrične teste samo za tiste primere vedenj, kjer so testi pokazali statistične razlike.

Kot je razvidno iz Preglednic 7 in 8, ima izobrazba pomemben vpliv na splošno vprašanje o spremembah nakupovalnih navad in na štiri oblike samozaznavnih vedenj: pazljivost pri poreklu hrane, pazljivost pri tem, koliko hrane zavržejo, pogostejše kupovanje hrane neposredno od kmeta, pogostejše naročanje hrane prek spleta. Anketiranci z višjo izobrazbo so pogosteje naročali prek spleta in neposredno od kmeta. Pri drugih oblikah učinkov pa razlike med skupinami anketirancev glede na njihovo izobrazbo niso tako enostavno začrtane v smeri višje izobrazbe (Preglednica 8). Npr. pri tem, koliko hrane zavržejo, so anketiranci s srednjo šolo ali manj in anketiranci z univerzitetno izobrazbo odgovarjali podobno, medtem ko so bili najmanj previdni anketiranci z najvišjo izobrazbo, najbolj previdni pa anketiranci z višješolsko izobrazbo.

Laborde in sod. (2020) opozarjajo, da bodo po COVID-19 krizi najbolj na udaru ekonomsko ranljive skupine. V naš vzorec smo uspeli zajeti predvsem skupine srednjega in višjega srednjega razreda. Kljub temu je število anketirancev, ki se sami uvrščajo v nižji srednji razred ali v delavski razred dovolj veliko, da podamo orientacijske primerjave razlik po skupinah. Pri skoraj vseh spremembah vedenja ni bilo razlik glede na samouvrstitev v družbeni razred. Izjema je zgolj vprašanje o nakupu hrane neposredno od kmeta - samo pri tem vprašanju se kažejo razlike po skupinah: s samouvrstitvijo v višji družbeni razred so anketirani pogosteje odgovarjali, da so zaradi COVID-19 pogosteje kupovali neposredno od kmetov.

Primerjava glede na kraj prebivanja (Preglednica 10) pokaže, da kraj prebivanja večinoma nima vpliva na spremembe potrošnih vedenj zaradi COVID-19. To je

Preglednica 9: Kruskal-Wallisov test, povprečje rangov glede na samouvrstitev v družbeni razred

	Samouvrstitev v družbeni razred	N	Povprečje rangov
Zaradi epidemije COVID-19 sem bolj pogosto kupoval-a hrano neposredno od kmeta bodisi prek spleta oziroma na kmetiji.	Nižji srednji razred, nižji razred ali delavski razred	77	170,95
	Srednji razred	231	214,06
	Višji srednji razred ali višji razred	116	236,97
	Skupaj	424	

Preglednica 10: Kruskal-Wallisov test, povprečje rangov glede na velikost kraja

	Velikost kraja prebivanja	N	Povprečje rangov
Moje nakupovalne navade se zaradi epidemije COVID-19 niso popolnoma nič spremenile. (rekodirano)	Do 2.000 prebivalcev	188	229,89
	Več kot 2.000 do 50.000 prebivalcev	178	221,24
	Več kot 50.000 prebivalcev	96	253,69
	Skupaj	462	

Preglednica 11: Ustvarjanje prehranskih zalog in zmanjševanje prehranskih odpadkov. Mann-Whitneyev U test neodvisnih vzorcev, statistično značilne razlike glede na spol

		N	Povprečje rangov	Vsota rangov
Zaradi epidemije COVID-19 sem si naredil-a več prehranskih zalog kot ponavadi.	Moški	98	189,67	18587,50
	Ženska	357	238,52	85152,50
	Skupaj	455		
Zaradi epidemije COVID-19 sem bil-a bolj pazljiv-a, koliko hrane zavržem.	Moški	98	190,37	18656,00
	Ženska	357	238,33	85084,00
	Skupaj	455		
Moje nakupovalne navade, se zaradi epidemije COVID-19 niso popolnoma nič spremenile. (rekodirano)	Moški	97	201,85	19579,50
	Ženska	356	233,85	83251,50
	Skupaj	453		

sicer v nasprotju z rezultati Kušar (2020). Izjema je odgovor na splošno trditev "Moje nakupovalne navade se zaradi COVID-19 niso popolnoma nič spremenile". Pri tej trditvi je najmanjše spremembe izrazila skupina anketirancev, ki prebivajo v krajih velikih med 2.000 in 50.000 prebivalcev. Za njimi so ljudje iz manjših krajev in najbolj so se po samonavajanju spremenile navade prebivalcev večjih mest.

Primerjava glede na spol pokaže statistično značilne razlike pri splošni trditvi o spremembah navad in dveh oblikah sprememb vedenja zaradi COVID-19 (Preglednica 11): ustvarjanju prehranskih zalog in zmanjševanju prehranskih odpadkov. V vseh treh primerih ženske izražajo višjo raven sprememb kot moški. Ne zasledimo pa nobenega vpliva spola na spremembe pri nakupnih kanalih.

4 SKLEPI

COVID-19 je kriza s pomembnimi posledicami, ki segajo v vse družbene pore. Obravnava posledic izrednih razmer zaradi epidemije COVID-19 zahteva na vseh ravneh - od dobavnih verig, prekinitev v trgovinski dejavnosti, do brezposelnosti in naraščajoče stopnje revščine, hitro, učinkovito in ciljno usmerjeno socialno zaščito za najbolj ranljive skupine prebivalstva (IFPRI, 2020). Vprašanje prehranske varnosti je v njej postalo pomembna točka razprave tako vladnih politik kot medijskega poročanja. Vprašanje o tem, v kolikšni meri vse to vpliva na strah potrošnikov o prehranski varnosti, do sedaj še ni bil odgovorjeno. Pričujoča raziskava v prvi vrsti podaja poglobljen in testiran merski instrument za spremljanje stopnje strahu o prehranski varnosti, ki ga je smiselno uporabiti za spremljanje sprememb odzivov potrošnikov v prihodnje, glede na spremembe, ki jim še bomo priča s COVID-19 krizo ter v obdobju po krizi.

Rezultati pričujoče raziskave so pokazali, da lahko samozaznane spremembe vedenj zaradi COVID-19 pojasnimo s stopnjo strahu o prehranski varnosti trenutno in v prihodnjih podobnih krizah. Obe merjeni obliki strahu: tako strah o prehranski varnosti med COVID-19 krizo in strah o prehranski varnosti v prihodnosti, sta namreč statistično značilno, srednje močno in pozitivno povezani s skoraj vsemi oblikami samozaznanih sprememb vedenja zaradi COVID-19 krize.

Prehranska varnost je v prvi vrsti povezana s prehransko suverenostjo neke države. Pri tem je pozitiven odnos lokalnih potrošnikov do nakupa hrane lokalnega izvora še posebnega pomena. V Sloveniji smo tako v zadnjem času priča številnih promocijskim kampanjam za ozaveščanje in spodbujanje potrošnikov za nakup slovenskih pridelkov in izdelkov (MKGP, 2020b, MKGP, 2020c, MKGP, 2020č in GZS-ZKŽP, 2020a, GZS-ZKŽP, 2020b). Kar po naših podatkih še posebej izstopa pri anketiranih potrošnikih je to, da so se anketiranci po lastnem navajanju zaradi epidemije COVID-19 najbolj osredotočili na nakup živil, ki so pridelana in predelana v Sloveniji. O vzrokih lahko v tem trenutku samo špekuliramo. Po eni strani bi morebiti lahko bilo zaradi povečanih javnih pozivov potrošnikov k nakupu lokalno pridelanega, po drugi strani pa tega, da so lokalni proizvajalci v prvem valu COVID-19 imeli težave z izvozom in so bile v prvih tednih vsaj deloma prekinjene nekatere uvozne verige za agro-živilske proizvode. So pa ti rezultati v nasprotju z napovedmi Boumphrey (2020), da bo poreklo hrane manj pomembno v COVID-19 krizi. Raziskava, ki vključuje pregled odziva potrošnikov na povpraševanje lokalnih prehranskih proizvodov iz 13 držav tako v Evropi kot izven, potrjuje tezo, da so potrošniki predvsem v prvih tednih po razglasitvi epidemije COVID-19 prednostno posegali po proizvodih lokalnega izvora, saj so le-te dojemali kot varne in trajnostne (Nemes in sod., 2021). Povečano povpraševanje in podporo potrošnikov lokalnim pridelovalcem gre med drugim pripisati vpeljavi strogih omejitev gibanja prebivalstva in zaprtju držav.

Pričujoča raziskava nadgrajuje opažanja, da je v preteklih desetih letih pri nakupu slovensko poreklo pridobilo na pomenu in v nasprotju s pričakovanji podpira tezo, da je COVID-19 kriza pripomogla k večji ozaveščenosti o nakupu hrane s slovenskim poreklom. Podrobnejša analiza pokaže, da je v analiziranem vzorcu ta odziv odvisen od starosti in izobrazbe, ne pa tudi od spola, samouvrstitve v družbeni razred ali kraja prebivanja. Bolj izobraženi in starejši prebivalci so statistično značilno bolj pogosto izrazili, da so zaradi COVID-19 nakupovali hrano s slovenskim poreklom.

Glede na napovedi, da bo epidemija COVID-19 prehransko najbolj prizadela najrevnejše dele prebivalstva, predlagamo v prihodnjih analizah večji poudarek na analizi ekonomskih faktorjev in odzivov, ki jih sproža finančna ogroženost. V prihodnosti je tudi treba primerjati te slovenske podatke z drugimi državami in analizirati, ali je pri tovrstnem odzivu Slovenija edinstvena, in na tak način med drugim analizirati uspešnost tovrstnih promocijskih kampanj za časa COVID-19.

Drugi najpogostejši odziv na COVID-19 krizo je bilo ustvarjanje večjih prehranskih zalog, kar je v skladu s podobnimi raziskavami (Gerhold 2020, Kušar 2020, Wang in sod. 2020, Janssen in sod., 2021). V nadaljnjih raziskavah bi bilo pomembno izvedeti, v kolikšni meri so pred COVID-19 krizo potrošniki sploh delali zaloge hrane (npr. urbani prebivalci). Pričujoča raziskava nadalje pokaže, da se glede ustvarjanja zalog različne skupine anketirancev niso razlikovale med seboj, edina izjema je spol, kjer so ženske delale več zalog - slednje je v skladu s pričakovanji tako glede družbenih vlog kot glede raziskav, ki so pokazale, da so moški manj prestrašeni v zvezi s COVID-19 (Turnšek in sod., 2020).

Podobno pričakovano je na tretjem mestu po pogostosti bila večja pazljivost pri tem, koliko hrane anketiranci zavržejo, kar je prav tako v skladu s predhodnimi raziskavami tako v Evropi (Kušar, 2020, Janssen in sod., 2021, Vidal-Mones in sod., 2021, Theodoridis in Zacharatos, 2022, Masotti in sod., 2023), kot izven nje (Jribi in sod. 2020, Everitt in sod., 2021, Chang in sod., 2022, Allahyari in sod., 2022, Deliberador in sod., 2023). Pazljivost glede prehranskih odpadkov je glede na pričujoče podatke odvisna od spola, starosti in deloma tudi izobrazbe. Ženske in starejši so bolj previdni glede tega, koliko hrane zavržejo. To je verjetno odraz družbeno določenih spolnih vlog v gospodinjstvu in zgodovinskih razlik, ki so oblikovale starejše generacije. Slednje se verjetno bolj spominjajo drugih podobnih kriz v zgodovini. Najvišje izobraženi anketiranci izkazujejo najmanjšo previdnost pri tem, koliko hrane zavržejo, pri čemer pa presenetljivo ni statistično značilnih razlik glede samouvrstitve v družbeni razred, in tudi ne glede na kraj bivanja.

Na zadnjem mestu pri spremembah vedenja, ki jih je po navedbah anketirancev povzročila COVID-19 kriza, pa so spremembe nakupnih kanalov. Starejši, višje izobraženi in ti, ki se samouvrščajo v višji družbeni razred so zaradi COVID-19 pogosteje nakupovali neposredno od kmeta. Kar bi ta rezultat lahko pomenil, je da za razliko od ostalih vedenj, nakup neposredno od kmeta izrazito statusni simbol, kar je v prid neposredni prodaji. Po drugi strani pa nakazuje razredne razlike v smeri, da si ekonomsko prikrajšani težje privoščijo nakup neposredno od kmetov in lažje nakupujejo v večjih trgovinah. Pri tem je treba opozoriti, da ni razlik glede na kraj prebivanja - da torej to, kjer anketiranci živijo ni imelo vpliva na povečanje neposrednega nakupa od kmetov zaradi COVID-19, kar je deloma presenetljivo, saj prebivalci v ruralnih okoljih načeloma lažje dostopajo do neposrednih nakupov. V prihodnje priporočamo pozornejše spremljanje trenda neposrednih nakupov in učinka COVID-19 krize na ta trend.

V pričujoči raziskavi presenečajo rezultati o spremembah nakupovanja hrane prek spleta. Medtem ko so v slovenskih medijih poročali, da se je večina potrošnikov odločila za nakup prehranskih izdelkov prek spleta (celo v tako množičnem številu, da je bilo spletno naročanje pri večjih trgovcih z živili v določenem obdobju onemogočeno (Siol.net, 2020), pa rezultati pričujoče raziskave tega ne potrjujejo. Večina anketirancev se namreč ni strinjala s trditvijo, da so zaradi epidemije COVID-19 hrano pogosteje naročali prek spleta; tovrsten odziv je bil med najredkejšimi odzivi na krizo. Manjša pogostost je bila samo še pri vprašanju, če so anketiranci začeli kupovati v trgovinah, kjer poprej niso kupovali.

Pričujoči rezultati nakazujejo potencialne pomembne razlike pri tem, kako anketiranci gledajo na prehransko varnost. Največjo stopnjo strahu so izražali na kolektivni globalni ravni in na ravni Slovenije, manjšo stopnjo pa na individualni ravni, kjer se nakazuje miselnost, da medtem ko je družba ogrožena, se bo posameznik »sam že znašel«. V prihodnje bi bilo treba primerjati te rezultate z rezultati drugih držav, analizirati vpliv posameznikovega ekonomskega položaja in tudi v kolikšni meri sta vrtnarjenje in možnost pridelave hrane v urbanih naseljih mehanizma, s katerim si ljudje zmanjšujejo individualno raven strahu o prehranski varnosti v prihodnosti (Lal, 2020, Pulighe in Lupia, 2020). V prihodnje je potrebno nasloviti tudi dve osrednji omejitvi pričujoče raziskave: nereprezentativnost vzorca in samozaznavanje učinkov krize. Medtem ko je prvo omejitev smiselno nasloviti z raziskavo, ki bo zajela večji in bolj reprezentativen vzorec, drugo omejitev naslovimo tako, da v prihodnosti pričujoče podatke kombiniramo z analizami dejanske potrošnje na ravni države – pričujočo raziskavo pa uporabimo kot vpogled v razmišljanje uporabnikov za časa zgodovinsko relevantnega obdobja prvega vala COVID-19 v Sloveniji.

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doi:10.14720/aas.2023.119.1.1105 Original research article / izvirni znanstveni članek

Evaluating land suitability for *Rhus coriaria* L. (Sumac) by habitat suitability model

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Received March 30, 2019; accepted February 28, 2023.
Delo je prispelo 30. marca 2019, sprejeto 28. februarja 2023

Evaluating land suitability for *Rhus coriaria* L. (Sumac) by habitat suitability model

Abstract: The cultivation of *Rhus coriaria* has become necessary to preserve their wild populations. To be competitive in the international market, it is important to develop an efficient production chain to reduce costs and improve the quality of the products. The main objective of this study is to provide a method to determine the suitable areas to develop the *R. coriaria* cultivation with a case study in Gonabad County of Iran. A habitat suitability model (HSM) was applied to survey the distribution of *R. coriaria* and to identify the best areas the growing of its. Three different main criteria including environmental suitability, agronomic suitability, and social-economical suitability selected for the HSM. Then, each of the three main criteria and their multi-specific indicator was defined in Analytic Hierarchy Process (AHP) and the weights of them were calculated by pairwise comparison matrix. In the next stage, the weights are applied to their layers such as hypsometry, slope, slope aspect, mean annual precipitation, mean annual temperature, soil texture, landuse, water resource type, water resource quality and quantity, road network, and land ownership as roaster layers. The results of the HSM showed a weighted map of land suitability for the *R. coriaria* that included the maximum and minimum potential of areas for its planting. Based on these results, the areas with the highest suitability for the *R. coriaria* are strictly associated with precipitation, soil texture, and water resources type.

Key words: AHP; cultivation; HSM; SMCDM; spice; Sumac

Ovrednotenje primernosti zemljišč za uspevanje strojilnega octovca (*Rhus coriaria* L.) z modelom primernega habitata

Izvleček: Gojenje strojilnega octovca (*Rhus coriaria* L.) postaja nuja za ohranjanje njegovih populacij v naravi. Za obstoj konkurenčnosti na mednarodnih trgih je pomembno razviti učinkovito proizvodno verigo za zmanjšanje stroškov in izboljšanje produktov iz te rastline. Namen te raziskave je bil razviti metodo določanja primernih območij za gojenje strojilnega octovca z vzorčno študijo v provinci Gonabad, Iran. Pri popisu razširjenosti strojilnega octovca je bil uporabljen model primernega habitata (A habitat suitability model -HSM) za določitev najboljših območij za njegovo uspevanje. Za model HSM so bili izbrani trije glavni kriteriji, ki so obsegali okoljsko, agronomsko in socio-ekonomsko primernost. Nato so bili vsem trem glavnim kriterijem določeni multispecifični indikatorji v analitičnem hierarhičnem procesu (AHP), kjer so bile njihove uteži izračunane na osnovi relativnih prioritet. V naslednji fazi so bile uteži uporabljene za parametre kot so razgibanost reliefa (hipsometrija), nagib terena, poprečna letna količina padavin, poprečna letna temperatura, tekstura tal, raba tal, vrsta, količina in kakovost vodnih virov, razvitost cestnega omrežja in lastništvo zemljišča kot glavne plasti. Rezultati HSM modela so pokazali uravnoteženo karto primernosti zemljišč, ki je vsebovala maksimalno in minimalno potencialno primerna območja za gojenje te vrste. Na osnovi teh rezulatov so najbolj primerna območja za gojenje strojilnega octovca tesno povezana s padavinami, teksturo tal in vrsto vodnega vira.

Ključne besede: AHP; gojenje; HSM; SMCDM; dišava; strojilni octovec

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1 INTRODUCTION

Rhus coriaria L. which is commonly known as Sumac is widely growing throughout Middle Eastern countries such as Iran. Sumac is a very popular spice in food production. It gives a sour lemon taste to food and is consumed for various foods, spatially meat dishes (Morshedloo et al., 2018).

The cultivation of *R. coriaria* has become necessary to preserve their wild populations. Also, to be competitive in the international market, it is important to develop an efficient production chain to reduce costs and improve the quality of the products. For developing a wild crop in a particular area, such as *R. coriaria*, land suitability analysis is a requisite to achieve optimum exploitation of the available land resources for sustainable agricultural production (Nisar et al., 2000).

To evaluate land suitability for a specific species, it is important to know its specific habitat of it (Barbaro et al., 2011). The habitat is an area with a combination of resources (such as food, cover, water) and environmental conditions (temperature, precipitation, presence or absence of predators and competitors) that promotes the occupation of individuals of a certain species (or population) and allows to those individuals to survive and reproduce (Morrison et al., 2006). For plants, the habitat suitability models (HSMs) are tools to analyze the best areas for growing using land knowledge (Hirzel et al., 2001). The HSMs are used both to predict the distribution of a specific plant species and to identify the best areas for its growth (Guisan & Zimmermann, 2000). HSMs allow being evaluated the quality of the habitat for a species within its study area. In GIS, HSMs apply land suitability to layers such as land use, elevation, slope, slope aspect, roads network, water sources, and other important factors as a raster-based layer (Barbaro et al., 2011). One of the common ways to build the LSM is a literature review and expert opinion. The procedure requires expert knowledge to assign a weight to each factor and a land suitability score to each class within a factor. Suitability scores for all factors are then combined to form a single land suitability map with a suitability score for each pixel (Barbaro et al., 2011).

One of these solutions for this purpose is using the spatial multi-criteria decision making (SMCDM) methods. Integrating the geographical information systems (GIS) with multi-criteria decision making (MCDM) methods leads to SMCDM methods (Malczewski, 1999).

The analytic hierarchy process (AHP) is one of the main methods of the MCDM which can be used for allocating weights to indicators. The AHP is a mathematical model which was developed for solving the multi-criteria decision making by Saaty in 1977. The most important abilities of it consider both quantitative and qualitative criteria (Taslicali & Ercan, 2006). In general, the AHP model is composed of a goal, criteria, sub-criteria, and alternatives (Buyukyazc & Sucu, 2003).

A GIS-based model can be applied for conservation planning and regional management and determining the best growing areas (Barbaro et al., 2011). Several GIS models have been developed to evaluate the suitability of cropland. Sicat Rodrigo et al. (2004) introduced a fuzzy modeling method to evaluate farmers' knowledge to create a cropland suitability classification. Liu et al. (2006) showed methods to analyze the land suitability in the Qinling Mountains of China. Another method was developed by Pirbalouti (2009) to select the best patterns of cropping at a regional level. Recently, a methodology was developed to analyze land suitability for forest plantations (Dengiz et al., 2010). Also, Hua et al. (2010) introduced a GIS-based prediction methodology for the conservation planning of medicinal plant distributions.

Many types of research have been done in the field of environmental management based on MCDM methods. Recently, the AHP-Fuzzy method was used to evaluate rangeland suitability for livestock grazing in the Bagheran Birjand watershed of Iran (Rouhi-Moghaddam et al., 2017). Two methods of MCDM (AHP and analytic network process) were used to estimate the potential areas of flooding in Kakhk paired catchment in Iran and were compared with each other (Eshghizadeh, 2017). Also, the AHP method was used to prioritize and determine the most important factors that affected sediment yield in a semi-arid region of Iran (Eshghizadeh et al., 2015). The groundwater artificial recharge suitable area was determined by GIS and AHP methods in the Silakhor, Borujerd of Iran (Mehrabi et al., 2012). All results showed that the AHP method can prioritize the environmental criteria.

R. coriaria, commonly called sumac, is a deciduous shrub to a small tree in the Anacardiaceae family. The *R. coriaria* grows up to 5 meters and has composite leaves of 9 to 15 leaflets that are covered with cracks. It is in flower from March to April and is hermaphrodite. It can grow in all three types of sandy, loamy, and clay soil texture but prefers well-drained soil. Suitable soil reaction is from acid to neutral and basic. It is not shade-tolerant. It can grow in both dry and moist soil. The *R. coriaria* is a native shrub in southern Europe and western Mediterranean and Iran (Shahrokhi, 2015).

Due to the occurrence of perennial droughts in the east of Iran, many tree and shrub species such as figs, almonds, pomegranates, and grapes were destroyed or severely damaged. While the *R. coriaria* showed good resistance to drought. Based on local farmers' experience and knowledge, this species is one of the most suitable species for development in this area. In the northeastern regions of Iran, which are affected by drought, identifying areas that are suitable for cultivation of the *R. coriaria*, especially in the form of biological and biomechanical watershed management plans, or replacing them as a compatible species by watershed stakeholders can be effective in improving socio-economic status and soil and water protection. Therefore, the main objective of this study is to provide a method to determine the suitable areas to develop the *R. coriaria* cultivation with a case study in Gonabad County, which has the highest area of the *R. coriaria* in the east of Iran. For this purpose, the HSM by integrating GIS and AHP has been developed to survey the current distribution of the *R. coriaria* and to determine the land suitability for its cultivation.

2 MATERIALS AND METHODS

A habitat suitability model of *R. coriaria* was developed by a procedure including three steps: i) the definition of an analytical hierarchical model; ii) the preparation of data; iii) the implementation of the procedure on the raster layers in the GIS.

2.1 DEFINITION OF THE ANALYTICAL HIERARCHICAL MODEL

An analytical hierarchical model is defined in an analytic hierarchy process (AHP). In general, an AHP model is composed of a goal, criteria, sub-criteria, and alternatives (Buyukyazc and Sucu, 2003). Because of the success a supply chain of *R. coriaria* relies on the satisfaction of different criteria, three different main criteria have been evaluated from different aspects:

- environmental suitability criteria
- agronomic suitability criteria
- socio-economic suitability criteria.

For each of the three main criteria, multi-specific indicators (sub-criteria) were defined in the AHP model as shown in Figure 1. This classify was done based on the main factors that control the growth of *R. coriaria* in the study area (Shahrokhi, 2015; Saghari et al., 2017).

In the AHP model, the weight of each main criteria and their sub-criteria were calculated by a pairwise comparison matrix. In a pairwise comparison matrix, the elements of one level of the hierarchy model are compared as a pairwise judgment based on Table 1. For each level of the hierarchy, a matrix of relative weights is generated based on the results of the pairwise comparisons. Since pairwise comparisons are based on personal judgments, consistency among pairwise comparisons has to be verified. This verification is done by determining consistency ratios computed for each pairwise comparison (Saaty, 1980).

The matrices of the pairwise comparisons were entered into the Expert choice (EC) program. The EC program presents a graph of the weights and shows their inconsistency. In general, if the inconsistency rate is less than 0.1, the inconsistency is acceptable. If more than 0.1 should be revised in the judgments (Saaty and Vargas, 2006).

Table 1: Saaty's fundamental scale (Saaty, 1980)

Intensity of importance	Definition
1	Equal importance
3	Moderate importance
5	Strong importance
7	Very strong importance
9	Extreme importance
2, 4, 6, 8	Intermediate values

2.2 PREPARATION AND ANALYSIS OF DATA

Data for three main criteria and their multi-specific indicator (sub-criteria) were prepared in a GIS environment (ILWIS 3). The hypsometry, slope, and aspect maps were created by a digital elevation model (DEM). The source of DEM was topographic maps that were prepared from the database of the natural resources and watershed management department of Gonabad County. The original spatial resolution of the map was 30 x 30 m that due to the large volume of data and area of the study area became 100 x 100 meters to run the calculations in GIS layers.

The main source of climatic data was the national meteorological station located in the northeast of Iran. For this purpose, stations located up to a radius of 100 km in the study area, including 15 synoptic, climatological, and evaporative stations were used. After examining the correlation relationships between stations, the mean annual precipitation and temperature maps were created by calculating the correlations between temperature and precipitation with the altitude of each station and applying their gradient equation on the DEM map of the study area.

The soil, landuse, water resources, road network, and land ownership maps were obtained from the database of the department of natural resources and watershed management in Gonabad County. All the layers were classified based on the optimal conditions for *R. co-*

riaria (Figure 1) in the ILWIS 3. The final weight of each class for criteria and sub-criteria was calculated via multiplying the relative importance of criteria by the relative importance of their sub-criteria and classes. Then, the final calculated weights in the AHP model were imported to the classes of each layer in the ILWIS 3. In the next stage, the weight map of each criterion was prepared by applying the sum operator on their sub-criteria layers in ILWIS 3. In the final, a value map of land suitability was calculated by summing of the weight maps of environmental, agronomic, and social-economical layers.

2.3 STUDY AREA

The study area was Gonabad County, in Razavi Khorasan province, northeast of Iran. The study area is part of the Dasht-e Kavir of Iran and is located in the east of it (Figure 2). The elevation range is between 839 and 2830 meters above sea level. The general slope of the area is from south to north in the mountain. The average annual precipitation is 148 mm in the Gonabad synoptic station. Also, the average annual evaporation is 1800 mm and the temperature is 17.5 °C. The current climate in the study area is arid in the north to semi-arid in the south.

3 RESULTS AND DISCUSSION

The evaluation procedure aimed to create an HSM by MCDM method at a regional scale, following the principle of the best available data. The implementation of the land evaluation procedure allowed the most suitable areas for cropping *R. coriaria* on the considered territory to be identified.

In HSM must be considered the ecologic, agronom-

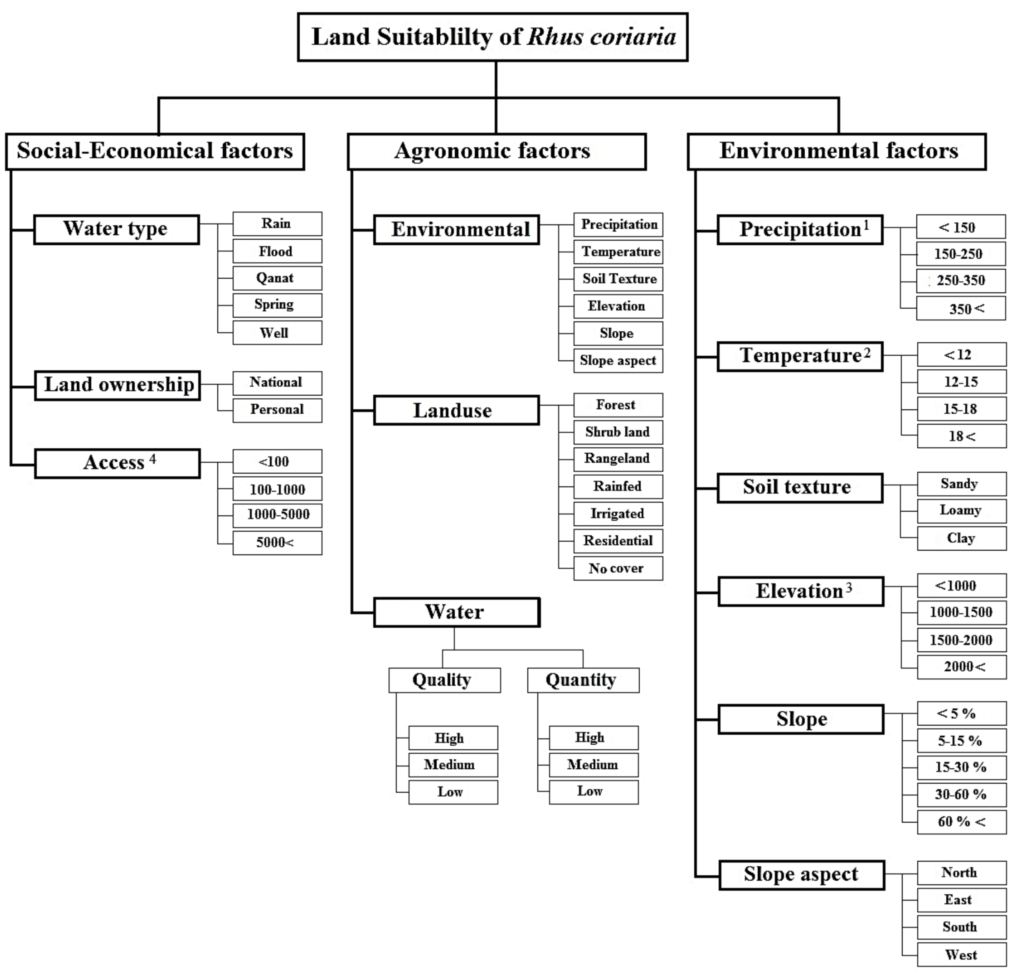

Figure 1: Analytical hierarchical model of the three main criteria and their specific indicator and classes in the AHP model for evaluating land suitability of *R. coriaria*, [1]: Average annual precipitation in mm, [2]: Average annual temperature in °C, [3]: Elevation range is in meter above sea level, [4]: Distance in meters (Saaty, 1980)

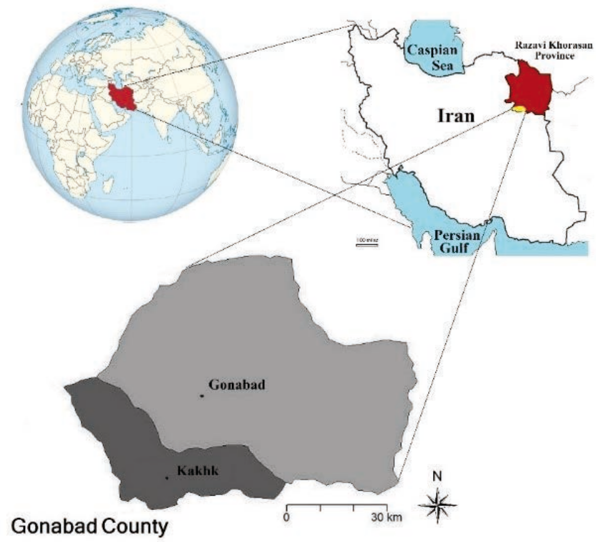

Figure 2: Location of the studied area

ic, logistic, and socio-economic aspects of a plant species for development (Barbaro et al., 2011). In this study, the main factors of them were also considered for cropping *R. coriaria*. Then, the synthesis weights them calculated by the AHP model. The synthesis weights of sub-criteria and classes are shown in Table 2. The results indicate the priority of the main factors in the development of *R. coriaria* planting. Table 3 shows the priority of the sub-criteria for cropping *R. coriaria* in the study area. Based on the results, the most important sub-criteria for cropping *R. coriaria* is precipitation (0.306) and the lowest importance of them was landuse (0.009).

Based on the results, the synthesis weights were imported on the classes of sub-criteria layers in ILWIS 3 and were created synthesis weighting maps (Figure 3). This figure shows the variation of the weight for cropping *R. coriaria* in the study area. For some sub-criteria such as precipitation, temperature, elevation, water resources, and slope a uniform and directional distribution can be considered for them, which shows that they are more influenced by the physiographic characteristics of the region. But some other sub-criteria such as soil texture, slope aspect, water resource type, land ownership, access, and landuse with non-distributed and no specific direction can be predicted for them.

This study, like the study of Barbaro et al (2011), provided a method for the decision-making process to develop a product on a regional scale. The results of Barbaro et al. (2011) for the development of medicinal plants in Italy, the parameters of elevation (above sea level) and distance of road had the greatest impact on locating suitable places for the development of medicinal plants. In this method, the role of elevation as a direct effect to calculate precipitation through the precipitation gradient and digital elevation model can be seen in the precipitation parameter. Therefore, it can be said that elevation is one of the most important parameters in the cultivation of medicinal plants such as *R. coriaria*. The results of Ghasemi Pirbalouti et al. (2011) confirmed that elevation alone did not affect land suitability, because this factor affected climatic, soil, and agronomic management variables. Also, Hirzel et al. (2001) has confirmed that climatic parameters are among the most important factors that control species' distribution. Therefore, topography mostly affects species indirectly through its correlation with climatic parameters.

As a main result, the factors derived from Digital Elevation Model (e.g. slope, aspect, precipitation, and temperature) are often crucial for the growth of plants, because they control local conditions of light, soil moisture, temperature, soil stability, and nutrient leaching, etc.

After integrating synthesis weights maps, the suitability map was created in the ILWIS 3. Figure 4 shows the final suitability map for *R. coriaria* in Gonabad County. This map showed that the maximum integrated weight for the growth of *R. coriaria* located in the south of the study area. Based on the results, the potential cropping surface for the *R. coriaria* was about 61410 ha (11.9 %), whereas 12819 ha (2.5 %) of these have been classified with very high and high suitability for *R. coriaria*.

Using the analytical hierarchical model showed that the areas that were more suitable for *R. coriaria* cropping had a higher weight in precipitation, soil texture, and water resources type sub-criteria (Figure 5). In particular, it can be seen that the most suitable areas for *R. coriaria* are the mountain areas. The studied was done by Morshedloo et al. (2018) showed that the habitat of the *R. coriaria* is a mountain of an arid, semi-arid, and inferior semi-arid region.

According to studies, the value of a factor can affect other parameters (Eshghizadeh et al., 2016). For example, Saghari et al, (2017) have expressed that the growth and development of *R. coriaria* at altitudes less than 2000 meters in the study area, make clear the effect of the slope aspect on it. Therefore, the method used in this research can be a suitable method for integrating different factors to calculate the final map of land suitability for *R. coriaria*. In similar research, Ghasemi Pirbalouti et al. (2011) were used a GIS-based suitability model by agroecological variables such as soil, climate, and topographical environmental components to identify suitable areas for chamomile (*Matricaria chamomilla* L.) production in Iran. Also, this result, like the studies conducted by Eshghizadeh et al. (2015), Eshghizadeh and Noura (2013), Hajkowicz and Collins (2007), showed that the AHP can be used in the studies of natural resources.

Table 2: Calculated synthesis weights of sub-criteria and classes by AHP model

sub-criteria and classes	synthesis weight	sub-criteria and classes	synthesis weight
Precipitation (mm) = 0.306		Slope aspect = 0.064	
> 350	0.168	North	0.041
250-350	0.099	East	0.015
150-250	0.028	South	0.004
< 150	0.011	West	0.004
Soil texture = 0.261		Elevation (m) above sea level = 0.057	
Sandy	0.128	> 2000	0.033
Loamy	0.116	1500-2000	0.018
Clay	0.018	1000-1500	0.004
		< 1000	0.002
Temperature °C = 0.042		Slope % = 0.021	
> 15	0.002	> 60	0.001
15-18	0.004	30-60	0.008
12-18	0.018	15-30	0.018
< 12	0.018	5-15	0.002
		< 5	0.001
Environmental of agronomy = 0.078		Water = 0.038	
Soil texture	0.035	Quality = 0.019	
Precipitation	0.023	High	0.013
Slope aspect	0.002	Medium	0.005
Elevation	0.004	Low	0.001
Temperature	0.007	Quantity = 0.019	
Slope	0.006	High	0.013
		Medium	0.004
		Low	0.001
Landuse = 0.009		Water resource type = 0.099	
Forest	0.003	Rain	0.048
Irrigation agriculture	0.002	Flood	0.037
Shrubland	0.002	Spring	0.007
Rainfed	0.001	Qanat	0.006
Range	< 0.001	Well	0.003
Residential	< 0.001		
Rocky and unusable	< 0.001		
Land ownership = 0.015		Access (m) = 0.010	
National land	0.013	> 5000	< 0.001
Personal land	0.002	1000-5000	0.002
		100-1000	0.002
		<100	0.006

Table 3: Priority of the sub-criteria for cropping R. coriaria in the study area

Priority	Sub-criteria	Priority	Sub-criteria
1	Precipitation	7	Temperature
2	Soil texture	8	Water
3	Water resource type	9	Slope
4	Environmental of agronomy	10	Land ownership
5	Slope aspect	11	Access
6	Elevation	12	Landuse

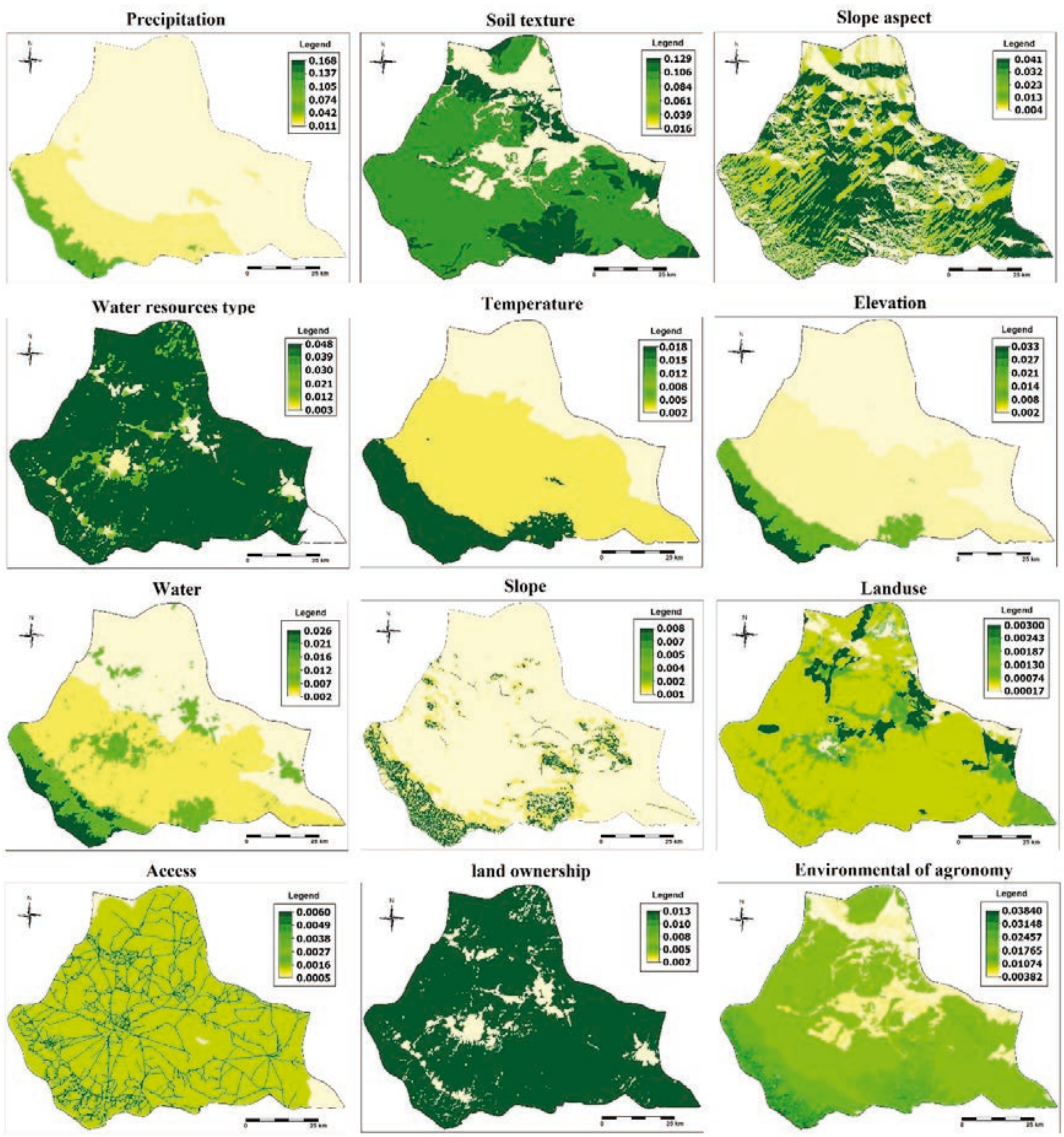

Figure 3: Synthesis weight maps of multi-specific indicator (sub-criteria) for *Rhus coriaria* in the study area. A higher value represents more suitable conditions for the growth of *Rhus coriaria*

The total area of *R. coriaria* in the studied area has been reported 1050 ha (Ministry of Agriculture Jihad, 2017). Based on the results, the HSM showed that all lands in the studied area with the very high suitability for the planting of *R. coriaria* have been implemented before time by native stakeholders based on native knowledge. However, only 6.7 % of the lands with high suitability have been cultivated by them. The fruits of *R. coriaria* have great economic importance as a natural resource of bioactive compounds and its consumption has been increasing around the world (Abu-Reideh et al., 2014; Kizil & Turk, 2010; Shabbir, 2012).

However, the total production of *R. coriaria* in the studied area has been reported as 162.5 tons/year (Ministry of Agriculture Jihad, 2017). By cultivating *R. coriaria* in lands with high and very high suitability, the production of it can be reached up to 3200 tons/year. Moreover, vegetation, directly and indirectly, affects runoff, erosion, and sediment (Eshghizadeh et al., 2016). The canopy cover, litter, and roots of *R. coriaria* can reduce surface runoff and soil loss. To develop a spatial crop in a region, Barbaro et al. (2011) emphasize that a crop-land suitability analysis must be done as a prerequisite to achieving sustainable agricultural production. However, the results

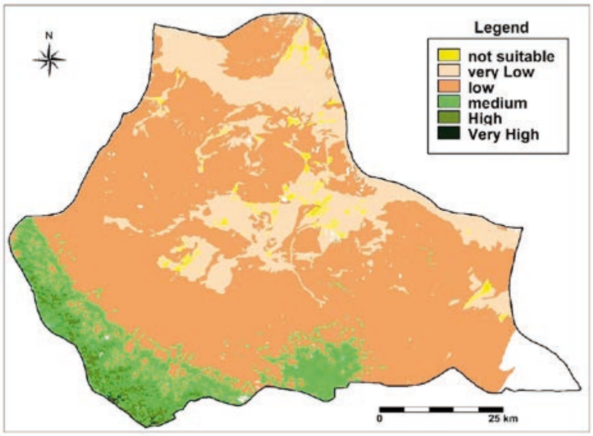

Figure 4: Suitability map for *Rhus coriaria* in the study area

of an HSM directly depend on the input data, selected factors, and evaluation procedure. Therefore, different factors and weights will determine different output habitat suitability maps. However, the main limiting factors in an HSM that can be considered are the geomorphology (slope and elevation), climate (precipitation), and agronomic management. Also, the evaluation model for this purpose does not include other aspects such as competition with other crops that could be considered in the assessment of the study area.

4 CONCLUSIONS

This research reports the creation of a methodology to evaluate land suitability of *R. coriaria* and can be used to biological management in natural resources. A weighted map of land suitability for *R. coriaria* shows the maximum and minimum potential of areas for its planting. This information helps to compare and rank subcatchments, catchments, basins, and watersheds for the development of its cultivation.

The presented method has some specific characteristics: i) It is not specific to a particular plant and can be used for different plant species; ii) it can be easily repeated in different areas; iii) the repeatability allows a reduction in the costs of the procedure implementation and easy repetition of the procedure in the case of missing or incorrect input data.

This methodology, based on data layers, can consider environmental adaptation, productivity, quality of the production, and logistics requirements for specific plant production. Also, it can provide information for local governments to select optimum landuse plans at a regional scale. The method used in this research can be easily adapted to different plants. For the application of it, a dataset of grid layers of land characteristics and defining the special parameters for a specific plant are required as

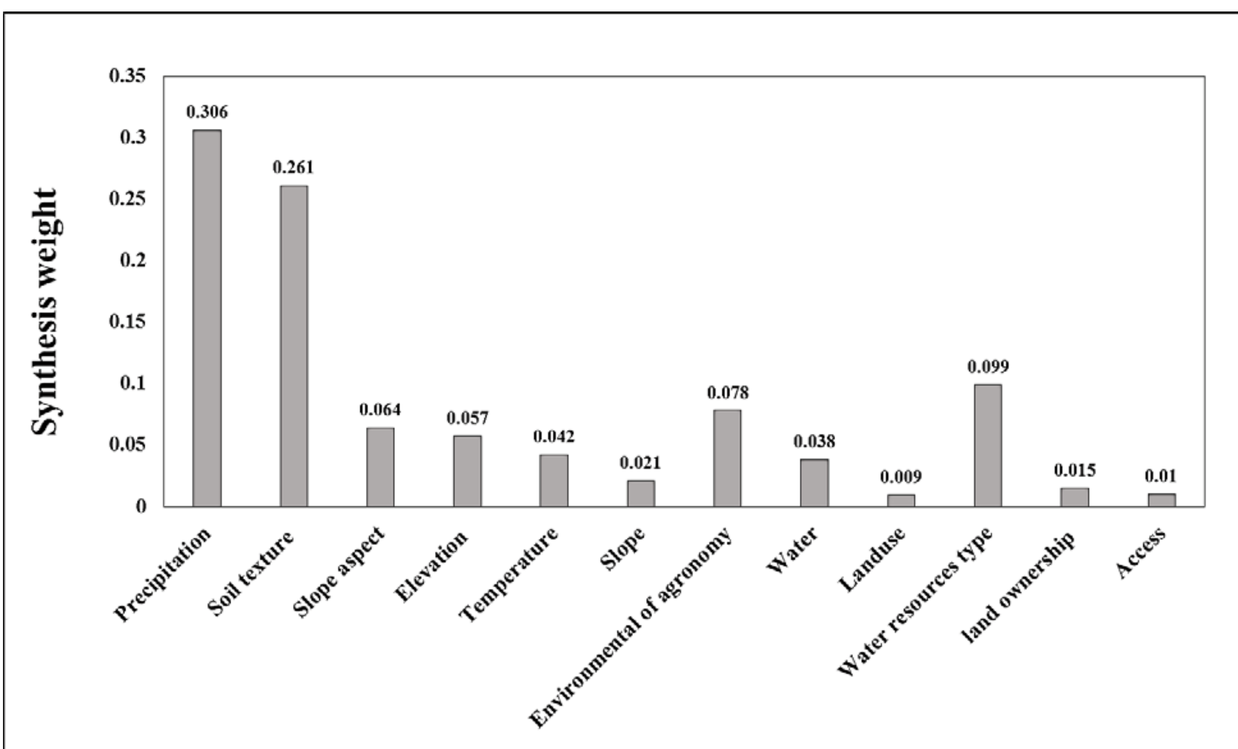

Figure 5: Synthesis weight of multi-specific indicator (sub-criteria) of the three main criteria

inputs. Spatially, biological measures in watershed management plans can be used to select and prioritize the lands for planting species. Therefore, the success rate of these projects can be increased.

5 CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this manuscript.

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doi:10.14720/aas.2023.119.1.2994 Original research article / izvirni znanstveni članek

Kakovost kosil in večerij v dijaškem domu v Ljubljani

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Received January 05, 2022; accepted March 03, 2022.
Delo je prispelo 5. januarja 2022, sprejeto 3. marca 2022

The quality of lunches and dinners in the dormitory in Ljubljana

Abstract: Due to intensive growth and numerous physiological changes durning adolescence a healthy diet is essential. Meals in dormitories must be planned in accordance with the Guidelines for Healthy Eating in Educational Institutions (Guidelines). The purpose of the research was to determine the energy and nutritional value of lunches and dinners in the dormitory in Ljubljana and to assess their compliance with the Guidelines. We weighed each ingredient of the meal and determined the energy and macronutrient content using the Open Platform for Clinical Nutrition. The students assessed the sensory acceptability of the meals using a 9-point hedonic scale. The average energy values of the lunches were in the line with the recommendations for energy intake for girls and lower than recommended for the boys. The average energy value of dinners was too high for girls in all three weeks and too low for boys in first week, but in the line in the last two weeks. The values of energy consumed with proteins in lunches were higher than recommended. The values of energy consumed with carbohydrates in lunches and dinners were lower than recommended. The intake of energy with fats was higher than recommended in lunches of the second week and in all three weeks' dinners. On average, lunches and dinners contained a sufficient amount of dietary fibre. The average hedonic score was 6.5 ± 1.9 for the lunches and 6.6 ± 1.9 for the dinners.

Key words: adolescent nutrition; dormitory; energy value; nutritional value; sensory acceptability; online tool OPKP

Kakovost kosil in večerij v dijaškem domu v Ljubljani

Izvleček: Zdrav način prehranjevanja je v obdobju mladostništva, zaradi intenzivne rasti in številnih fizioloških sprememb, izjemno pomemben. Obroki v dijaških domovih morajo biti načrtovani skladno s Smernicami za prehranjevanje v vzgojno-izobraževalnih zavodih (Smernice). Cilj raziskave je bil ovrednotiti energijsko in hranilno vrednost kosil in večerij v dijaškem domu v Ljubljani ter oceniti njihovo skladnost s Smernicami. Posamezne jedi v ponujenih obrokih smo stehtali ter s spletnim orodjem Odprta platforma za klinično prehrano ovrednotili vsebnost energije in makrohranil. Dijaki so z 9-točkovno hedonsko lestvico ocenjevali senzorično sprejemljivost obrokov. Rezultati so pokazali, da so bile povprečne energijske vrednosti kosil skladne s priporočili za energijski vnos za dekleta, za fante pa so bile premajhne. Povprečna energijska vrednost večerij je bila v vseh treh tednih večja od priporočene za dekleta, medtem ko je bila za fante premajhna v 1. tednu in skladna v drugih dveh tednih. Povprečni vnosi energije z beljakovinami s kosili so bili preveliki, v nasprotju pa so bili povprečni vnosi energije z ogljikovimi hidrati s kosili in večerjami premajhni v vseh treh tednih. Dijaki so v povprečju preveč energije zaužili z maščobami. Povprečna hedonska ocena kosil na 9-točkovni hedonski lestvici je bila 6,5 ± 1,9, povprečna hedonska ocena večerij pa 6,6 ± 1,9.

Ključne besede: prehrana mladostnikov; dijaški dom; energijska vrednost; hranilna vrednost; senzorična sprejemljivost; spletno orodje OPKP

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1 UVOD

V obdobju mladostništva je zdrav način prehranjevanja izjemno pomemben, saj je to čas intenzivne rasti in razvoja. V tem času mladostniki pridobijo 50 % končne telesne mase, znatno pa se poveča tudi njihova kostna masa (do 40 %) (Das in sod., 2017; Baxter-Jones in sod., 2011). Hitra rast in mnoge fiziološke spremembe povzročijo povečane potrebe po energiji in hranilih (Blunt in sod., 2020). Nezadosten energijski vnos lahko upočasni rast ter zakasni spolni razvoj in dozorevanje, hkrati pa energijski vnos ne sme biti prevelik, saj ta vodi v čezmerno hranjenost in debelost (Savarino in sod., 2021). Priporočen dnevni energijski vnos za mladostnike stare od 15 - 18 let, z normalno telesno maso in višino ter starosti prilagojeno zmerno telesno dejavnostjo je 12552 kJ (3000 kcal) za fante in 9623 kJ (2300 kcal) za dekleta (NIJZ, 2020). Razširjenost čezmerno hranjenih otrok ter mladostnikov, starih od 5 - 19 let, se je v zadnjih 50 letih s 4 % povzpela na nekaj več kot 18 % (WHO, 2021). Rezultati raziskave Z zdravjem povezano vedenje v šolskem obdobju (angl. Health Behaviour in School-Aged Children (HBSC)), ki so jo v Sloveniji petič izvedli leta 2018, kažejo, da je kar 18 % slovenskih mladostnikov čezmerno hranjenih in debelih (Jeriček Klanšček in sod., 2019). Predpostavimo lahko, da je današnje stanje še slabše, saj je Poročilo o telesnem in gibalnem razvoju otrok in mladine v šolskem letu 2019/20 pokazalo, da je bila telesna masa osnovnošolcev največja v zgodovini spremljanja, trend naraščanja telesne mase v šolskem letu 2019/20 pa je bil neprimerljivo večji od trendov med posameznimi leti v zgodovini spremljanja. Pomembna ugotovitev je bila tudi, da je bil porast maščobne mase za več kot tretjino bolj izrazit od porasta skupne mase, kar nakazuje na povečevanje zamaščenosti pri fantih in dekletih. Nastala situacija je negativna posledica vplivov ukrepov za zajezitev koronavirusa (Starc in sod., 2020).

V obdobju mladostništva posamezniki prevzamejo tudi večjo odgovornost za lastne prehranske navade in velika verjetnost je, da bodo pridobljene prehranske navade ohranili tudi kasneje v življenju (Blunt in sod., 2020). Vzgojno-izobraževalni zavodi, kamor sodijo tudi dijaški domovi, imajo lahko pri oblikovanju dobrih prehranskih navad pomembno vlogo (Bosanac in sod., 2016). V Sloveniji področje šolske prehrane ureja Zakon o šolski prehrani (Ur. l. RS, št. 3/13). V 4. členu je navedeno, da se pri organizaciji šolske prehrane upoštevajo Smernice za prehranjevanje v vzgojno-izobraževalnih zavodih, ki so bile sprejete na Strokovnem svetu Slovenije za splošno izobraževanje. Smernice vsebujejo strokovne usmeritve in navodila, ki opredeljujejo merila za izbor živil, načrtovanje sestave, količinske normative in način priprave šolske prehrane in časovni okvir za njeno izvedbo, ki jih določi javni zdravstveni zavod, pooblaščen s strani ministrstva, pristojnega za zdravje (Zakon o šolski prehrani, 2013).

Na podlagi Smernic zdravega prehranjevanja v vzgojno-izobraževalnih zavodih iz leta 2005 (Gabrijelčič Blenkuš in sod., 2005) je bil leta 2008 izdan Praktikum jedilnikov zdravega prehranjevanja za otroke in mladostnike v vzgojno-izobraževalnih ustanovah (Hlastan Ribič in sod., 2008) in Priročnik z merili kakovosti za živila v vzgojno-izobraževalnih ustanovah (Pograjc in sod., 2008). Namen Praktikuma je bil približati Smernice zdravega prehranjevanja v vzgojno-izobraževalnih ustanovah (Smernice 2005) (Gabrijelčič Blenkuš in sod., 2005) organizatorjem prehrane v šolah in vrtcih. Služi kot pomoč pri količinskem odmerjanju živil za pripravo jedi in obrokov hrane ter porcioniranju hrane glede na priporočila zdravega prehranjevanja, saj so ta ponujala izključno teoretična prehranska izhodišča, kar pa pogosto ne zadostuje za praktično planiranje, pripravo in ponudbo zdravih obrokov (Toth in sod., 2019). Nove posodobljene Smernice za prehranjevanje v vzgojno-izobraževalnih zavodih (Smernice) (Gregorič in sod., 2020), ki vsebinsko dopolnjujejo šolsko zakonodajo in podajajo konkretne ukrepe za izvajanje organizirane šolske prehrane, so v zadnji fazi priprave. Osrednje podporno okolje predstavlja spletni portal Šolski lonec (Šolski lonec, 2022), ki je nastal na podlagi medsektorskega sodelovanja med zdravstvom in šolstvom. Na portalu so na enem mestu zbrani praktični napotki ter uradne, strokovne in znanstveno utemeljene informacije za lažje uresničevanje Smernic v praksi. Vključuje tudi informacijska orodja, ki lahko služijo kot pripomoček organizatorjem prehrane v vzgojno-izobraževalnih zavodih.

Vprašanje je, kako ustrezna je dejansko prehrana v dijaških domovih, tako količinsko kot tudi po sestavi in deležih makro- in mikrohranil. Glede na to, da so mladostniki pogosto neješči in nagnjeni k zavračanju določenih vrst živil in jedi, je smiselno preveriti tudi, kako jim ponujena hrana ugaja (Bosanac in sod., 2016; Heide in sod., 2019). Cilj raziskave je bil ovrednotiti energijsko in hranilno vrednost ponujenih kosil in večerij v Dijaškem domu (DD) v Ljubljani ter oceniti njihovo skladnost s Smernicami (Gregorič in sod., 2020). Eden izmed ciljev raziskave je bil tudi ugotoviti senzorično sprejemljivost ponujenih obrokov s strani dijakov. Raziskavo smo izvedli v obdobju epidemije koronavirusa, ko so veljali protikoronski ukrepi, ki so med drugim vključevali tudi občasna zaprtja dijaških domov. Izvedba raziskave je tako potekala v več obdobjih, kar lahko predstavlja eno izmed ključnih omejitev raziskave.

2 MATERIAL IN METODE

2.1 VZOREC

Vzorec je v raziskavi predstavljalo 12 kosil in 12 večerij, skupaj 24 obrokov, ki so bili pripravljeni v kuhinji DD (preglednica 1). Prvi del raziskave smo izvedli v obdobju od 12. do 15. oktobra 2020 (1. teden). Sledilo je večmesečno zaprtje dijaških domov in sicer do spomladi 2021, zato smo z raziskavo nadaljevali od 29. do 30. marca 2021, čemur je sledilo ponovno zaprtje dijaških domov. Zadnji del raziskave smo izvedli po ponovnem odprtju dijaških domov, od 12. do 15. (3. teden) in od 21. do 22. aprila. 2. teden pa je vključeval dejansko obroke iz dveh različnih časovnih obdobij, in sicer: od 29. do 30. marca 2021 (ponedeljek in torek) in od 21. do 22. aprila (sreda in četrtek). Skupaj smo zajeli tri tedne, od ponedeljka in četrtka (12 dni), skupaj 24 obrokov. Dijakom je bilo kosilo na voljo vsak dan od 12.00 do 16.00, večerja pa od 18.30 do 21.00. Glede na to, da ob petkih večina dijakov odhaja domov že takoj po dopoldanskih šolskih obveznosti, na ta dan nismo vzorčili obrokov. Vodstvo DD se je strinjalo z izvedbo raziskave, prav tako vsi v raziskavo vključeni dijaki. Seznanjeni so bi bili tudi s tem, da lahko od raziskave (senzorično ocenjevanje in anketa) kadarkoli odstopijo. Sodelovanje v raziskavi je bilo prostovoljno.

Poleg jedi iz jedilnika so imeli dijaki pri kosilu vedno na razpolago še kruh, različne vrste solat iz solatnega bara, sadje, vodo, sok in nesladkan čaj, pri večerji pa kruh, mleko, sadje, vodo, sok in nesladkan čaj. V raziskavi so sodelovali dijaki, ki bivajo v DD. Zaradi protikoronskih ukrepov in obolelih dijakov, je bilo dnevno prisotnih različno število dijakov, kar nam je onemogočilo enoten vzorec dijakov skozi celotno raziskavo, kar predstavlja drugo omejitev naše raziskave.

Omejitev raziskave je bila tudi to, da nekateri dijaki niso zaužili vsega, kar jim je bilo ponujeno, pri kosilu ali večerji. Nekateri niso vzeli vseh ponujenih jedi, drugi niso zaužili vzete količine obroka ali pa so posamezno

Preglednica 1: Jedilnik
Table 1: Menu

		1. teden		
Obrok	1. dan	2. dan	3. dan	4. dan
Kosilo	Goveja juha, svinjski paprikaš, njoki, solatni bar, sadje	Zeljna juha, goveji stroganov, dušen riž, solatni bar, sadje	Fižolova juha, puranji zrezek piazzolla, pire krompir, solatni bar, sadje	Kokošja juha, golaž, gluhi štruklji, solatni bar, sadje
Večerja	Piščančji kebab, sadni jogurt, hruška	Carski praženec s čokoladnim prelivom, mandarina	Pečenica, testeninska solata, jogurt, solatni bar	Testenine s paradižnikovo omako in sirom, solatni bar, jabolko
		2. teden		
Obrok	5. dan	6. dan	7. dan	8. dan
Kosilo	Goveja juha, mesni polpet, zeljne krpice, solatni bar, sadje	Milijonska juha, pečen piščanec, krompirjeva pogača, solatni bar, sadje	Boranja, jogurtova strjenka na biskvitu z gozdnimi sadeži, solatni bar, sadje	Kokošja juha, krompir s peteršiljem in maslom, frigani lignji, solatni bar, sadje
Večerja	Hot dog, dodatki (kečap, majoneza, gorčica), žitna ploščica, sadni jogurt, kivi	Carski praženec s čokoladnim prelivom in orehi, hruška	Svedrčki z omako po kraško, solatni bar	Hrenovka s prilogo (gorčica, majoneza, kečap), probiotični jogurt, jabolko
		3. teden		
Obrok	9. dan	10. dan	11. dan	12. dan
Kosilo	Zeljna juha, puranji zrezek na žaru, ocvrt krompir, ajvar, čebula, solatni bar, sadje	Kokošja juha, špageti z bolonjsko omako, solatni bar, sadje	Korenčkova juha, kruhov cmok, goveji zrezek v omaki, solatni bar, sadje	Goveja juha, ocvrt svinjski kotlet, pire krompir, brokoli, solatni bar, sadje
Večerja	Mlečni zdrob, kakav, riževa čokolada, banana	Topli sendvič, pomaranča, jogurt	Umešana jajca, žitna ploščica, jogurt, jabolko	Enolončnica s hrenovko, grmada, hruška

živilo, ki je bilo sestavni del obroka, nesli s seboj v sobo in ga zaužili kasneje. Da bi pridobili natančne podatke o hranilni vrednosti obrokov, ki so jih dijaki zaužili pri posameznem obroku, bi morali stehtati vsako porcijo jedi in nato odšteti morebitne ostanke jedi in jedi, ki jih dijaki niso zaužili pri obroku.

Pri vsakem obroku smo anketirali od 50 do 88 dijakov obeh spolov, starih od 15 do 19 let. Pri anketi je najmanj fantov (31 od skupno 60 dijakov) sodelovalo 7. dan raziskave in najmanj deklet (16 od skupno 56 dijakov) 8. dan raziskave. Dijaki so bili pred anketo seznanjeni s potekom raziskave. Vprašanja so se nanašala na senzorično sprejemljivost obroka.

2.2 METODE

2.2.1 Tehtanje in vrednotenje ponujenih kosil in večerij

Iz servirne linije smo vsak dan naključno vzeli po 4 ponujene obroke. Vsako ponujeno jed iz obroka, ki je bila sestavni del kosila ali večerje, smo stehtali s kuhinjsko tehtnico. Iz dobljenih meritev smo izračunali povprečno maso posamezne jedi v obroku tistega dne. Pri tem smo pazili na natančno uporabo kuhinjske tehtnice in na vestno zapisovanje količin. Energijsko in hranilno vrednost ponujenih obrokov smo ovrednotili s spletnim orodjem Odprta platforma za klinično prehrano (OPKP, 2022) in sicer tako, da smo vanj vnesli recepte za vsako jed in izmerjeno povprečno maso te jedi. Količino izkoristljivih ogljikovih hidratov smo izračunali tako, da smo od količine skupnih ogljikovih hidratov odšteli količino prehranske vlaknine. V jedilniku smo preverili tudi kako pogosto so vključena priporočena in odsvetovana živila ter dobljene rezultate primerjali s priporočili iz Smernic (Gregorič in sod., 2020) in Smernic 2005 (Gabrijelčič Blenkuš in sod., 2005).

2.2.2 Ocenjevanje senzorične sprejemljivosti kosil in večerij s panelom dijakov

Dijaki so pri vsakem obroku na vprašalniku ocenili stopnjo ugajanja na opisni 9-točkovni hedonski lestvici (Golob in sod., 2006). Opisne ocene smo nato pretvorili v številčne ocene od 1 (izredno ne ugaja) do 9 (izredno ugaja). Za vsak teden smo izračunali povprečno hedonsko oceno kosil in večerij. Povprečno hedonsko oceno kosil in večerij smo izračunali posebej za dekleta in posebej za fante ter za dekleta in fante skupaj (skupna povprečna hedonska ocena). Dodatno smo jim postavili še vprašanje, kaj jim je pri obroku všeč in kaj bi pri obroku spremenili.

2.2.3 Vključenost priporočenih in odsvetovanih živil v obrokih

V Smernicah je navedena pogostost vključitve posameznih skupin živil za kosila oziroma tople malice ter za zajtrke oziroma malice za obdobje štirih tednov (20 dni) (Gregorič in sod., 2020). Glede na to, da je naša raziskava potekala 12 dni, smo priporočeno pogostost vključitve priporočenih skupin živil v obroke in mesečno še dopustno vključitev odsvetovanih skupin živil v obroke preračunali v odstotke. V Smernicah ni navedenih pogostosti vključevanja posameznih skupin živil za večerje, zato smo pri večerjah upoštevali priporočila, ki veljajo za kosila oziroma tople malice. V primeru, da je bilo živilo pripravljeno na način, ki ga Smernice odsvetujejo, smo ga uvrstili v kategorijo odsvetovanih živil. Skupine priporočenih in odsvetovanih skupin živil v obrokih smo sistematično označili, in sicer od P1 do P9 za priporočena živila in od O1 do O15 za odsvetovana živila.

2.2.4 Statistična obdelava podatkov

Rezultate, pridobljene s spletnim orodjem OPKP, smo uredili in statistično obdelali z računalniškim programom Microsoft Excel. Izračunali smo naslednje opisne statistike: povprečno vrednost ($\bar{x}$), minimalno vrednost (min.), maksimalno vrednost (max.) in standardno deviacijo (SD). Z ANOVA testom (analiza variance) pri stopnji tveganja $p \leq 0{,}05$ smo ob izpolnjenem pogoju normalne porazdelitve preverili, če se povprečne absolutne hranilne in energijske vrednosti jedilnikov 1., 2. in 3. tedna med seboj statistično značilno razlikujejo. Če obstajajo statistično značilne razlike med hedonskimi ocenami fantov in deklet smo preverili s t-testom pri stopnji tveganja $p \leq 0{,}05$. Z izračunom Pearsonovega korelacijskega koeficienta (r; stopnja tveganja $p \leq 0{,}05$) smo preverili, ali obstaja povezava med hedonsko oce-

Preglednica 2: 9-točkovna hedonska lestvica
Table 2: The 9-point hedonic scale

izredno ne ugaja	zelo ne ugaja	dokaj ne ugaja	rahlo ne ugaja	niti ugaja niti ne ugaja	rahlo ugaja	dokaj ugaja	zelo ugaja	izredno ugaja

no obrokov in vsebnostjo energije, maščob, enostavnih sladkorjev, prehranske vlaknine ter soli v obrokih. Pri tem smo upoštevali vrednosti za koeficient r-korelacije (Keršič, 1997):

r od 0,00 do ± 0,20: ni povezanosti ali le neznatna povezanost,

r od ± 0,20 do ± 0,40: lahka povezanost,

r od ± 0,40 do ± 0,70: pomembna povezanost,

r od ± 0,70 do ± 1,00: velika ali zelo velika povezanost.

3 REZULTATI

Dobljene rezultate glede vsebnosti energije, hranil ter vključenost priporočenih in odsvetovanih živil v obroke, smo primerjali s priporočili iz Smernic (Gregorič in sod., 2020).

3.1 ENERGIJSKA VREDNOST KOSIL IN VEČERIJ

Povprečna energijska vrednost kosil je bila 1. teden 2824 ± 479 kJ, 2. teden 2755 ± 553 kJ in 3. teden 3201 ± 263 kJ (slika 1), kar ni ustrezalo priporočeni energijski vrednosti kosil za fante (3762 – 4389 kJ). V povprečju so kosila v 1. tednu ustrezala priporočeni energijski vrednosti kosil za dekleta (2886 – 3367 kJ). Povprečna energijska vrednost kosil za dekleta je bila v 1. in 2. tednu rahlo pod priporočeno, medtem ko je bila v 3. tednu skladna s priporočili iz Smernic. Fantje so s kosili v povprečju 1. teden pokrili 75 %, v 2. tednu 73 % in v 3. tednu 85 % spodnje priporočene energije za kosilo.

Povprečna energijska vrednost večerij v 1. tednu (2762 ± 685 kJ) ni ustrezala niti priporočilom za fante (3135 kJ), niti za dekleta (2405 kJ). Fantje so z večerjami v 1. tednu v povprečju pokrili 88 % priporočene energije za večerjo, medtem ko je bila za dekleta povprečna energijska vrednost večerij v 1. tednu za 15 % prevelika. V 2. tednu je bila povprečna energijska vrednost večerij 3176 ± 1276 kJ, v 3. tednu pa 3124 ± 914 kJ, kar je skladno s Smernicami. Za dekleta so bile večerje energijsko prebogate, in sicer v povprečju za 32 % v 2. tednu in za 30 % v 3. tednu.

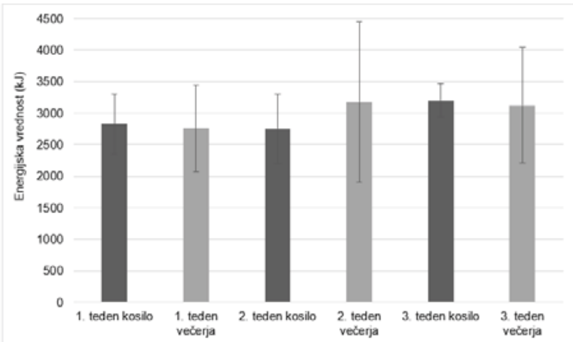
Rezultat analize variance (ANOVA test) pri stopnji tveganja $p \leq 0,05$ je pokazal, da se povprečne energijske vrednosti kosil (p = 0,4245) v 1., 2. in 3. tednu med seboj niso statistično značilno razlikovale, kakor se med seboj niso statistično značilno razlikovale tudi energijske vrednosti večerij v 1., 2. in 3. tednu.

Primernost prehrane v dijaških domovih so raziskovali tudi Bosanac in sod. (2016). V devetih dijaških domovih na Hrvaškem so ovrednotili 14 celodnevnih jedilnikov, in sicer v dveh različnih regijah - primorski in celinski. Preiskovanci so bili mladostniki, stari od 15 do 22 let (n = 978). Dijaki so z obroki v dijaških domovih dnevno zaužili približno 12931 ± 997 kJ, kar je preseglo njihova nacionalna priporočila (10180 kJ). Izkazalo se je, da so bili energijsko najbolj bogati obroki v celinski regiji, in sicer v pomladno-jesenskem času, najmanj pa v jesensko-zimskem času v primorski regiji. Do nasprotnih rezultatov so prišli Fidler Mis in sod. (2012), ki so pri 2224 slovenskih mladostnikih v okviru zdravniškega pregleda v različnih slovenskih regijah, z vprašalnikom o pogostosti uživanja živil, ugotavljali vnos energije, makro in mikrohranil. Ugotovili so, da tako fantje kot tudi dekleta na dnevni ravni uživajo nekoliko manj energije, kot je priporočeno za njihovo starost. Fantje so na dan zaužili 98 %, dekleta pa 92 % priporočene energije. Do zaključka, da mladostniki zaužijejo manj energije, kot je priporočeno za njihovo starost, so prišli tudi Hoppu in sod. (2010) pri 726 mladostnikih na Finskem. Fantje so v povprečju s šolskimi kosili zaužili 1687 ± 724 kJ, dekleta pa 1269 ± 547 kJ, kar je manj od povprečnih energijskih vrednosti kosil, ki smo jih ovrednotili v naši raziskavi. Mladostniki na Finskem so večji delež energije zaužili z obroki večerij (fantje 2315 ± 838 kJ in dekleta 1986 ± 978 kJ). Kljub temu je bila energijska vrednost večerij pri finskih mladostnikih manjša od povprečnih energijskih vrednosti večerij, ki smo jih ugotovili v naši raziskavi. Dnevni energijski vnos med evropskimi mladostniki so preučevali tudi Diethelm in sod. (2013) v okviru presečne študije HELENA (angl. Healthy Lifestyle in Europe by Nutrition in Adolescence). Rezultati študije so pokazali, da je bil vnos energije pri fantih nekoliko večji (109 %), pri dekletih pa skladen (101 %) z Referenčnimi vrednostmi Nemškega prehranskega društva.

3.2 VNOSI ENERGIJE Z MAKROHRANILI

Iz količine zaužitih hranil in uporabo ustreznega energijskega faktorja smo izračunali energijo posameznega hranila v kosilih, oziroma v večerjah. Zaradi primerjave s priporočili v Smernicah smo energijo posameznega makrohranila preračunali v odstotek skupne energije zaužite z obrokom. V nadaljevanju predstavljamo prispevek energije posameznih makrohranil k skupni energijski vrednosti obroka.

V vseh treh tednih so bili povprečni vnosi energije z beljakovinami s kosili podobni, in sicer je energijska vrednost beljakovin v kosilih 1. tedna v povprečju predstavljala 19,6 ± 7,1 %, v 2. tednu 18,6 ± 4,7 % in v 3. tednu 19,2 ± 3,8 % skupne energijske vrednosti, kar je več od priporočil v Smernicah (10 - 15 %) (slika 2). Smernice priporočajo, da naj energijska vrednost maščob v obroku



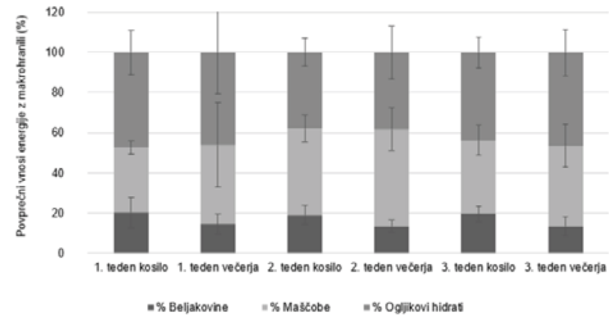
Slika 1: Povprečna energijska vrednost kosil in večerij za 1., 2. in 3. teden
Figure 1: The average energy values of the lunches and dinners for the 1st, 2nd and 3rd week

predstavlja 30 – 35 % energije. Priporočilom so ustrezala kosila v 1. tednu (31,3 ± 3,0 %), medtem ko so kosila v 2. tednu vsebovala prevelik delež energije iz maščob (41,7 ± 6,5 %). Kosila v 3. tednu so vsebovala v povprečju 35,7 ± 7,3 % energije iz maščob, torej na zgornji meji priporočene vrednosti. Povprečni vnosi energije z ogljikovimi hidrati s kosili so bili premajhni v vseh treh tednih, in sicer je bil njihov delež manjši od priporočenih 50 % skupne energije obroka. Priporočilom so se najbolj približala kosila v 1. tednu, v katerih je bilo 45,7 ± 10,6 % energije iz ogljikovih hidratov. Najmanj energije iz ogljikovih hidratov (36,8 ± 6,8 %) so dijaki v povprečju zaužili s kosili v 2. tednu.

Povprečni vnosi energije z beljakovinami z večerjami so bili v 1. tednu 14,3 ± 4,9 %, v 2. tednu 13,3 ± 2,8 % in v 3. tednu 13,3 ± 4,5 % skupne energijske vrednosti, kar je skladno s Smernicami (slika 2). V povprečju so večerje v vseh treh tednih vsebovale prevelik delež energije iz maščob. V vsebnosti maščob izstopajo večerje iz 2. tedna, ki so v povprečju vsebovale 47,3 ± 10,5 % energije iz maščob. Povprečni vnosi energije z maščobami z večerjami so v 1. tednu znašali 38,8 ± 20,6 %, v 3. tednu pa 39,2 ± 10,3% skupne energijske vrednosti. V vseh treh tednih so večerje vsebovale energijsko bogata in gosta živila, zaradi česar je bil vnos energije iz maščob prevelik. Čeprav je vnos maščob v prehrani mladostnikov esencialnega pomena, saj so med drugim tudi strukturna enota celičnih membran, predstopnja za sintezo bioloških molekul in sodelujejo pri regulaciji aktivnosti encimov in pri regulaciji izražanja genov ter olajšajo absorpcijo drugih snovi, pa njihov čezmeren vnos v času mladostništva ne sme biti prevelik, saj poveča tveganje za razvoj kroničnih nenalezljivih bolezni v odrasli dobi (Ozdemir, 2016; EFSA, 2010; Savarino in sod., 2021). Tako kot kosila, so tudi večerje v povprečju vsebovale premalo energije iz ogljikovih hidratov. Povprečni vnosi energije z ogljikovimi hidrati z večerjami so bili v 1. tednu 45,3 ± 20,4 %, 2. tednu 37,6 ± 13,0 % in v 3. tednu 45,5 ± 11,2 %.

S pomočjo analize variance (ANOVA test) pri stopnji tveganja $p \leq 0{,}05$ smo ugotovili, da se povprečni vnosi energije z beljakovinami s kosili (p = 0,9291) in večerjami (p = 0,7256), vnosi energije z maščobami s kosili (p = 0,4518) in večerjami (p = 0,8525) ter vnosi energije z ogljikovimi hidrati s kosili (p = 0,0887) in večerjami (p = 0,9714) v 1., 2. in 3. tednu med seboj niso statistično značilno razlikovali. Kosila in večerje iz prvega tedna so bila glede vsebnosti makrohranil najbolj skladna s Smernicami, saj so se najbolj približala priporočilom glede vnosa energije z makrohranili. Eden izmed razlogov za hranilno manj ustrezne obroke v 2. in 3. tednu bi lahko bila tudi bolniška odsotnost vodje prehrane.

Vnosi energije z beljakovinami, ki smo jih ovrednotili v obrokih DD, so bili podobni vnosu energije z beljakovinami pri mladostnikih, ki so bili vključeni v raziskavo o prehranskem vnosu makro- in mikrohranil pri slovenskih mladostnikih (Fidler Mis in sod., 2012). Beljakovine so prispevale 15 % dnevnega energijskega vnosa za fante in 13 % dnevnega energijskega vnosa za dekleta. Tudi Bosanac in sod. (2016) so prišli do podobnih rezultatov. Obroki ponujeni v dijaških domovih na Hrvaškem so na dnevni ravni v povprečju vsebovali 15,2 % energije iz beljakovin. Nasprotno, je bil pri mladostnikih iz 10-ih evropskih držav vnos energije z beljakovinami prevelik, saj je znašal 185 % priporočene vrednosti (Diethelm in sod., 2013). Bosanac in sod. (2016) so v obrokih v dijaških domovih na Hrvaškem, prav tako kot mi, ugotovili prevelik vnos energije z maščobami, saj so maščobe predstavljale 36,3 % energijskega vnosa dnevno ponujenih obrokov. Nasprotno so Fidler Mis in sod. (2012) pri slovenskih mladostnikih ugotovili nekoliko premajhen vnos energije z maščobami. Fantje so z maščobami pokrili manj kot 30 % dnevnih energijskih potreb, poleg tega so maščobe imele neustrezno maščobnokislinsko sestavo. Prehrana slovenskih mladostnikov je vsebovala preveč nasičenih in premalo večkrat nenasičenih maščobnih kislih. V naši raziskavi smo ugotovili, da obroki vsebujejo premalo energije iz ogljikovih hidratov. V nasprotju z našimi ugotovitvami so Fidler Mis in sod. (2012) pri slovenskih mladostnikih ugotovili zadosten vnos energije z ogljikovimi hidrati. Ti so prispevali 57 % dnevnega energijskega vnosa pri fantih in 58 % dnevnega energijskega vnosa pri dekletih. Obroki v dijaških domovih na Hrvaškem so v povprečju vsebovali 48,8 % energije iz ogljikovih hidratov, kar je prav tako pod priporočili (Bosanac in sod., 2016). Tako kot so povprečni vnosi energije z beljakovinami s kosili v naši raziskavi bili večji od priporočil, ki jih navajajo v Smernicah, so tudi Hoppu in sod. (2010) na Finskem ugotovili, da so mladostniki s šolskimi kosili zaužili več kot 15 % energije iz beljakovin. V našem primeru so bili povprečni vnosi energije z ogljikovimi hidrati s kosili premajhni, medtem ko so mladostniki na Finskem s šolskim kosilom zaužili dovolj energije iz ogljikovih hidratov. Njihova kosila so bila tudi skladna glede vsebnosti energije iz maščob, ki je bila v naši raziskavi prevelika. Večerje, ki so jih zaužili mladostniki na Finskem, so bile v primerjavi z večerjami, ki smo jih ovrednotili v DD, beljakovinsko bolj bogate. Prav tako kot smo mi ugotovili prevelik vnos energije z maščobami z večerjami, so tudi Hoppu in sod. (2010) prišli do rezultata, da mladostniki na Finskem z večerjami zaužijejo preveč energije iz maščob.



Slika 2: Povprečni vnosi energije z makrohranili s kosili in z večerjami (%), za 1., 2. in 3. teden
Figure 2: The average values of energy consumed with macronutiens in lunches and dinners (%) for the 1st, 2nd and 3rd week

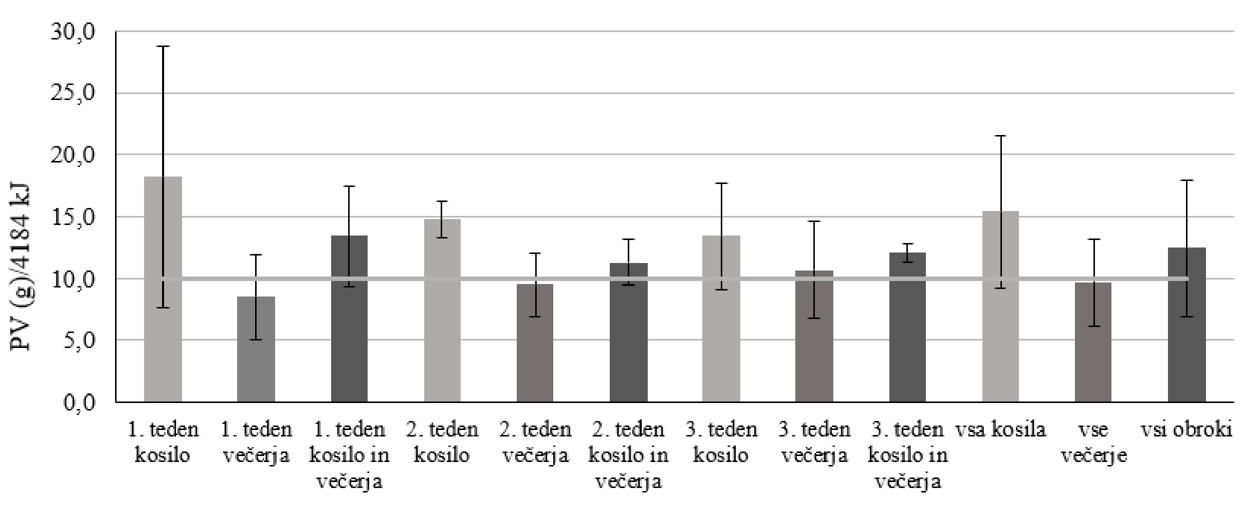
3.3 VSEBNOST PREHRANSKE VLAKNINE V OBROKIH

Povprečna vsebnost prehranske vlaknine v kosilih in večerjah skupaj je bila skladna s priporočili iz Smernic 2005 (Gabrijelčič Blenkuš in sod., 2005) (10 g na 4184 kJ oz. nad 28 g prehranske vlaknine na dan) v vseh treh tednih (13,4 ± 4,1 g/4184 kJ v 1. tednu, 11,3 ± 1,9 g/4184 kJ v 2. tednu in 12,1 ± 0,7 g/4184 kJ v 3. tednu) (slika 3).

Povprečna vsebnost prehranske vlaknine v kosilih je bila v vseh treh tednih skladna s Smernicami 2005 (Gabrijelčič Blenkuš in sod., 2005). Največjo povprečno vsebnost prehranske vlaknine so imela kosila v 1. tednu (18,2 ± 10,5 g/4184 kJ). V 2. tednu je povprečna vsebnost prehranske vlaknine v kosilih znašala 14,8 ± 1,5 g/4184 kJ, medtem ko so najmanjšo povprečno vsebnost prehranske vlaknine imela kosila v 3. tednu (13,4 ± 4,3 g/4184 kJ). K velikemu povprečju prehranske vlaknine v kosilih 1. tedna je prispevala predvsem fižolova juha, saj je vsebovala 12,5 g prehranske vlaknine na porcijo.

V nasprotju s kosili, so večerje vsebovale manj prehranske vlaknine, še posebej večerje v 1. tednu, saj je povprečna vsebnost prehranske vlaknine znašala 8,5 ± 3,4 g/4184 kJ, kar je manj od priporočene vrednosti. Skladna s priporočili ni bila tudi vsebnost prehranske vlaknine v večerjah 2. tedna (9,5 ± 2,6 g/4184 kJ). Večerje v 3. tednu so vsebovale največjo povprečno vsebnost prehranske vlaknine (10,7 ± 3,9 g/4184 kJ), kar je skladno s Smernicami 2005 (Gabrijelčič Blenkuš in sod., 2005).

Do podobnih ugotovitev so prišli tudi Hoppu in sod. (2010), ugotovili so, da so šolska kosila mladostnikov na Finskem vsebovala zadostno količino prehranske vlaknine. Skladne z našimi rezultati so tudi ugotovitve, da



Slika 3: Povprečna vsebnost prehranske vlaknine v kosilih in večerjah
Figure 3: The average amount of dietary fibre in lunches and dinners

so večerje, ki so jih zaužili mladostniki na Finskem, bolj revne s prehransko vlaknino. Količina prehranske vlaknine, ki so jih fantje in dekleta zaužili z obroki večerij, je bila namreč pod priporočeno vrednostjo. Rezultati vrednotenja 14-dnevnih jedilnikov v dijaških domovih na Hrvaškem (Bosanac in sod., 2016) kažejo, da so celodnevni obroki (12931 kJ) v povprečju vsebovali 22,2 g prehranske vlaknine, kar znaša 7,1 g/4184 kJ. Iz tega lahko sklepamo, da so bili obroki v dijaških domovih na Hrvaškem bolj revni s prehransko vlaknino v primerjavi z obroki iz naše raziskave.

3.4 OCENJEVANJE SENZORIČNE SPREJEMLJIVOSTI OBROKOV

Skupna povprečna hedonska ocena kosil v 1. tednu je znašala 6,6 ± 1,6, pri čemer so jih dekleta ocenila nekoliko slabše (6,3 ± 1,5) kot fantje (6,8 ± 1,7) (preglednica 3).

Najnižjo skupno povprečno hedonsko oceno so prejela kosila v 2. tednu (5,6 ± 2,1). Dijakom je bilo v 2. tednu najmanj všeč sredino kosilo (skupna povprečna hedonska ocena 4,8 ± 2,2). To je bila hkrati tudi najnižja skupna povprečna hedonska ocena kosila izmed vseh kosil v celotni raziskavi. Na ta dan je bila na jedilniku boranja in jogurtova strjenka na biskvitu z gozdnimi sadeži. Dijakom pri sredinem kosilu v 2. tednu ni bila všeč boranja, večina pa je pohvalila sladico. Pri sladici sicer nekaterim niso bile všeč borovnice.

Najvišjo skupno povprečno hedonsko oceno so prejela kosila v 3. tednu (7,1 ± 1,7). V 3. tednu so tako dekleta kot tudi fantje najvišjo povprečno hedonsko oceno dodelili ponedeljkovemu kosilu (skupna povprečna hedonska ocena 7,7 ± 1,3). Na ta dan je bil na jedilniku puranji zrezek na žaru in ocvrt krompir. Dijaki so navedli, da jim je bilo najbolj všeč meso, krompir ter kombinacija živil in okus. Na vprašanje »Kaj bi pri tem obroku spremenil?«, so bili najbolj pogosti odgovori: *dodal/a bi več zelenjave, nič, dodal/a bi omako* in *druga vrsta mesa*.

S t-testom pri stopnji tveganja $p \leq 0{,}05$ smo potrdili, da so se povprečne hedonske ocene kosil treh tednov statistično značilno razlikovale med dekleti in fanti (p = 0,0125). Z analizo variance (ANOVA test) pri stopnji tveganja $p \leq 0{,}05$ smo ugotovili, da so se skupne hedonske ocene kosil 1., 2. in 3. tedna med seboj statistično značilno razlikovale ($p < 0{,}001$).

Najvišjo skupno povprečno hedonsko oceno večerij so prejele večerje v 1. tednu, in sicer 7,0 ± 1,6 (preglednica 4). Dekleta so večerje v 1. tednu ocenile s povprečno hedonsko oceno 6,9 ± 1,6, fantje pa s 7,0 ± 1,7. Skupna povprečna hedonska ocena večerij je v 2. tednu znašala 6,8 ± 1,8. V 2. tednu so dekleta ocenile večerje z nižjo povprečno hedonsko oceno (6,7 ± 1,7), medtem ko so fantje večerje v 2. tednu ocenili s povprečno hedonsko oceno 6,8 ± 1,8. Najnižjo skupno povprečno hedonsko oceno so prejele večerje v 3. tednu (6,3 ± 2,2). Dekleta so 3. teden večerje ocenile s povprečno hedonsko oceno 6,4 ± 2,0, fantje pa z oceno 6,1 ± 2,3.

Najvišjo skupno povprečno hedonsko oceno med večerjami je v celotni raziskavi prejela večerja 6. dne oziroma torkova večerja v 2. tednu (7,5 ± 1,6). Na ta dan je bil na jedilniku carski praženec s čokoladnim prelivom. Dijakom je bil najbolj všeč čokoladni preliv, okus in kombinacija živil ter orehi, ki so bili potreseni po čokoladnem prelivu. Nekaterim se je preliv zdel

Preglednica 3: Povprečna hedonska ocena kosil
Table 3: The average hedonic score of lunches

Skupina	Statistični parameter	1. teden	2. teden	3. teden
Dekleta	$\bar{x}$	6,3	5,5	7,0
	max.	9,0	9,0	9,0
	min.	2,0	1,0	1,0
	SD	1,5	2,3	1,8
Fantje	$\bar{x}$	6,8	5,7	7,2
	max.	9,0	9,0	9,0
	min.	1,0	1,0	1,0
	SD	1,7	2,0	1,6
Dekleta in fantje skupaj	$\bar{x}$	6,6	5,6	7,1
	max.	9,0	9,0	9,0
	min.	1,0	1,0	1,0
	SD	1,6	2,1	1,7

presladek. Dijaki so izrazili željo, da bi pri obroku bilo na izbiro več vrst prelivov. Dijaki so imeli carski praženec za večerjo tudi 2. dan raziskave oziroma v torek v 1. tednu. Omenjena večerja je v celotni raziskavi dosegla 2. najvišjo skupno povprečno hedonsko oceno (7,3 ± 1,7). Dekletom je najmanj ugajala večerja 12. dne (5,9 ± 1,8) oziroma četrtkova večerja v 3. tednu (enolončnica s hrenovko, grmada, hruška). Dekleta in fante je pri obroku zmotila enolončnica, katero bi nadomestili z drugo jedjo oziroma bi izbrali drugačno kombinacijo živil. Nekateri dijaki bi želeli uživati enolončnico brez hrenovke. Večini deklet in fantov je bila pri obroku všeč sladica, vendar so nekatere zmotile rozine. Fantje so z najnižjo povprečno hedonsko oceno ocenili ponedeljkovo večerjo v 3. tednu (5,4 ± 2,7), ko je bil na meniju mlečni zdrob, kakav, riževa čokolada in banana. Omenjena večerja je v celotni raziskavi prejela tudi najnižjo skupno povprečno hedonsko oceno (5,8 ± 2,6). Kljub najnižji skupni povprečni hedonski oceni, večina dijakov pri obroku ne bi spremenila ničesar. Pri obroku jim je bil všeč okus, kakav in riževa čokolada. Štirje dijaki so navedli, da bi mlečni zdrob nadomestili z drugačnim tipom jedi.

S t-testom smo potrdili, da se povprečne hedonske ocene večerij treh tednov statistično značilno niso razlikovale med dekleti in fanti (p = 0,8724). Prav tako smo ugotovili statistično značilne razlike med skupnimi hedonskimi ocenami večerjami 1., 2. in 3. tedna (ANOVA test, p < 0,001).

3.5 POVPREČNA HEDONSKA OCENA V POVEZAVI Z ENERGIJSKO VREDNOSTJO IN VSEBNOSTJO HRANIL

Z izračunom Pearsonovega koeficienta korelacije smo preverili ali je vsebnost energije, maščob, enostavnih sladkorjev, prehranske vlaknine in soli povezana s hedonsko oceno obroka. Koeficiente korelacije ob stopnji značilnosti $p \leq 0{,}05$ predstavljamo v preglednici 5.

Rezultati so pokazali, da energijska vrednost kosil (r = -0,0300) niti večerij (r = -0,1427) ni bila povezana s hedonskimi ocenami obrokov, saj je bil korelacijski koeficient izredno majhen, kar kaže na neznatno povezanost.

Med vsebnostjo maščob v kosilih in med hedonskimi ocenami kosil pa smo ugotovili, da obstaja lahka povezanost (r = -0,2935). Pričakovali bi, da je višja hedonska ocena povezana z večjo vsebnostjo maščob, vendar so dekleta in fantje višje hedonske ocene dodelili kosilom z manjšo vsebnostjo maščob. Vsebnost maščob v večerjah ni bila povezana s hedonskimi ocenami večerij (r = 0,0037).

Zanimiv je tudi rezultat povezave hedonske ocene z vsebnostjo enostavnih sladkorjev (r = -0,4332 za kosilo in r = -0,5395 za večerjo), ki kaže na lahko povezanost. Razlog za nižje hedonske ocene obrokov, ki so imeli večjo vsebnost enostavnih sladkorjev, so najverjetneje enolončnice, ki so bile skupaj s sladicami sestavni del obrokov. Rezultati raziskave so pokazali, da dijaki niso ljubitelji enolončnic, medtem ko sladice prispevajo k veliki vsebnosti skupnih sladkorjev v obrokih.

S hedonskimi ocenami kosil ni bila povezana vsebnost prehranske vlaknine v kosilih (r = 0,0746), medtem ko je med hedonskimi ocenami večerij in vsebnostjo prehranske vlaknine v večerjah obstajala zmerna povezanost (r = -0,4004).

Pričakovali smo, da bo večja vsebnost soli povezana z višjimi hedonskimi ocenami, saj sol prispeva k okusu živil, ga poudari in izboljša (Leshem, 2009), vendar naši rezultati kažejo na obratno zvezo. Vsebnost soli v večerjah in hedonske ocene večerij so bile lahko povezane (r = -0,5022). Vsebnost soli ni bila povezana s hedonskimi ocenami kosil (r = - 0,0337).

3.6 VKLJUČENOST PRIPOROČENIH IN ODSVETOVANIH ŽIVIL V OBROKIH

3.6.1 Vključenost priporočenih živil v obroke

Vsak obrok bi, glede na priporočila v Smernicah, moral vključevati zelenjavo in/ali sadje (P1). V obdobju 20 dni, bi morala biti na jedilniku vsaj 16-krat zelenjava in od tega naj bi bila vsaj 8-krat surova (sveža) zelenjava. Naši obroki so bili skladni s priporočili iz Smernic, saj smo ugotovili, da sta bila zelenjava in/ali sadje vsak dan vključena v obroke (slika 4). Zelenjava je bila redno vključena v glavne jedi kosil, poleg tega so dijaki imeli na voljo pri kosilu vsak dan tudi svežo zelenjavo iz solatnega bara. Poleg juh, ki so navedene na jedilniku, je bila zraven pogosto ponujena še zelenjavna juha. Tudi sveže sadje je bilo ponujeno ob vsakem obroku. Rezultati o stalni vključenosti sadja in zelenjave so še posebej spodbudni, saj je bilo v okviru raziskave HBSC ugotovljeno, da manj kot polovica (39,8 %) slovenskih mladostnikov vsak dan uživa sadje in le nekaj več kot tretjina (35,9 %) uživa zelenjavo vsak dan (Jeriček Klanšček in sod., 2019).

Izdelki iz žit, kruh, kaše, in druga pretežno škrobna živila (P2) so bila vključena v vsak obrok, kar je skladno s Smernicami. Po priporočilih v Smernicah bi morali biti polnozrnati izdelki ali kaše v obroke vključeni vsaj 6-krat v obdobju 20 dni. Naši rezultati niso skladni s Smernicami, saj v celotni raziskavi polnozrnati izdelki ali kaše (P3) niso bili nikoli sestavni del kosila in/ali večerje. V Smernicah je navedeno priporočilo, da se krompirjeve jedi oz. krompir kot dodatek (P4) v obroke vključi do 8-krat v obdobju 20 dni. Naši rezultati kažejo, da so bile

Preglednica 4: Povprečna hedonska ocena večerij
Table 4: The average hedonic score of dinners

Skupina	Statistični parameter	1. teden	2. teden	3. teden
Dekleta	x̄	6,9	6,7	6,4
	max.	9,0	9,0	9,0
	min.	3,0	2,0	1,0
	SD	1,6	1,7	2,0
Fantje	x̄	7,0	6,8	6,1
	max.	9,0	9,0	9,0
	min.	1,0	2,0	1,0
	SD	1,7	1,8	2,3
Dekleta in fantje skupaj	x̄	7,0	6,8	6,3
	max.	9,0	9,0	9,0
	min.	1,0	2,0	1,0
	SD	1,6	1,8	2,2

Preglednica 5: Pearsonov koeficient korelacije za povezanost hedonske ocene obrokov z vsebnostjo energije oz. hranil v obrokih
Table 5: Pearson's correlation coefficient for the hedonic score of the meals and the energy value or nutrients content in meals

	Pearsonov koeficient (r)	
Energijska vrednost oz. hranilo	Kosilo	Večerja
Energijska vrednost	-0,0300	-0,1427
Maščobe	-0,2935	0,0037
Enostavni sladkorji	-0,4332	-0,5395
Prehranska vlaknina	0,0746	-0,4004
Sol	-0,0337	-0,5022

krompirjeve jedi 5-krat ponujene za kosilo, kar je skladno s Smernicami.

V Smernicah je navedeno, da bi morale biti stročnice (P5) vsaj 6-krat vključene v obroke v obdobju 20 dni. Naši rezultati so skladni s Smernicami, saj so bile stročnice vsak dan ponujene v solatnem baru, 1-krat pa je bila za kosilo tudi fižolova juha. Vprašanje pa je, koliko dijakov si je postreglo s stročnicami iz solatnega bara in fižolovo juho. Stročnice so bile v večerje vključene 4-krat, od tega 3-krat v solatnem baru, 1-krat pa so bile sestavni del enolončnice.

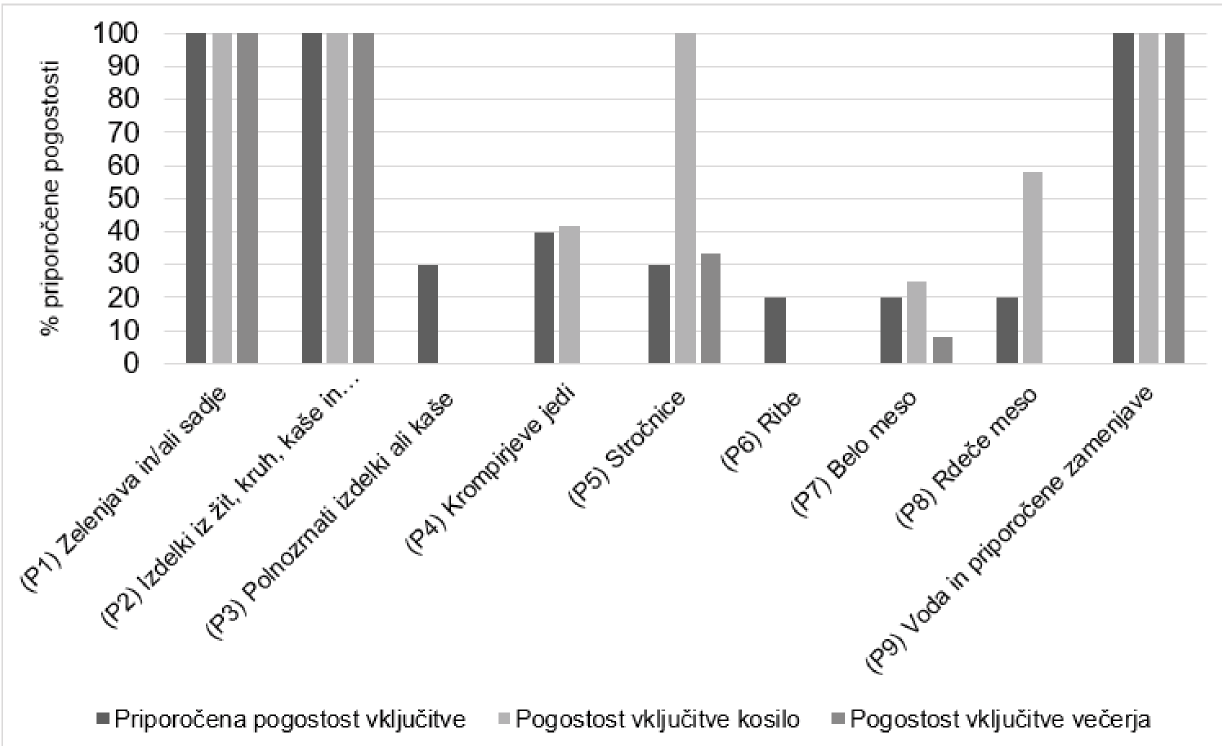
Po priporočilih v Smernicah bi morale biti ribe (P6) v kosila oziroma tople malice vključene 4-krat, od tega mastne ribe 2-krat v obdobju 20 dni. V kolikor ni mogoče dobaviti ustreznih in kakovostnih mastnih rib, se lahko te vključi le 2-krat na mesec. Ribe v dneh, ko smo izvajali našo raziskavo, niso bile nikoli ponujene, vendar je potrebno izpostaviti, da so-le te bile na jedilniku izven terminskega okvira naše raziskave. Na jedilniku so bile najpogosteje ob petkih, vendar petkovih obrokov v našo raziskavo nismo vključili, saj je večina dijakov odhajala domov že takoj po dopoldanskih šolskih obveznostih.

V Smernicah navajajo, da se belo (P7) in rdeče meso (P8) v obroke vključi 4 do 6-krat v obdobju 20 dni. V terminskem okviru naše raziskave je bilo belo meso v obroke kosil vključeno 3-krat, medtem, ko je bilo to za večerjo le enkrat. Rdeče meso je bilo v kosila vključeno 7-krat, kar je pogosteje kot ga priporočajo v Smernicah.

Glede na priporočila v Smernicah, bi morala biti voda in priporočene zamenjave (P9) med obroki vedno na voljo. Naši rezultati so skladni s Smernicami, saj so si dijaki lahko pri vsakem kosilu in večerji natočili vodo in nesladkan čaj v neomejenih količinah.

3.6.2 Vključenost odsvetovanih živil v obroke

V Smernicah je navedeno, da so lahko skupine živil: sladkani in mastni žitni izdelki (O1), sladkani mlečni izdelki/deserti (O2), mesni izdelki in pripravki s homogeno strukturo oz. večjo vsebnostjo maščob (O3), sladkani kakavovi/žitni/zeliščni/sadni napitki in poparki (O4) ter ocvrta živila v globoki maščobi (O5), vključeni v največ petino oz. 20 % vseh organiziranih obrokov, vendar ne več kot 8-krat na mesec. Omejitev se nanaša na vsa živila, ne glede na to iz katere odsvetovane skupine prihaja posamezno živilo (Gregorič in sod., 2020). Naši rezultati niso skladni s Smernicami, saj so se omenjene skupine živil v obrokih pojavljale preveč pogosto. Med njimi iz-



Slika 4: Pogostost vključitve priporočenih skupin živil v kosila in večerje glede na priporočene vrednosti
Figure 4: Frequency of inclusion of recommended food groups in lunches and dinners in relation to recommended values

stopajo predvsem mesni izdelki in pripravki s homogeno strukturo oz. večjo vsebnostjo maščob (O3), ki so bili 5-krat vključeni v večerje in ocvrta živila v globoki maščobi (O5), ki so bila 3-krat vključena v kosila (slika 5).

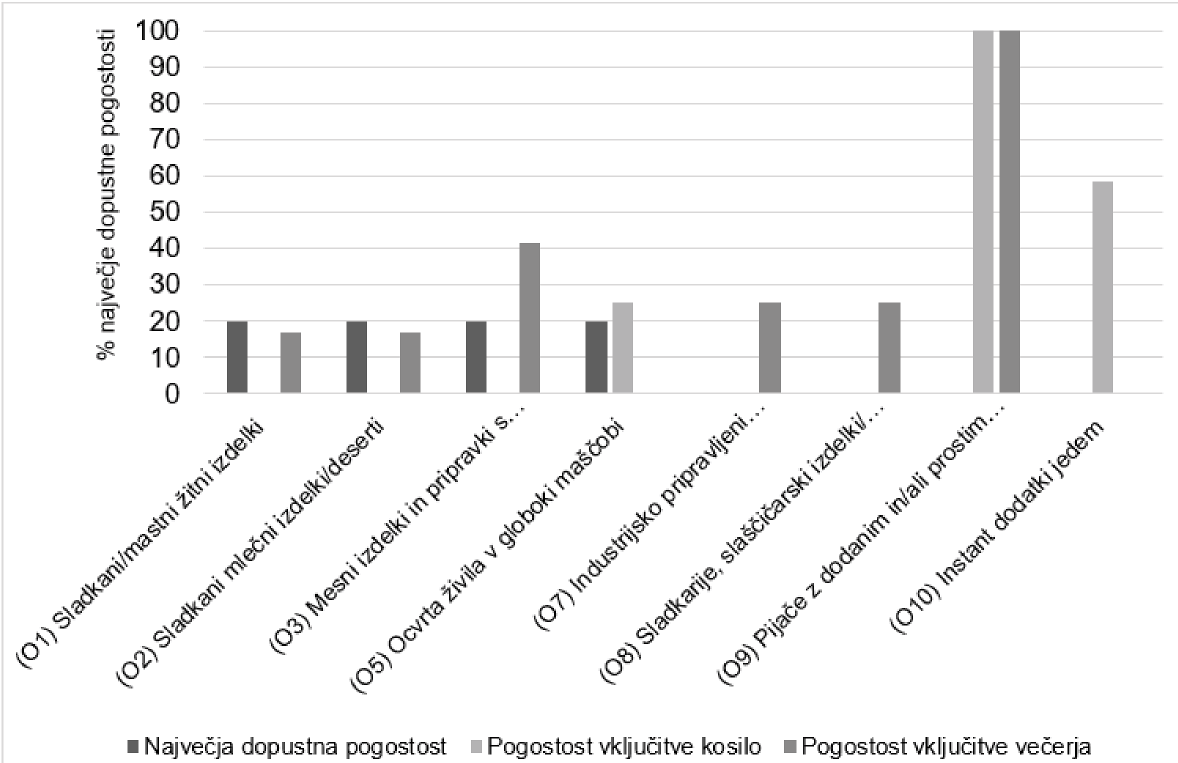
Skupini živil: industrijsko pripravljeni sladki/mastni namazi/prelivi (O7) in sladkarije, slaščičarski izdelki/deserti, sladki/soljeni prigrizki (O8), naj bi v obroke vključevali le nekajkrat letno. Obe skupini živil sta bili 3-krat vključeni v večerje, kar ni skladno s Smernicami (Gregorič in sod., 2020).

Glede na priporočila v Smernicah, se v obroke odsvetuje vključevati skupine živil: pijače z dodanim in/ali prostim sladkorjem ali drugimi sladili (O9), instant dodatki jedem (O10), sladkani mlečni/rastlinski napitki (O11), kremni polnomastni siri, siri s plemenito plesnijo, topljeni siri (O12), maščobe z neugodno prehransko sestavo (O13) ter izdelki s sestavinami neprimernimi za otroke (O14). Naši rezultati niso skladni s Smernicami, saj so bili instant dodatki (O10) v kosila vključeni 7-krat, in sicer v obliki jušne kocke. Obroki v času naše raziskave niso nikoli vključevali naslednjih skupin živil: sladkani mlečni/rastlinski napitki (O4), kremni polnomastni siri, siri s plemenito plesnijo, topljeni siri (O12), maščobe z neugodno prehransko sestavo (O13) in izdelki s sestavinami neprimernimi za otroke (O14).

Dijaki so si lahko pri vsakem obroku postregli z vodo ali nesladkanim čajem, a zaskrbljujoče je, da so bili v neomejenih količinah vedno na voljo tudi napitki iz skupine pijač z dodanim in/ali prostim sladkorjem ali drugimi sladili (O9), za katere je v Smernicah navedeno, da se jih naj v obroke ne vključuje. Menimo, da ponudba pijač z dodanim sladkorjem pri obrokih ni primerna, saj se z uživanjem tovrstnih pijač poveča tveganje za čezmerno telesno maso in zobni karies. Lahko so vzrok za manj raznoliko prehrano in slabšo oskrbo s hranili ter so povezane s povečanim tveganjem za razvoj sladkorne bolezni tipa 2, srčno-žilnih bolezni in drugih nezaželenih vplivov na zdravje (Fidler Mis in sod., 2017).

V Smernicah izjemoma dovoljujejo v obroke vključiti industrijsko (pred-)pripravljene jedi in že sestavljene izdelke (O15), kot so na primer: omake, lazanje, polnjene testenine, pripravljeni sendviči in podobne gotove jedi, v primeru, ko obstajajo nepremostljive ovire oziroma ko dobavitelj trenutno ne more zagotoviti drugega izdelka ali je to neizogibno zaradi posebne formulacije živila (dietni izdelki). Naši rezultati so skladni s Smernicami, saj ta skupina živil ni bila nikoli v 12 dneh sestavni del kosila ali večerje.

Ne gre zanemariti podatka, da v celotnem obdobju ni bilo večerje, ki ne bi vsebovala živil iz seznama odsve-



Slika 5: Pogostost vključitve odsvetovanih skupin živil v kosila in večerje glede na Smernice
Figure 5: Frequency of inclusion of not recommended food groups in lunches and dinners according to the Guidelines

tovanih živil, le dve večerji pa sta bili taki, ki poleg pijač z dodanim sladkorjem, nista vsebovali tudi drugih odsvetovanih živil. Nekateri obroki so vsebovali celo več kot eno odsvetovano živilo. Tak primer je bila ponedeljkova večerja v 2. tednu, ki je vključevala hrenovko, omake (kečap, majonezo, gorčico), sadni jogurt in žitno ploščico.

4 ZAKLJUČEK

Mladostništvo je življenjsko obdobje z intenzivno rastjo in s številnimi fiziološkimi spremembami, ki zahtevajo povečane potrebe po energiji in hranilih. Poleg tega je še posebej pomembno, da se v tem času vzpostavijo dobre prehranske navade, saj je velika verjetnost, da se bodo takšne navade ohranile kasneje v življenju (Blunt in sod., 2020). Vzgojno-izobraževalni zavodi, kamor sodijo tudi dijaški domovi, imajo lahko pri oblikovanju dobrih prehranskih navad pomembno vlogo (Bosanac in sod., 2016). Prav zato smo v okviru naše raziskave želeli ugotoviti, kako skladna je energijska in hranilna vrednost kosil ter večerij v dijaškem domu s priporočili iz Smernic za prehranjevanje v vzgojno-izobraževalnih zavodih. Naši rezultati so pokazali, da je bila povprečna energijska vrednost kosil skladna s priporočili iz Smernic za dekleta, medtem ko je bila za fante premajhna. Povprečna energijska vrednost večerij je bila skladna s priporočili iz Smernic, in sicer za fante v 2. in 3. tednu, za dekleta pa je bila prevelika v vseh treh tednih. V splošnem so bili povprečni vnosi energije z ogljikovimi hidrati s kosili premajhni. V povprečju so dijaki s kosili zaužili večji delež energije z beljakovinami in maščobami kot je priporočajo Smernice, medtem ko so bili z večerjami povprečni vnosi energije z beljakovinami skladni s priporočili iz Smernic in povprečni vnosi energije z maščobami preveliki. V povprečju so kosila v vseh treh tednih in večerje le prvega tedna vsebovale zadostne količine prehranske vlaknine. Povprečna vsebnost prehranske vlaknine v kosilih in večerjah skupaj je bila skladna s Smernicami 2005 v vseh treh tednih. Povprečna hedonska ocena kosil na 9-točkovni lestvici je bila 6,5 ± 1,9, kar je na meji med »rahlo ugaja« in »dokaj ugaja«. Nekoliko višja je bila povprečna hedonska ocena večerij, in sicer 6,6 ± 1,9, oziroma »dokaj ugaja«. Energijska vrednost kosil in večerij ni bila povezana z njihovimi hedonskimi ocenami. Pri pregledu jedilnikov smo ugotovili, da je z izjemo polnozrnatih izdelkov in kaš ter rib večina priporočenih skupin živil bila dovolj pogosto vključena v jedilnik. Neugoden pa je podatek, da so bile odsvetovane skupine živil na jedilniku preveč pogosto. Na rezultate raziskave je lahko vplivalo tudi epidemiološko stanje v državi, zaradi katerega smo morali raziskavo izvesti v več delih.

Komentar: Članek je nastal na osnovi podatkov magistrskega dela Erike Jesenko, pod mentorstvom doc. dr. Tanje Pajk Žontar in somentorstvom izr. prof. dr. Mojce Korošec.

Comment: This article is based on the Master‘s thesis Erika Jesenko, by the mentorship of assist. prof. Tanja Pajk Žontar, Ph.D., and co-mentorship of assoc. prof. Mojca Korošec, Ph.D..

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doi:10.14720/aas.2023.119.1.2564 Original research article / izvirni znanstveni članek

Potential effect of intercropping in the control of weeds, diseases, and pests in a wheat-faba bean system

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Received February 22, 2022; accepted March 10, 2023.
Delo je prispelo 22. februarja 2022, sprejeto 10. marca 2023

Potential effect of intercropping in the control of weeds, diseases, and pests in a wheat-faba bean system

Abstract: Intercropping has proved to be a promising alternative in the biological control of biotic factors by reducing the excessive use of plant protection products that are harmful to the environment and human health. In this study, aimed to examine the effect of intercropping systems on diseases, weeds and pests control in organic field experiments in Western Morocco. Two field experiments were conducted during 2017-2018 and 2018-2019. Three cropping regimes (monocropped wheat, monocropped faba bean, and intercropped wheat-faba bean) and three nitrogen levels N_0 (0 kg N ha^{-1}), N_1 (50 kg N ha^{-1}), and N_2 (100 kg N ha^{-1}) were evaluated. Compared with monocropping, intercropping (N_0 level) reduced the incidence of stripe rust by 71–120 % and severity by 244–337 % in 1st and 2nd experiments respectively. In addition, the incidence of septoria was reduced by 236 % and severity by 276 %. Obviously, the intercrops significantly decreased the total weed biomass by more than 40 % in both experiments. Black aphid populations in faba bean were reduced by 80 %. In contrast, the nitrogen fertilizer increased the attack of diseases and black aphids. It is concluded that wheat-faba bean intercrops can be used as a method of reduction of inputs, reduction of environmental impacts of crops, and stability in the face of biotic factors.

Key words: diseases; faba bean; intercropping; nitrogen treatment; pests; sole crops; weeds; wheat

Potencialni učinek medsetve na nadzor plevelov, bolezni in škodljivcev v sistemu krušna pšenica-bob

Izvleček: Medsetev se je izkazala kot obetajoča alternativa pri biološkem nadzoru biotičnih dejavnikov za zmanjšanje prekomerne uporabe sredstev za zaščito, ki so škodljiva okolju in zdravju ljudi. Namen raziskave je bil preučiti učinek medsetve na plevele, bolezni in škodljivce v poljskem poskusu organske pridelave v Zahodnem Maroku. V obdobjih 2017-2018 in 2018-2019 sta bila izvedena dva poljska poskusa. Ovrednoteni so bili trije načini setve (čist posevek pšenice, čist posevek boba in mešani posevek pšenice in boba) in trije odmerki dušikovih gnojil: N_0 (0 kg N ha^{-1}), N_1 (50 kg N ha^{-1}), in N_2 (100 kg N ha^{-1}). V primerjavi s čistimi posevki je medsetev pri načinu gnojenja N_0 zmanjšala pojavljanje progaste rje za 71–120 % in jakost okužbe za 244–337 % v prvem in drugem obdobju poskusa. Dodatno je bila pojavnost listne pegavosti pšenice zmanjšana za 236 % in jakost njene ukužbe za 276 %. Zelo očitno je medsetev značilno zmanjšala celokupno biomaso plevelov za 40 % v obeh poskusih. Populacija črnih fižolovih uši na bobu se je zmanjšala za 80 %. Nasprotno je povečano dodajanje dušikovih gnojil povečalo napad bolezni in črnih fižolovih uši. Zaključimo lahko, da bi z mešanim posevkom krušne pšenice in boba zmanjšali stroške pridelave in negativne vplive pridelave na okolje kot tudi izboljšali stabilnosti pridelave glede na biotske dejavnike

Ključne besede: bolezni; bob; medsetev; obravnavanja z dušikovimi gnojili; škodljivci; čisti posevki; pleveli; krušna pšenica

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1 INTRODUCTION

In terms of ecology and environment, monospecific crops have caused a series of serious problems. Excessive use of chemical products promotes the development and extreme spread of biotic factors. The agricultural system should not only meet the needs of todays and future generations, but they should also be environmentally friendly, logistically feasible and economically worthwhile. It, therefore, seems essential to achieve sustainable agriculture. One of the key strategies of sustainable agriculture is the diversity of agricultural ecosystem restoration and its effective management. Various researches have made it possible to highlight the advantages of intercropping both in terms of productivity and ecological services (increase of biodiversity, control of bio-aggressors and weeds, limitation of pollution) (Konlan et al., 2013; Corre Hellou et al., 2015; Lopes et al., 2015; Luo et al., 2021). In fact, according to the farmers, these systems would be more competitive vis-a-vis weeds because of a better soil cover allowed by the intercropping compared to the sole crops. Intercropping can actually be seen as a way of securing crop production against biotic factors, which is particularly interesting in biological systems where the use of synthetic phytosanitary products is not allowed (Boyeux and Magnard, 2013; Mamine and Farès, 2020; Victoria et al., 2022).

In intercropping, the arrangement of plant stems and leaves is different in the vertical and horizontal directions. This allows agricultural crops to better utilize solar radiation, while weeds receive less light and thus are suppressed (Yadollahi et al., 2014; Li et al., 2019). Combination crops are often less invasive than single crops. By planting a mixture of plants, more ecological niches can be filled, providing fewer opportunities and resources for weeds to thrive (Sturm et al., 2018). Mennan et al. (2020) also point out that crop association, particularly the inclusion of plants with allelopathic properties, can be an ecological alternative to chemical weed control.

Reductions in plant diseases and pests have also been recorded for varietal mixtures. Zhu et al. (2000) reported a 94 % reduction in rice plant diseases in the intercropping. Previous studies have shown that the intercropping of faba bean and wheat reduces yield losses associated with powdery mildew and yellow rust in wheat (Jiang et al., 2012; Xiao et al., 2018). In addition, crop association is a practicable alternative that controls crop pests (Rao et al., 2012; Sharaby et al., 2015; Sulvai et al., 2016). Letourneau et al. (2011) reported positive effects of crop association on pest management based on a meta-analysis of 522 experiments. It was suggested that intercropping can potentially be used to improve the abundance and diversity of natural enemies (natural enemy hypothesis) or cause a reduction in pest food concentration, thereby reducing their numbers (resource concentration hypothesis).

Nitrogen is not only an important nutritional factor that promotes crop growth and increases yield, but it is also known to have a direct impact on disease severity (Devadasa et al., 2014; Zhu et al., 2017a; Luo et al., 2022). These results are attributed to the increase in canopy density resulting from the application of nitrogen fertilizer, providing a favorable microclimate for the development and spread of pathogenic fungi (He, 2009). Other studies have suggested that the effects of nitrogen on pathogenic fungi are mediated by increasing the nitrogen content of the host tissue by acting as a substrate for pathogen growth (Chen et al., 2013; Zhu et al., 2017a). Obviously, the rational use of nitrogen fertilizers is a key factor in the control of powdery mildew and stripe rust in wheat, thereby increasing yields in these cropping systems (Chen et al., 2013; Yang et al., 2013; Zhu et al., 2017a). Therefore, it is relevant to analyze the interaction between the effects of intercropping and nitrogen fertilizers on the performance of the crop association.

Therefore, the objective of this study was to evaluate the effects of nitrogen fertilization and wheat-faba bean intercropping on the regulation of diseases occurrence, weeds and pests control efficiency.

2 MATERIALS AND METHODS

2.1 CROPPING SYSTEMS AND EXPERIMENTAL DESIGN

The field experiments were carried out over two cropping seasons in the same region but in different soils in 2017–2018 (1st experiment) and 2018–2019 (2nd experiment). The 1st experiment was located in the Saada station of National Institute of Agronomic Research (INRA) in Marrakesh, Morocco, about 7 km to the south of Marrakesh, Morocco; the 2nd experiment was located in the experimental station (31°37′46.7″ N; 8°09′23.4″ E) of the National Institute of Agronomic Research (INRA) in Marrakesh, Morocco. The wheat (W) (*Triticum aestivum* L.) cultivar 'Wafia' and faba bean (F) (*Vicia faba* L.) cultivar 'Alfia' were grown as sole crops (SC) in full density, half density sole crops (SC/2) and as intercrops (IC). Three nitrogen (N) treatments: N_0 – 0 kg N ha^{-1}, N_1 – 50 kg N ha^{-1} and N_2 – 100 kg N ha^{-1}, were evaluated on IC, wheat SC and SC/2, while faba bean SC and SC/2 were grown without N application. It was effectively hypothesized that N is not a limiting resource for legumes because of their ability to increase the symbiotic N fixation from the air to meet their needs; SC and SC/2 were considered as controls. No herbicides or fungicides were applied; the weeding was done manually. The irrigation system was gravity type. The soil plots undergoing the two experiments have never been before cultivated or treated by chemical fertilizers or organic manures.

The experimental design was a randomized complete block with three replicates (Table 1). The dimension of each elementary plot was 1.60 × 1.20 m. The seedlings were planted manually in January for both experiments and crops, 8 rows of wheat and 6 rows of faba bean (inter-rows 20 cm) for full density SC, whereas 4 rows of wheat and 3 rows of faba bean for IC (inter-rows 20 cm) and SC/2 (inter-rows 40 cm). The harvesting of the whole plots was done manually in July for wheat and in May for faba bean for both experiments. The seeding density was 320 plants per m^2 for soft wheat as a SC, 40 plants per m^2 for faba bean as a SC, and 160 per m^2 plants for soft wheat as SC/2 and 20 plants per m^2 for faba bean as SC/2 and IC.

Before the field experiment, the soil chemical properties in 0–30 cm layer were analysed (Table 2). Soil texture was determined by Robinson's method (Baize, 2018); soil organic carbon (SOC) and soil organic matter (SOM) were determined according to Aubert (1978); total nitrogen (N_{tot}) – by the Kjeldhal method (ISO 11261:1995. Soil quality - Determination of total nitrogen - Modified Kjeldahl method); available phosphorus (P_2O_5) content was measured by the method of Olsen and Sommers (1982); available (K_2O) was determined according to Gueguen and Rombauts (1961).

During the experiments, the dry season was from May to October. The annual average rainfall was 232 mm (1st experiment) and 100 mm (2nd experiment). The average air temperature was 20 °C and 18.7 °C in autumn, 11.6 °C and 15.3 °C in winter, 19.2 °C and 22.8 °C in spring and 26.5 °C and 27.8 °C in summer, respectively (Figure 1).

2.2 EVALUATION OF BIOTIC FACTORS PRESSURE

2.2.1 Diseases

The diseases studied were those usually encountered on the territory for these crops. For each plot of wheat, each plant was inspected for the presence of various leaf cryptogamic diseases. Septoria and stripe rust of wheat were studied on the same plot at the same time, and the incidence and severity of both diseases were recorded at the wheat heading stage for each plot. The severity of each disease was evaluated according to grades 1-9 (ICARDA, 1986). Grade 0 indicated the absence of visible spore spot symptoms on wheat leaves; grade 1 indicated spore spots covering 5 % of the total leaf area; grade 3 indicated spore spots covering 6-25 % of the total leaf area; grade 5 indicated spore spots on 26-50 % of the total leaf area; grade 7 indicated spore spots on 51-75 % of the total leaf area; and grade 9 indicated extensive spore spots on leaves and stems (76 %). Cryptogamic diseases were diagnosed and identified based on their typical symptoms (Zillinsky, 1983), and on the microscopic observation of spores. In 2018-2019, only yellow rust was observed in the experimental station.

These data were used to calculate the disease incidence, severity, and relative control efficacy for each plot, as follows (Luo et al., 2021):

$$\%\,Incidence = \frac{Number\ of\ infected\ plants}{Total\ number\ of\ plants\ assessed} \times 100$$

$$\%\,Severity = \frac{Number\ of\ diseased\ leaves\ in\ each\ grade \times value\ of\ the\ corresponding\ grade}{Total\ number\ of\ leaves\ scored \times maximum\ disease\ grade} \times 100$$

$$\%\,Relative\ control\ efficacy = \frac{Incidence\ or\ severity\ of\ monocrops - Incidence\ or\ severity\ of\ intercrops}{Incidence\ or\ severity\ of\ monocrops} \times 100$$

Table 1: Planting patterns in the field experiments at different N levels

Crops	Treatment
Faba bean sole crops in full density	N_0F-SC
Faba bean sole crops in half density	N_0F-SC/2
Wheat sole crops in full density at three N levels	N_0W-SC, N_1W-SC, N_2W-SC
Wheat sole crops in half density at three N levels	N_0W-SC/2, N_1W-SC/2, N_2W-SC/2
Wheat faba bean intercrops at three N levels	N_0IC, N_1IC, N_2IC

Table 2: Chemical characteristics of soils during the two experimental seasons (2017-2018 and 2018-2019)

Treatment	Depth cm	Texture	pH	SOC %	SOM %	N_{tot} %	P_2O_5 mg kg^{-1}	K_2O mg kg^{-1}
1st experiment	0–30	clay-loam	8.7	0.95	1.64	0.15	20	220
2nd experiment	0–30	clay-loam	8.5	0.77	1.34	0.11	14	850

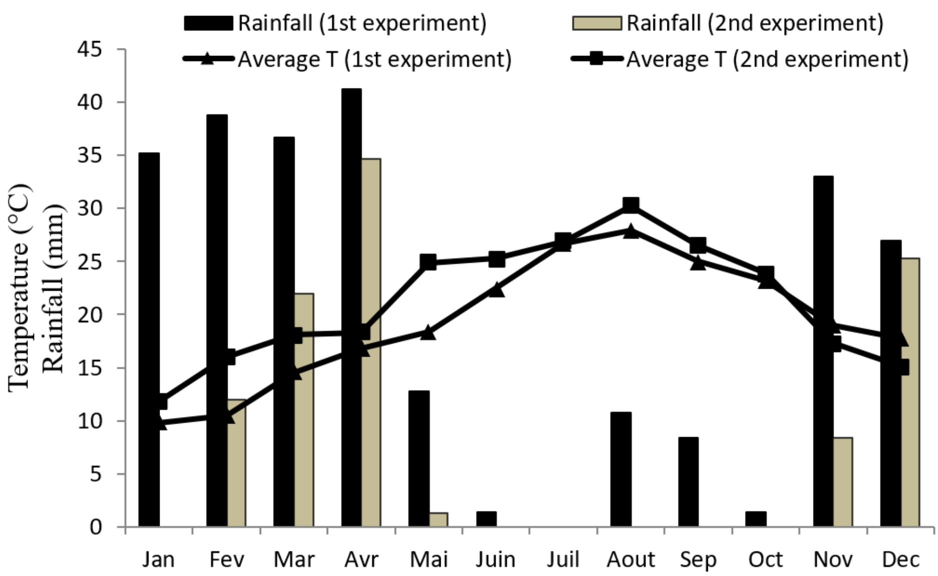

Figure 1: Precipitation and average air temperature during the 1st and 2nd experiments (data of the Marrakesh Meteorological Station)

2.2.2 Weeds

Weeds were studied during the crop, in all plots. They were characterized by their distribution (homogeneous, intermediate or heterogeneous) at the scale of the observation area, their percentage of cover, estimated from a photograph of the plot, and by the number of species present, for each plot. During the 1st experiment, there were six weed species, while in the 2nd experiment, only one species was observed at the field level. These species were counted in each plot and identified by the Laboratory of Microbial Biotechnologies, Agrosciences and, Environment, Cadi Ayyad University.

2.2.3 Pests

During the 1st experiment, no pest population was detected. However, during the 2nd experiment, an estimation of the population of black aphids on faba bean (*Aphis fabae* Scopoli, 1763) was performed. For this purpose, aphid counts were conducted on all plants in each plot.

3 RESULTS

3.1 EFFECT OF N LEVELS AND INTERCROPPING ON WHEAT DISEASE DEVELOPMENT

In the first experiment, stripe rust (*Puccinia striiformis* Westend f.sp. *tritici* Erikss.) and septoria (*Septoria tritici* Desm.) were observed on soft wheat. In the 2nd experiment, only stripe rust was observed on soft wheat. While faba bean was not affected by any disease in both experiments.

Without fertilizer application, the intercropping compared to the monoculture (B-SC) reduced the incidence of stripe rust by 71.55 % and 120.90 % for 1st and 2nd experiments respectively, and the severity by 244.55 % and 337.85 % for 1st and 2nd experiments (Figure 2). In the case of septoria, the intercropping without nitrogen application reduced significantly ($p < 0.05$) the incidence by 236.48 % and the severity by 276.50 % compared to the N_0B-SC (Figure 3). In addition, the soft wheat intercrops without nitrogen supply had a lower level of diseases than the treated ones. Therefore, faba bean-wheat intercropping regressed the symptoms of septoria and stripe rust, indicating a positive effect of intercropping compared to sole crops.

In the two-year experiment, the incidence and severity of both diseases in monoculture and intercropping wheat showed an increasing trend with increasing N levels. Compared to N_0, N_1-N_2 treatments increased the incidence of wheat stripe rust in monoculture (SC) and intercropped plants by 19.14-41.82 % and 16.37-25.21 %, respectively (2017-2018); and by 17.20-42.43 % and 20.34-36.07 %, respectively (2018-2019). Compared to N_0, N_1-N_2 treatments increased wheat stripe rust severity in monoculture and intercropped plants by 37.75-21.25 % and 37.57-60.48 %, respectively (2017-2018); and 39.54-61.81 and 47.95-70.46 %, respectively (2018-2019)

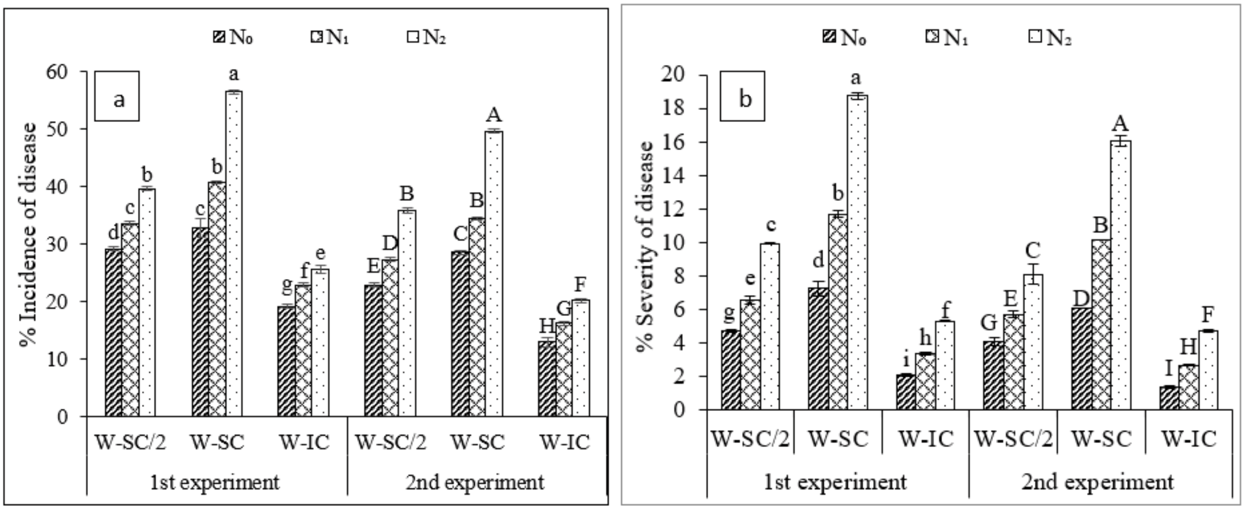

Figure 2: Incidence (a) and severity (b) of stripe rust in sole crops soft wheat (W-SC and W-SC/2) and intercropping (IC) under three nitrogen treatments in 1st and 2nd experiments. Values represent the mean of three replicates ± standard errors within the same graphic followed by different letters are statistically significant ($p < 0.05$)

(Figure 2). In addition, compared to N_0, N_1-N_2 treatments increased the incidence of septoria in wheat in monoculture and intercropped plants by 10.37-16.40 % and 10.26-29.36 %. respectively. As well as the severity was increased by 41.20-54.45% and 42.16-50.88% (Figure 3). The effects of nitrogen fertilizer application on disease severity were greater than the incidence of wheat stripe rust. Compared to the monoculture, the intercropping reduced the incidence and severity of wheat diseases caused by nitrogen application.

The control effect of two experiments intercropping wheat on stripe rust was 41.71–54.62 % and 52.94–59.24 % when based on incidence, for which the N_2 level was relatively superior. The corresponding values were 70.98–71.54 % and 70.47–77.16 % when based on severity, for which the N_0 level was superior. Moreover, the control effect of wheat intercropping on septoria was 64.82-70.32 % for incidence, which N_0 scored well, and 73.00-75.36 % for severity which N_2 scored well (Table 3). After evaluate the overall effects of nitrogen level and

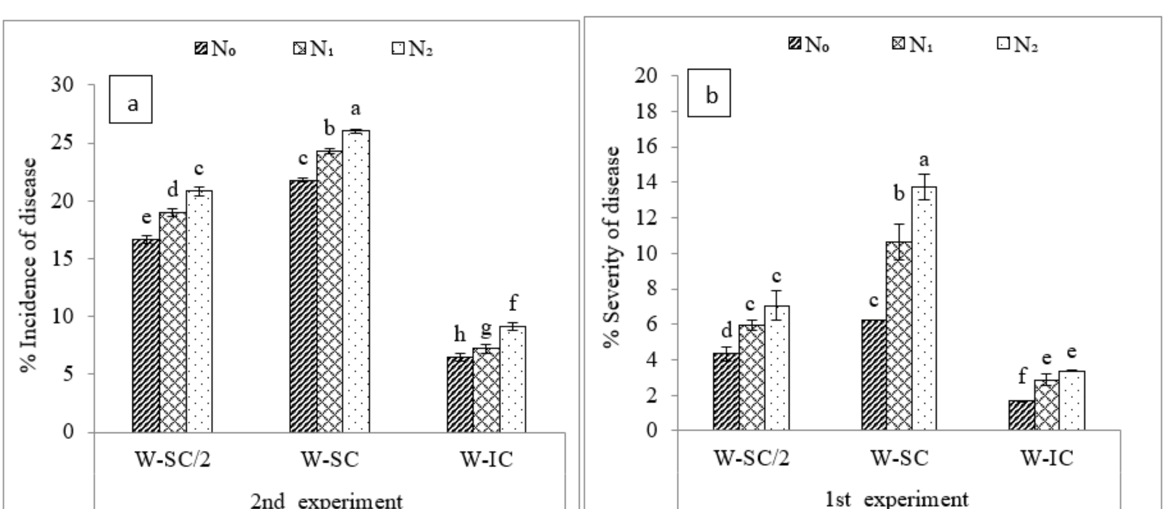

Figure 3: Incidence (a) and severity (b) of septoria in sole crops soft wheat (W-SC and W-SC/2) and intercropping (IC) under three nitrogen treatments in 1st experiment. Values represent the mean of three replicates ± standard errors within the same graphic followed by different letters are statistically significant ($p < 0.05$)

Table 3: Relative control efficacy (%) for wheat stripe rust and Septoria at different N levels in 1st and 2nd experiments

	1st experiment				2nd experiment	
	Stripe rust		Septoria		Stripe rust	
N level	Incidence	Severity	Incidence	Severity	Incidence	Severity
N_0	41.71±2.00b	70.98±0.52a	70.28±0.53a	73.44±0.05b	54.73±1.08b	77.16±0.05a
N_1	43.64±0.63b	71.06±0.25a	70.32±0.56a	73.00±1.37b	52.94±0.54b	73.47±0.05b
N_2	54.62±0.99a	71.54±0.25a	64.82±0.54b	75.36±0.77a	59.24±0.72a	70.47±0.39c

Letters represent significant differences between different N levels at $p < 0.05$

Table 4: Effects of N levels and cropping system on stripe rust and septoria

	1st experiment				2nd experiment	
	Stripe rust		Septoria		Stripe rust	
Factors	Incidence	Severity	Incidence	Severity	Incidence	Severity
Cropping system	0.93**	3.36**	3.14**	3.90**	1.52**	3.79**
N levels	0.32**	1.64**	0.14**	0.97**	0.52**	1.89**
Cropping system × N levels	0.017**	0.010**	0.006*	0.021ns	0.006**	0.039**

The data in the table are F-values of the interaction between N levels and planting patterns (two-way ANOVA, $p < 0.05$). *Represents significant difference ($p < 0.05$), **represents significant difference ($p < 0.001$), ns represents no significant difference

mode of intercropping on the occurrence of diseases, there was a significant interaction between cropping system and N levels ($p < 0.01$) (Table 4).

3.2 EFFECT OF N LEVELS AND INTERCROPPING ON WEEDS DEVELOPMENT

In the case of wheat sole crops, there was no significant difference between the two densities seeded for certain weed species (*Spergularia flaccida* (Madden) I.M. Turner, *Malva sylvestris* L., *Chenopodium album* L., *Aizoon hispanicum* L.). However, in the case of faba bean sole crops, the infestation was higher on half density compared to full density seedlings, except *Spergularia flaccida*, which was largely the majority in both cases. It should be noted that soft wheat and faba bean intercrops decreased significantly the total biomass of weeds ($p < 0.001$).

An infestation of all test plots was observed, with differences in weed species and biomass. *Spergularia flaccida, Malva sylvestris, Sinapis arvensis* L., *Chenopodium album, Aizoon hispanicum* and *Mesembryanthemum nodiflorum* L. were the predominant species (Figure 4). In the case of wheat sole crops, there was no significant difference between the two seeded densities for some weed species (*Spergularia flaccida, Malva sylvestris, Chenopodium album, Aizoon hispanicum*). However, in the case of the faba bean sole crops, the infestation was higher on the half density than on the full density seedlings, except *Spergularia flaccida*, which was largely the majority in both cases.

Compared to the wheat and faba bean sole crops, the intercropping significantly ($p < 0.001$) reduced the development of *Spergularia flaccida*. The intercropping reduced the development of *Malva sylvestris, Chenopodium album and Aizoon hispanicum* compared to F-SC/2. While *Sinapis arvensis* and *Mesembryanthemum nodiflorum* were decreased under the intercropping compared to B-SC/2.

During the 2nd experiment, soft wheat and faba bean sole crops showed a higher degree of infestation in the case of crops sown at half density compared to crops sown at full density. However, weed biomass in the intercropping was significantly lower than that of the monospecific legumes and cereals. It decreased by 92.14 %; 91.51 %; 49.01 % and 51.03 % compared to F-SC/2; B-SC/2; F-SC and B-SC respectively (Figure 5). Finally, we observe that the number of weeds was lower in B-SC/2 compared to F-SC/2 for both experiments. It should be noted that the soft wheat and faba bean intercrops decreased very significantly ($p < 0.001$) the total weed biomass. This de-

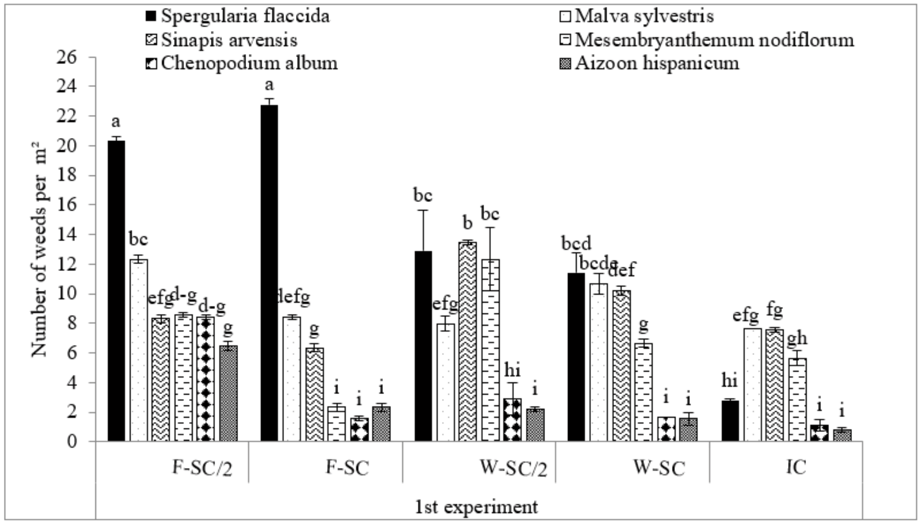

Figure 4: The number of weeds developed per m^2 in sole crops and intercrops of soft wheat and faba bean in 1st experiment. Values represent the mean of three replicates ± standard errors within the same graphic followed by different letters are statistically significant at ($p < 0.05$)

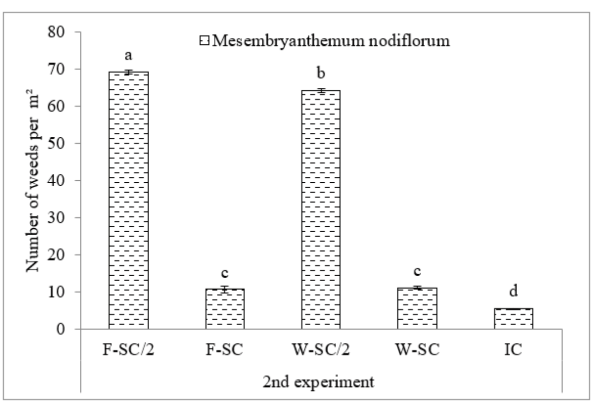

Figure 5: The number of weeds developed per m^2 in sole crops and intercrops of soft wheat and faba bean in 2nd experiment. Values represent the mean of three replicates ± standard errors within the same graphic followed by different letters are statistically significant ($p < 0.05$)

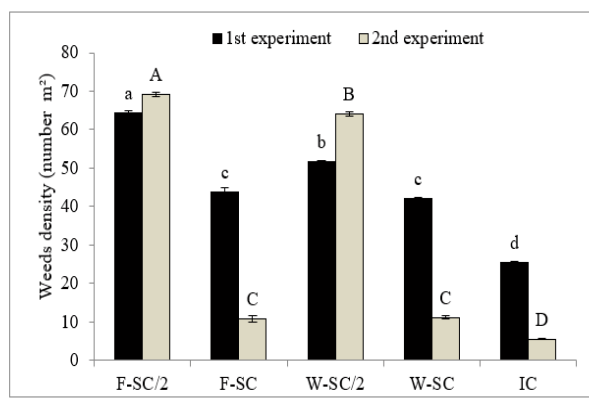

Figure 6: Total number of weeds developed per m^2 in sole crops and intercropping of soft wheat and faba bean. Values represent the mean of three replicates ± standard errors within the same graphic followed by different letters are statistically significant ($p < 0.05$)

crease was of the order of 60.38 %; 41.66 %; 50.68 % and 39.51 % compared to the F-SC/2; F-SC; B-SC/2 and B-SC respectively in the 1st experiment (Figure 6).

3.3 EFFECT OF N LEVELS AND INTERCROPPING ON FABA BEAN PEST DEVELOPMENT

Figure 7 shows the number of black faba bean aphids measured by average accounts made on March 25, 2019 and April 25, 2019 expressed as aphids per plant and aphids per square meter. Faba bean sole crops and faba bean half density sole crops had higher population levels than faba bean intercropped with soft wheat, both in terms of the number of aphids per plant and per square meter. Indeed, under nitrogen treatment, the significant differences were found between intercrops treated, both per plant and per square meter. Finally, in none N-fertilized plots, the intercrops significantly reduced the attack by aphid populations than sole crops.

4 DISCUSSION

Intercropping is known to have interesting effects in terms of productivity, especially when available resources

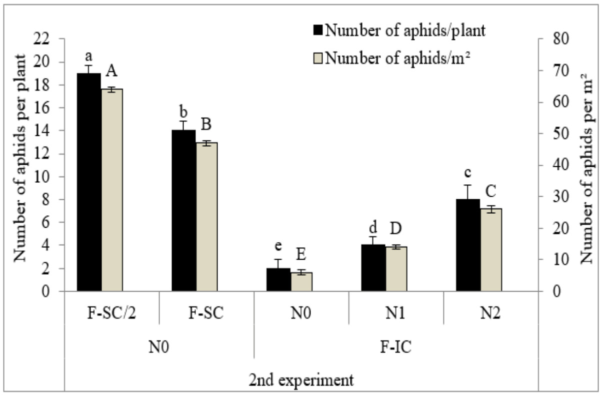

Figure 7: The number of black aphids per plant per m^2 in faba bean sole crops (F-SC and F-SC/2) and intercropping (IC) under three nitrogen treatments in 2nd experiment. Values represent the mean of ten replicates ± standard errors within the same graphic followed by different letters are statistically significant ($p < 0.05$)

are limited, and good weed, disease and pest management (Shtaya et al., 2021). The reduction of weed density and biomass in intercropped plants compared to monocrops has long been mentioned in the literature (Bedoussac et al., 2015; Hamzei and seyedi, 2015). Several studies have explained weeds reduction by two phenomena: (i) higher interspecific competition combined with complementarity between species grown in intercrops, which allows them to use the available resources more efficiently and thus limits the access of weeds to those resources. (ii)the depressive effect of some species in intercrops on weeds through allelopathy by producing phenolic compounds and root exudates toxic for their growth (Norsworthy et al., 2011; Amosse et al., 2013; Saudy, 2015).

At the plot level, intercropping would also make it possible to reduce diseases compared to pure crops. Recent study reported that intercropping reduced stripe rust and powdery mildew diseases in wheat, and chocolate spot and fusarium wilt diseases in faba bean incidence by 45 % on average (Zhang et al., 2019). The effectiveness of this control depends on microclimate conditions such as ventilation conditions, which weaken the disease conditions and reduce its reproduction and spread (Guo et al., 2020). Moreover, the effect of host dilution by changing the planting ratio and arrangement of the cereal in intercropping. The difference in plant height between wheat and faba bean forms a "ventilation corridor", which alters the uniform canopy structure of the cereal in sole crops, enhances airflow, and effectively improves moisture and temperature in the field (Zhu et al., 2020). In addition, modification of host physiology and resistance, as well as direct inhibition of pathogens by antagonistic chemical exudates (Boudreau, 2013; Corre-Hellou et al., 2014).

The intercropping was an effective way to reduce the black bean aphid populations in faba bean compared to pure crops. Similar findings were reported by Ndzana et al. (2014) in pea-wheat intercrops. Plant diversity promotes pest regulation using several mechanisms. Non-host crops grown in intercropping can emit volatile chemicals that harm insect pests, thus providing a degree of protection (Ninkovic et al., 2013; Shalaby and Fouad, 2016; Sulvai et al., 2016). In addition, natural enemies can exert top-down control over pests (Song et al., 2013; Dassou and Tixier, 2016). Other mechanisms can also affect the visual location of host plants, such as greener and/or taller non-host plants, which can camouflage the host plant, or even lead to its physical obstruction (Parker et al., 2013; Gardarin, et al., 2021). All of these factors therefore make it more difficult to recognize the host plant and reduced both the aphid's settlement and mobility in the canopy, affecting its spread dynamics.

On the other hand, nitrogen fertilizer is generally considered a key force leading to disease development due to its effect on plant nutritional status and their ability to multiply pathogens (Dordas, 2008). Along this line, Zhang et al. (2019) and Zhu et al. (2020) indicated that nitrogen fertilizer greatly increased the incidence of wheat powdery mildew. The higher N application can lead to a denser canopy, resulting in a more favorable microclimate for infection (Chen et al., 2013; Guo, 2016; Zhu et al., 2017a). This indicates that the disease occurrence was related to the colonization and spore transmission conditions during the season (Guo et al., 2020). Furthermore, high nitrogen content in plants leaves leads to a reduction in total phenols, flavonoids and peroxidase activity, which affects the epidermal characteristics, cell wall structure and metabolic activity of host crop leaves (Li et al., 2006; Lu et al., 2008). Nevertheless, potassium is beneficial in increasing phenolic and polyphenol oxidase activity in crop leaves. It promotes protein synthesis, sugar and starch synthesis and transport, reduces the source of carbon and nitrogen required by phytopathogenic bacteria and fungi. Therefore, improves the disease resistance of crops (Zhu et al., 2017b). In our most recent publication, the intercropping increased the potassium content of wheat leaves (Sammama et al., 2021), thus, the incidence and severity of diseases in intercropping at various nitrogen levels were significantly lower than those in sole crops. It is deduced, that intercropping is an effective control measure for these two diseases at low input levels, indicating that intercropping should be used as a management strategy for disease control.

5 CONCLUSION

The present study reveals the great resilience of intercropping in reducing weeds, diseases, and pests. Compared to the wheat and faba bean sole crops, the intercropping significantly ($p < 0.001$) reduced the total weed biomass by more than 40 %. Moreover, it reduced the attack of the two diseases in wheat and the black aphid populations in faba bean. When nitrogen was applied to intercropping an increase in the incidence (+ 10 %) and severity (+ 40 %) of yellow rust and septoria was remarkable compared to the untreated intercropping. Thus, the effect of nitrogen fertilizer application on disease severity was greater than incidence. The use of intercropping systems adapted to difficult conditions could be an interesting sustainable agriculture tool to help plants tolerate environmental constraints.

6 ACKNOWLEDGEMENTS

The author gratefully thanks Professor OUHAMMOU Ahmed from the Laboratory of Microbial Biotechnologies, Agrosciences and, Environment, Cadi Ayyad University for identifying the weed species.

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doi:10.14720/aas.2023.119.1.2136 Original research article / izvirni znanstveni članek

The effect of fruit position and bagging treatment on Gamboge disorder in mangosteen (*Garcinia mangostana* L.)

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Received March 21, 2021; accepted March 15, 2023.
Delo je prispelo 21. marca 2021, sprejeto 15. marca 2023

The effect of fruit position and bagging treatment on Gamboge disorder in mangosteen (*Garcinia mangostana* L.)

Abstract: Gamboge disorder has a detrimental effect on mangosteen. The leakage of the gamboge may result from water availability. Thus, modifying the transpiration of the fruit by bagging might minimize the inappropriate leak of the gamboge produced by the fruit. The study's objective was to understand the relationship between different fruit positions and bagging treatment on the gamboge disorder in mangosteen. The experiment was conducted on 10-years old trees by tagging young fruits, five replicates with two fruit positions (inside and outside), and bagging treatment (no bagging, transparent and black plastic bagging). The result showed that bagging the fruits inside the canopy does not affect fruit mass. However, bagging with transparent and black plastic of the fruits inside the canopy decreases fruit size. The fruit quality improves by black bagging on the inside canopy fruits. These findings demonstrate that bagging fruits outside the canopy lowers their quality. Black bags used to package fruits inside the canopy improve fruit quality. However, the treatment also causes more fruit to fall from the tree and decreases the nutrient content.

Key words: canopy architecture; fruit position in canopy; gamboge; fruit characteristics; mangosteen; nutrients

Učinek položaja plodov in ovijanja z vrečkami na pojav fiziološke bolezni gamboge pri mangostinu (*Garcinia mangostana* L.)

Izvleček: Fiziološka bolezen "gamboge"ima škodljiv učinek na kakovost plodov mangostina. Iztekanje grenkih smol (bolezen gamboge) je lahko posledica pomanjkanja vode, zato bi s spreminjanjem transpiracije plodov z ovijanjem z vrečkami lahko zmanjšali neprimerno iztekanje smol iz plodov mangostina. V raziskavi je bilo preučevano razmerje med različnimi položaji plodov v krošnji, njihovim ovijanjem z vrečkami in pojavom motnje gamboge na plodovih mangostina. Poskus je potekal na desetletnih drevesih z označevanjem in ovijanjem mladih plodov v petih ponovitvah dveh položajev v krošnji (znotraj in na obodu krošnje) in načini ovijanja (brez ovoja, prozoren in črn plastični ovoj). Rezultati so pokazali, da ovijanje plodov znotraj krošnje ne vpliva na maso plodov, vendar pa ovijanje s prozornimi in črnimi plastičnimi vrečkami znotraj krošnje zmanjša velikost plodov. Ovijanje plodov s črnimi plastičnimi vrečkami znotraj krošnje izboljša njihovo kakovost, a povzroča tudi njihovo odpadanje in upad nekaterih hranil. Ovijanje plodov na obodu krošnje zmanjšuje njihovo kakovost.

Ključne besede: arhitektura krošnje; položaj plodov v krošnji; lastnosti plodov; mangostin; hranila

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1 INTRODUCTION

Mangosteen (*Garcinia mangostana* L.) is distributed widely in India, Indonesia and Thailand (Mansyah et al., 2013; Parthasarathy and Nandakishore, 2014; Makhonpas et al., 2015); and becomes important source of antioxidant in the tropic (Tjahjani et al., 2014). However, the presence of gamboge or yellow latex disorder in mangosteen fruit becomes a barrier for global promotion (Sdoodee and Limpun-Udom, 2002; Affandi et al., 2008; Qosim, 2013; Rai et al., 2014).

The incident of gamboge disorder has been reported widely across places, seasons, culture practices and tree segments (Setiawan, 2005; Primilestari, 2011; Apiratikorn et al., 2012; Mansyah et al., 2013; Kurniadinata et al., 2016). For example, gamboged-fruits in Java-Indonesia was 30–83 % (Primilestari, 2011; Kurniadinata et al., 2016), in Sumatra-Indonesia 17–53 % (Mansyah et al., 2013; Kurniadinata et al., 2016), while in Songkhla-Thailand 3–5 % (Sdoodee and Chiarawipa, 2005).

There are two forms of gamboge disorder in mangosteen fruits, i.e., in fruit peel and fruit aril. Gamboge spot on the fruit peel is easily removed during post-harvest handling, but the spot on the aril is almost undetectable without open the fruit. Aril with gamboge is less palate because it taste bitter by consumers; although the gamboge contains α-mangosteen and γ-mangosteen a kind of xanthone as anti-inflammatory effects (Sukatta et al., 2013). A device for selecting gamboged-aril using near infra-red detector has been developed (Novita et al., 2011), however the accuracy is still low. Therefore, study to reduce gamboge is important.

Gamboge is common in Gutiferaceae family (Richards, 1990; Te-chato, 2007). The gamboge disorder arises when the latex duct leakages due to fluctuation of water, calcium (Ca) deficiency, disease infection and physical impacts (Asano et al., 1995; Sdoodee and Chiarawipa, 2005; Affandi et al., 2008; Dorly et al., 2008; Poerwanto et al., 2010; Diczbalis, 2011; Irianto et al., 2013; Purnama, 2014; Kurniadinata et al., 2016; Kurniawan et al., 2016). Among potential causes, status of Ca is one of the most tributal among scientists (Dorly, 2008; Poerwanto et al., 2010; Primilestari, 2011; Kurniawan, 2016); because application of Ca has inconsistent effect on the gamboge reduction.

Setiawan (2005) has revealed that Ca status on the mangosteen fruit varies among tree canopy segments as well as among gamboged-fruits. It is well known that uptake of Ca in a plant is driven by transpiration power (Bangerth, 1979); and the transpiration rate through leaf stomata depends on environment conditions (Tjondronegoro et al., 1999; Setiawan et al., 2015). It is probable that different fruit position at canopy has different transpiration rate. Hence, in present experiment, transpiration rate was manipulated through bagging with impermeable plastic to maintain high air relative humidity around mangosteen fruit. We hypothesis that restriction transpiration leads low uptake of Ca, consequently the fruit severe gamboge disorder. Although Bangerth (1979) and White and Broadley (2003) have stated that Ca ions translocation to the fruit could be small because immobile characteristic of Ca ions through the phloem. Objective of present study was to evaluate gamboge disorder and nutrient status in mangosteen from different fruit position and bagging materials.

2 MATERIALS AND METHODS

2.1 STUDY SITE

Research was conducted at Pasir Kuda Experimental Station of Center for Tropical Horticulture Studies (PKHT), Bogor Agricultural University, Bogor, Indonesia from Mar 2016–May 2017. Soil is podzolic type and has water table about three meters below soil surface. Monthly rainfall during experiment ranged from 240–375 mm, temperature ranged from 27–37 °C (average 28.3 °C), and relative humidity ranged from 65–82 % (average 71 %). Wind speed ranged from 0.25 –2.03 m s^{-1} at daytime and sunny condition about 3–6 hour per day.

2.2 PLANT MATERIAL

Experiment used mangosteen trees about 10-year-old derived from seedling at which the previous fruits exhibited variation on gamboge incident. Individual tree were fertilized before flowering (Mar–Apr 2016) with nitrogen, phosphorus, potassium and dolomite at rate 0.5 kg, 0.5 kg, 0.5 kg and 2 kg, respectively. The experiment consisted of two factors, i.e., fruit position at canopy (inside and outside canopies) and bagging (control without bagging, bagging using transparent and black plastic), totaled six treatment combinations. We performed five replications and, in each replication, used one tree.

Initially, we tagged five replications of mangosteen flowers at anthesis to obtain uniform fruit age on 31 May 2016. On 20 Jun 2016 (20 days after anthesis), the young fruits approximately 3–4 cm in diameter, were bagged according to the treatment. Time to bagging followed Pludbuntong and Poovarodom (2013) to coop with critical time of nutrient uptake. At six weeks after anthesis, we determined the final fruits position and classified as outside and inside canopy. We selected healthy 5 fruits for each treatment combination. Outside-canopy (OC) fruit received direct sunshine or visible from horizontal observation, in contrast the sunshine to fruit was blocked by leaves for inside canopy (IC) (Fig. 1)

Water vapor around bagged fruits was theoretically in saturated condition during fruit development until harvest; thus, transpiration rate from the fruit (if any) was theoretically zero. Control unbagged fruits were exposed freely to ambient condition. The bag covered fruit loosely to ensure no restriction on the fruit growth (Fig. 1B–C).

Fruit drop was observed weekly for those of the treatment and non-treatment of a particular tree. The fruit was harvested at 3.5 months after anthesis at stage maturity for consumption indicating by red-purplish peel. Fruit size, color, specific gravity, aril mass and total soluble solid were measured immediately after fruit harvest. Gamboge disorder on peel was scored 1–5 according to Kartika (2004), i.e., 1-as free gamboge, 2-as smooth skin had 1–5 gamboge spots, 3-as smooth skin had 6–10 gamboge spots, 4-as skin dirty had many gamboge spot and present gamboge lines, and 5-as skin dull had severe gamboge spot and lines. Gamboge in aril was scored 1–5 according to Kartika (2004), i.e., 1-as free gamboge, 2-as one aril had gamboge, 3-as two arils had gamboge, 4-as three arils had gamboge, and 5-as more than three arils had gamboge. Levels of Ca, Mg, P and K in fruit peel were determined using AAS (Shimadzu AA 6400, Japan). Nitrogen content of same part was evaluated using Kjedahl method. Specific gravity was measured with Platform Scale method by divide the mass of material in the air by the mass of water displaced multiplied by the specific gravity of water.

Statistical analysis: Data was analyzed using Statistical Tool for Agricultural Research (STAR). We performed ANOVA to determine significant different between the treatment, further evaluation was performed using Duncan Multiple Range test (DMRT) at probability level 5 %.

3 RESULTS AND DISCUSSIONS

3.1 ANALYSIS OF VARIANT

Analysis of variant showed that bagging significantly affected fruit mass, gamboge incident on peel and aril, total soluble solid (°Brix), calcium, and phosphorus levels (Table 1). Characteristics of fruit size (diameter, mass, and specific gravity), gamboge disorder and aril mass varied among trees. We found interactions between fruit position and bagging on fruit mass and height, specific gravity, peel mass and gamboge spot on aril.

All the variables exhibited low coefficient of variant, except peel thickness and nitrogen level that had coef-

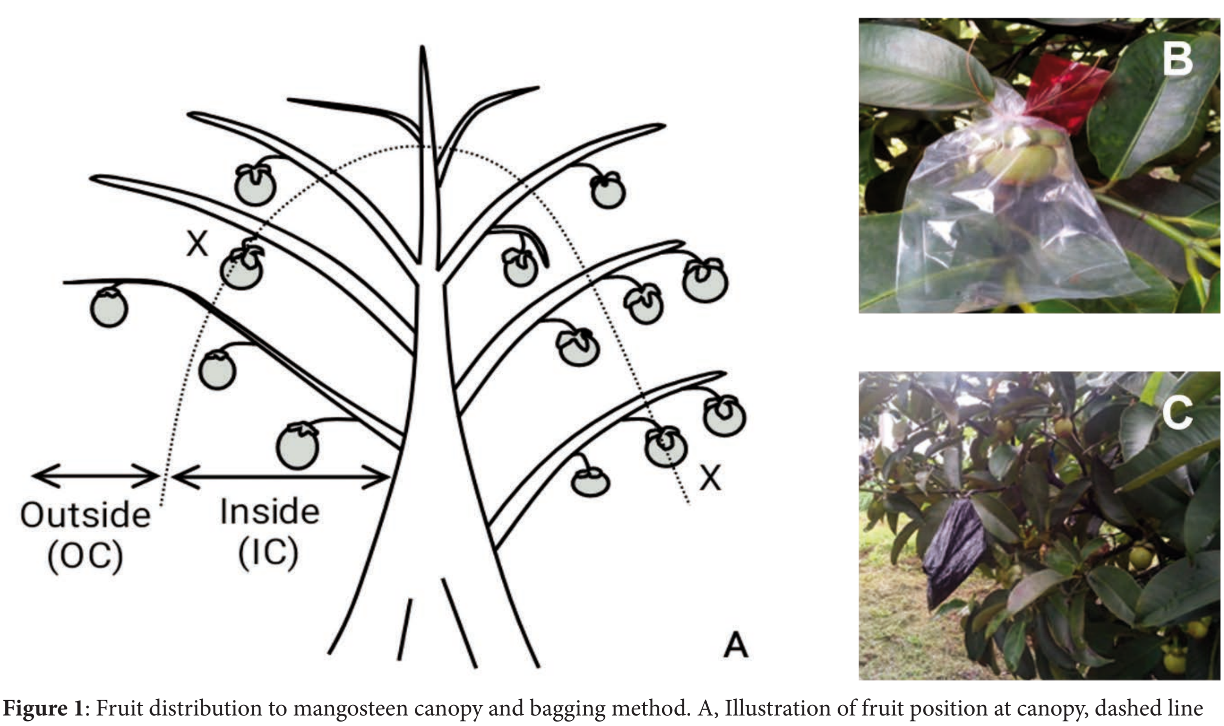

Figure 1: Fruit distribution to mangosteen canopy and bagging method. A, Illustration of fruit position at canopy, dashed line denotes penetration border of direct sunshine on canopy. B, Outside-canopy (OC) fruit is bagged with transparent plastic. C, OC fruit is bagged with black plastic

Table 1: Mean square of fruit characters of *Garcinia mangostana* L. based on ANOVA from repetition, bagging and fruit position at tree canopy, and its interaction

	Mean square				
Variable	Tree (repetition)	Bagging (B)	Fruit position (F)	B × F	cv (%)
Fruit drop	0.4908	0.2190	0.7933	0.1313	26.08
Fruit diameter (horizontal)	0.0010**	0.5532	0.5077	0.3868	3.86
Fruit height (vertical)	0.0197*	0.2535	0.2638	0.0409*	4.33
Fruit mass	0.0109*	0.5428*	0.5310	0.0945*	11.18
Fruit specific gravity	0.0341*	0.7680	0.5496	0.0651**	9.93
Peel mass	0.2007	0.2460	0.7246	0.0270**	11.06
Peel thickness	0.4065	0.3935	0.2842	0.3941	42.14
Peel color index	0.2826	0.4725	0.4870	0.3258	6.59
Gamboge spot on peel	0.0159*	0.0287*	0.3607	0.4966	28.80
Gamboge spot on aril	0.0004**	0.3787**	0.4673	0.0311*	28.01
Aril mass	0.0239*	0.8481	0.1971	0.1871	17.56
Sweet level (°Brix)	0.1362	0.0085**	0.7243	0.7043	1.22
Calcium level (Ca)	0.0756	0.0805*	0.2213	0.5844	22.11
Nitrogen level (N)	0.3956	0.1599	0.6579	0.6828	37.01
Phosphorus level (P)	0.2495	0.0028**	0.0101*	0.6806	9.45
Potassium level (K)	0.0916	0.3640	0.3297	0.4723	20.63
Magnesium level (Mg)	0.0844	0.9695	0.1889	0.9695	25.61

Significant at level of 5 %; ** significant at level of 1 %; cv-coefficient of variant

ficient of variant larger than 30 %. It indicates that the characters of peel thickness and nitrogen level were highly dependent on the characteristic of the mangosteen tree. According to Mansyah et al. (2013) and Sobir et al. (2013), genetic variation among seedlings is apparent in apomicts *Garcinia mangostana*. In present experiment, plants are originally from seeds, thus, high variation among trees was due to different genetic background. We had tried to observe subsequence fruiting period in Apr–May 2017, however, most trees failed to flower due to a lot of rain during period of Jun–Oct 2016. According to Sdoodee and Sakdiseata (2013), mangosteen expresses alternate bearing with index 0.25–0.57, indicates strong effect of climatic condition especially rain fall on the fruiting.

High coefficient of variance among trees could relate to the mangosteen physiology. Wiebel et al. (1993) states that mangosteen is shade-tolerant, however, the highest carbon gain is obtained for leaves grown in 50 % shade than 20 % or 80 % shade treatments. We observed different canopy shaped such as spherical, columnar, and irregular in the experiment, which might cause different shading level on leaves among the trees leads to different source capacity. Sdoodee and Phonrong (2006) concluded that a set of 17–18 leaves is required to support one mangosteen fruit to get optimum marketable size.

3.2 FRUIT DROP

Fruit drop was pronounced in all trees, in both treated-and untreated-fruits. A tree with a larger number of fruits tended to abort more fruits than smaller one.

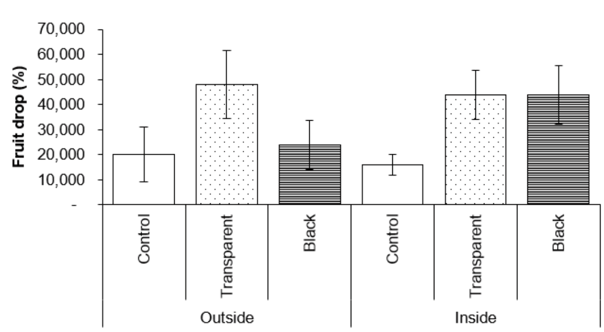

Figure 2: Total number of fruits drop in Garcinia mangostana L. as affected by bagging treatments. Bar ± S.E

On average, a tree aborted 4–7 fruits weekly from one month after anthesis until harvest. Fruit started to mature at 3.5 months after anthesis. Within bagged fruits, there was significant different on the drop rate among the treatments (Fig. 2). In OC, the drop rate of control fruits was statistically similar to the fruit bagged with black plastic. On the other hand, bagged-IC-fruit aborted by about three times larger than control, irrespective of bagging material. This finding implies that drop of IC fruit is more sensitive than OC fruits.

Fruit drop in mangosteen is still poorly studied. In mango, Hofman et al. (1999) stated that bagging increase fruit drop. Setiawan (2012) stated that fruit drop in mangosteen depends on canopy sector, i.e., at rate 44–50 % from outer canopy and 40–41 % from inner canopy. In present experiment, both young and mature fruits aborted in bagging treatments. Interestingly, all aborted fruits from control treatment were at the young age 5–10 weeks after anthesis. It is likely that bagging treatment stimulates fruit drop at later stage of fruit development.

There is a correlation between fruit drop and gamboge disorder. Aborted fruits from transparent plastic were higher than 90 %, irrespective the fruit position at canopy, severed from gamboge disorder on peel. At control, by less than 25 % of aborted fruits expressed gamboge spot. However, the number of aborted fruits with gamboge spot from control IC tended to be larger than those from control OC, although statistically similar (Fig. 2). Unexpectedly, bagging with black plastic increased aborted-young fruit noticed with gamboge disorder on peel. It is still unclear, why gamboge incident was high in aborted-fruits and why application of black plastic reduced drop in OC fruit.

3.3 FRUIT SIZE

Interaction between bagging treatment and fruit position on specific gravity, fruit and peel mass, and fruit diameter presented in Table 2. The maximum value of specific gravity was found in black plastic bagging in IC and in control in OC. in IC fruit bagging with black plastic increased specific gravity by 10.7 % contrary to IC that reduced by 11.2 %. Different fruit size of mangosteen among canopy sectors has been reported by Setiawan (2012).

In both canopy positions, the largest fruit mass was obtained in control bagging. Furthermore, the peel from the black plastic bagging was heavier than the peel from the OC. Fruit diameter was similar among treatments, irrespective of the position at canopy and bagging. It has been studied that fruit quality depends on fruit load (Sdoodee and Phonrong, 2006; Sdoodee et al., 2008) and fruiting seasons (Apiratikorn et al., 2012).

We speculated that different microclimate around IC and OC may affect the fruit size. At daytime, OC fruits received much direct sunshine causing fruit heating. Bagging with black plastic presumably increased air temperature around the OC fruit markedly caused high respiration. As a result, OC fruits had lower fruit mass. In Satsuma Mandarin, Daito et al. (1981) reported that respiratory rate varies among the fruiting locations within the canopy. In storage experiment, Castro et al. (2012) notified that mass loss of mangosteen fruit is accounted 34.4 % higher in 25 °C than 13 °C. In our simulation, temperature inside bag was 3-6 °C higher than ambient temperature. On the other hand, IC fruits received limited direct sunshine by about 10–25 % from full sunshine. Average temperature inside canopy recorded 0.5–11.6 °C lower than outside canopy. As consequences of increasing heat inside bagged-IC fruits, the fruits temperature was approximately similar to the temperature of control OC fruits that received direct sunshine. Therefore, specific gravity and peel mass of black plastic treatments of IC fruits are like control of OC fruits (Table 2).

3.4 FRUIT QUALITY

Fruit position and bagging treatments did not affect sweet level of aril (Table 3). This data is in line with previous finding (Apiratikorn et al., 2012; Pludbuntong

Table 2: Specific gravity, fruit and peel weight, and fruit diameter of mangosteen from bagging treatment and fruit position at tree canopy

	Specific gravity (g)		Fruit mass (g)		Peel mass (g)		Fruit diameter (cm)	
Bagging	IC	OC	IC	OC	IC	OC	IC	OC
Control	56.97b	61.97a	56.10b	61.15a	35.58b	41.29a	4.52ab	4.36b
Transparent	58.06ab	57.22ab	56.38b	55.07b	35.31b	36.47b	4.20b	4.59ab
Black plastic	63.06a	55.01b	54.30b	54.30b	41.52a	36.27b	4.42ab	4.75ab

Values in each column of parameter followed by different alphabet are significantly different based on DMRT at α 5 %; IC-inside canopy, OC-outside canopy and Poovarodom, 2013). Apiratikorn et al. (2012) stated that total soluble solid (TSS) and total acid (TA) of mangosteen fruits are stable among seasons. Peel color index was similar statistically among fruit position at canopy and bagging treatment (Table 1 and 3). Interestingly, fruits treated with black bag had bright red-brownish color while dull red brownish from control and transparent treatment, irrespective of the fruit position. Saengnil et al. (2005) has recommended to cover mangosteen fruit with bag to increase visual attrackness. This indicates that pigmentation in mangosteen peel is sensitive to bagging. In apple, Saure (1990) noted that temperature and light determine fruit pigmentation. Fruit bagging is common to maintain high quality fruit (Fallahi et al., 2001; Saengnil et al., 2005; Candra et al., 2013; Pludbuntong and Poovarodom, 2013).

3.5 GAMBOGE DISORDER

Gamboge disorder on peel and aril was marked in all fruits irrespective of fruit position at canopy and bagging treatment (Fig. 3). Irrespective of gamboge severity, number of fruits with gamboge-peel on control IC was approximately 0–60 % (average 36 %), while it was approximately 60–100 % (average 80 %) on control OC. Fruit with gamboge-aril was 0–40 % (average 28 %) and 40–80 % (average 64 %) in control OC and IC, respectively. It is contrary to persimmon fruit disorder that the least is in the middle canopy (George et al. 1996). Number of gamboge-fruit in present experiment is larger than that has been reported by Setiawan (2005). According to Apiratikorn et al. (2012) gamboge disorder in mangosteen depends on the fruiting season. In present experiment, however, the gamboge disorder seems unlikely due to different fruit position at canopy solely, as indicated by insignificant effect (Table 1).

Number of fruits with gamboge-aril was insignificantly different in OC fruit treated with transparent plastic, but significantly increased by about 57 % in black plastic than control. The gamboge-aril on IC fruit, on the other hand, decreased up to 44 % and 24 % in transparent and black plastic treatments, respectively. In peel, number of gamboge-IC fruit increased by 77.8 % from control when treated with black plastic. Interestingly, bagging with transparent plastic tended to reduce gamboge-peel in IC fruits, although statistically insignificant at level of 5 % to control. In OC fruit, bagging irrespective of the material decreased number of fruits with gamboge-peel, i.e., 35–45 %.

Here, we present manipulation of transpiration highly affects gamboge disorder in mangosteen fruit. The effect of blocking transpiration was more pronounce in IC than OC fruits in the incident of gamboge-aril; conversely, it is more pronounced in OC fruits in the presence of gamboge-peel. It is likely that mechanism that promote gamboge disorder on peel and aril could be different. We observed inconsistent relationship of gamboge-aril of OC fruit with by bagging treatment (Fig. 3B), unlike the gamboge-peel (Fig. 3A). On peel, gamboge severity increased by bagging treatment irrespective of fruit position; and IC fruits was more sensitive to bagging treatment. It seems that high gamboge disorder on peel is common on mangosteen fruit from outer canopy.

Individual tree produced different level of gamboge disorder in both peel and aril (Table 1). This matter could arise as consequences of different microclimate due to different canopy shape of each tree. Inside canopy had relative air humidity 1.98–2.71 times higher and wind speed 0.1–0.3 m s^{-1} lower than the outside canopy. Thus, microclimate inside canopy facilitates fruit to have lower transpiration rate, in absence of bagging treatment.

3.6 NUTRIENT STATUS

Calcium and phosphorus levels varied among different fruit position and bagging treatments, while potassium and magnesium levels were stable among treatments (Table 4). Nitrogen tended to decrease in bagged fruit than control, irrespective of the fruit position at canopy although statistically similar at 5 % level of con-

Table 3: Sweetness level (°Brix) and peel color index of mangosteen at harvest from bagging treatment and fruit position at tree canopy

	(°Brix)		Peel color index	
Bagging	IC	OC	IC	OC
Control	18.30a	18.22a	1.692a	2.044a
Transparent	17.80a	18.02a	1.740a	2.530a
Black plastic	17.96a	17.99a	2.560a	1.890a

Values in column followed by different alphabeth are significantly different based on DMRT at α 5 %; IC-inside canopy, OC-outside canopy

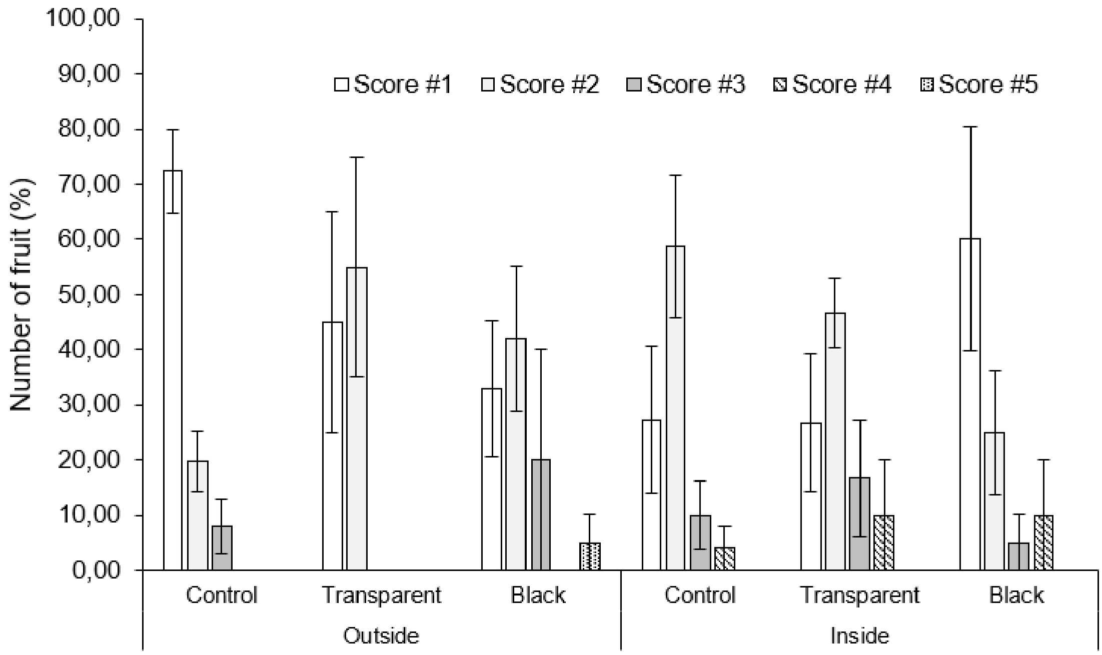

Figure 3: Percentage of fruit according to score of gamboge incident on peel (A) and on aril (B). Score #1, healthy fruit without gamboge while score #5 is very severe of gamboge. Bar ± S.E

fident of DMRT test. Bagging with black plastic consistently lowered Ca and P levels on mangosteen peel of OC fruits. In IC fruits, there was no marked different on Ca and P levels among bagging treatments. In control fruits, Ca level was significantly higher in OC than IC fruits, but the level of other nutrients was similar among OC and IC fruits. Consequently, transpiration manipulation in IC fruits reduce Ca level by 16.67 %. The reduction of Ca level was more marked in OC fruits; depends on bag color, i.e., 34.78 % in black plastic and 26.09 % in transparent plastic. This is in line with Pludbuntong and Poovarodom (2013) where blocking transpiration reduces Ca level on mangosteen fruit.

There were correlations between Ca level and gamboge disorder on aril ($R^2 = 0.135$; $p < 0.000$). These findings supported hypotheses that Ca plays important role on gamboge disorder. Limited transpiration and high fruit temperature in bagged treatment as the effect of high exposure to sunshine may increase the incident of gamboge-aril. It implies that increasing transpiration rate and maintaining fruit temperature could be important aspect in gamboge management, because according Setiawan (2012) 86–94 % mangosteen fruit is located inside canopy. Sdoodee and Hadloh (2007) have evaluated a pruning trial of mangosteen tree, reveals that topping at 3-meter height increases light transmission, photosynthetic rate by 48.55 % and root growth, and maintains continuation of fruit bearing.

4 CONCLUSIONS

Fruit position and bagging treatment in mangosteen trees influence the fruits' characteristics, quality, and

Table 4: Nutrient level of fruit peel of mangosteen from different bagging treatment and fruit position at tree canopy

	Ca (%)		N (%)		P (%)		K (%)		Mg (%)	
Bagging treatment	IC	OC	IC	OC	IC	OC	IC	OC	IC	OC
Control	0.18b	0.23a	0.64a	0.51a	0.09a	0.09a	1.65a	2.17a	0.05a	0.05a
Transparent	0.15c	0.17bc	0.43a	0.43a	0.08b	0.07bc	2.04a	1.99a	0.05a	0.04a
Black plastic	0.15c	0.15c	0.36a	0.39a	0.08b	0.06c	1.69a	1.60a	0.05a	0.04a

Values in each column of parameter followed by different alphabet are significantly different based on DMRT at α 5 %; IC-inside canopy, OC-outside canopy nutritional content. Fruits inside the canopy with black bagging improved quality by reducing the percentage of gamboge disorder on the peel and fruit aril compared to transparent bagging. However, both bagging treatments increased the number of fruit falls and reduced nutrient content. Outside canopy fruits with black bagging had a lower percentage of fruit health, size, and quality than transparent bagging. Therefore, there may be better options than bagging the fruits outside the canopy.

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doi:10.14720/aas.2023.119.1.2970 Original research article / izvirni znanstveni članek

Evaluation of yield and water use efficiency of quinoa under irrigation regimes, gamma aminobutyric acid, and vermicompost application

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Received December 22, 2022; accepted March 17, 2023.
Delo je prispelo 22. decembra 2022, sprejeto 17. marca 2023

Evaluation of yield and water use efficiency of quinoa under irrigation regimes, gamma aminobutyric acid, and vermicompost application

Abstract: The current study was aim to evaluate the interaction effects of gamma aminobutyric acid (GABA) and vermicompost on yield and yield components of quinoa under different levels of drought stress. For this, two experiments were similarly designed as the factorial-split with four replicates for evaluating this hypothesis. Irrigation regimes (50, 75, and 100 % of plant water requirement (PWR)) as the main plot and vermicompost V (0, 5 t ha^{-1}) × gamma aminobutyric acid GABA levels (0, 5, 10 mg l^{-1}) as the subplot were designed. Severe drought stress had a significant effect on plant height. Plant height reduced 31.8 % after using 50 % of PWR compared to the control conditions. Although drought stress negatively affected the 1000 seed mass and seed yield, GABA foliar application alleviated these effects. After using 50 % of PWR, 10 mg l^{-1} of GABA increased the seed yield and harvest index up to 21.22 and 15.5 %, respectively, compared to the non-foliar application. The reduction in PWR from 100 to 50 % led to increasing in P and K concentrations, as well as sugar and proline contents. In the same conditions, the use of GABA or V had a significant effect on improving these traits. A similar trend was also recorded in relation to water use efficiency. Therefore, using 10 mg l^{-1} of GABA and 5 t ha^{-1} of V can be effective in alleviating water stress.

Key words: *Chenopodium quinoa*; proline; seed yield; sugar; drought stress

Abbreviations: V: vermicompost, GABA: gamma aminobutyric acid, PWR: plant water requirement, WUE: Water use efficiency

Ovrednotenje pridelka in učinkovitosti izrabe vode kvinoje pri različnih načinih namakanja in dodatku gama aminomaslene kisline in komposta deževnikov

Izvleček: Namen raziskave je bil ovrednotiti vzajemne učinke gama amino maslene kisline (GABA) in komposta deževnikov (vermikomposta) na pridelek in njegove komponente kvinoje pri različnih ravneh sušnega stresa. V ta namen sta bila izvedena dva podobna poskusa kot poskusa z deljenkami s štirimi ponovitvami. Načini namakanja (50, 75 in 100 % potrebe rastlin po vodi (PWR)) so bili na glavni ploskvi in dodatki vermikomposta (V; 0, 5 t ha^{-1}) ter gama aminomaslene kisline (GABA; (0, 5, 10 mg l^{-1}) na podploskvah. Velik sušni stres je imel značilen učinek na višino rastlin. Višina rastlin se je zmanjšala za 31,8 % pri 50 % oskrbi rastlin z vodo v primerjavi s kontrolo. Čeprav je sušni stres negativno vplival na maso1000 semen in pridelek semena, je foliaren nanos GABA ublažil te učinke. Pri 50 % oskrbi z vodo je dodatek 10 mg l^{-1} of GABA povečal pridelek semena in žeteveni indeks za 21,22 in 15,5 % v primerjavi z obravnavanjem brez foliarnega dodatka GABA. Zmanjšanje PWR iz 100 na 50 % je vodilo k povečanju koncentracij P in K, kot tudi k povečanju vsebnosti sladkorja in prolina. Pod enakimi pogoji je imela uporaba GABA ali V značilen učinek na izboljšanje teh lastnosti. Podoben trend je bil zabeležen glede povezave z učinkovitostjo izrabe vode. Zaradi vsega naštetega je uporba 10 mg l^{-1} GABA in 5 t ha^{-1} V lahko učinkovita pri blažitvi sušnega stresa.

Ključne besede: *Chenopodium quinoa*; prolin; pridelek semena; sladkor; sušni stres

Okrajšave: V: vermikompost, GABA: gama amino maslena kislina, PWR: zahteva rastline po vodi, WUE: učinkovitost izrabe vode

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1 INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.), an annual herbaceous plant from the *Amaranthaceae* family, is grown in arid and semi-arid regions of the world (Hinojosa et al., 2018; Bedoya-Perales et al., 2018), especially in Iran (Razzaghi et al., 2012; Ahmadi et al., 2019). The crop is well-known for its nutritional values based on the carbohydrates, lipids, and proteins nutrients (Gómez-Pando et al., 2010). Leucine, phenylalanine, and valine (822, 669, 583 mg 100 g^{-1} of air-dried flour, respectively) were determined as the main amino acids of this crop (Agza et al., 2018). The grain flour of quinoa has been extensively used in the food industry due to its gluten free-based values (Wu et al., 2020) and highly nutritive quality based on the protein, Ca, Fe, and Zn contents (Ballester-Sánchez et al., 2019, Bazile et al., 2016; Präger et al., 2018), which can justify the quinoa as a suitable product for celiac patients (Peñas et al., 2014).

The appropriate tolerance of quinoa perceiving stressful and unfavorable environments were precisely reported (Talebnejad and Sepaskhah, 2015). Moreover, this plant can be properly considered as a pivotal candidate where water shortage is known as a crucial challenge (Talebnejad and Sepaskhah, 2015; Bedoya-Peraleset al., 2018). Ahmadi et al. (2019) reported that quinoa is a well-known tolerant crop against the drought stress conditions. However, water shortage and drought stress are considered as the serious problems for crop production, especially in the east and northeast of Iran (Bannayan et al., 2011; Koocheki et al., 2014), the total annual rainfall in which is recorded about 187 and 285 mm, respectively, indicating water deficiency as the main challenge against the successful crop production (Iran meteorological organization, 2020). Drought stress may significantly affect the plant growth and production by diminishing the leaf area (Feng et al., 2013), decelerating the photosynthesis rate (Saeidi et al., 2017; Zhao et al., 2020; Nadali et al.,2021), and retarding the enzymatic activity (Xu et al., 2015; Askary et al., 2018). Deficit irrigation regimes can negatively affect the dry matter production and seed yield of quinoa compared to the normal irrigation treatment (Talebnejad and Sepaskhah, 2015). Therefore, it is necessary to use the agronomic approaches to increase the plant tolerance against the stressful conditions, which maximizes the crop yield and water use efficiency (Razzaghi et al., 2012).

Water use efficiency (WUE) is a crucial indicator related to evaluating available or applied water that can determine an actual plant function, especially in arid and semi-arid areas. The concept of WUE indicates how much growth and performance changes for each unit of water used (Dong et al., 2011). Under dried conditions, plants generally use the available water with higher efficiency, although this may lead to a decrease in plant yield (Hosseinzadeh et al. 2018). Therefore, when the plant is faced with water limitation or suboptimal irrigation, the set of operations such as organic matter supply can directly affect the efficiency of consumed water by stimulating the growth behavior.

Soil fertility can be determined for mitigating the stress effects when the plant is affected by the adverse environmental conditions. In this regard, the use of vermicompost has been known as an appropriate media for improving the rhizosphere fertility and increasing the crop production (Joshi et al., 2015). Vermicompost can significantly promote the soil biological mechanisms (Hosseinzadeh et al., 2020) and simultaneously regulate the synchrony of minerals uptake during the growing seasons due to the balanced nutrient and high organic matter contents (Rezaei-Chiyaneh et al., 2020; Xu et al., 2016). Seyyedi et al. (2016) reported that the expanded root growth decreased soil pH, and increased microbial activity showed the positive consequences of vermicompost application.

Endogenous hormonal modifications are known as a plant self-mechanisms for repairing the cellular damage (Dobra et al., 2010), balancing the osmolytes uptake (Sarwat and Tuteja, 2017), stabilizing the cell membrane (Albacete et al., 2014), and proliferating the plant tolerance against the abiotic stresses such as drought conditions (Ni et al., 2013). However, the efficiency of internal self-mechanisms may be disturbed by increasing the severity of water shortage. Therefore, the use of exogenous phytohormone is possibly useful for mitigating the adverse consequence of water shortage. In this regard, γ-aminobutyric acid, a four-carbon non-protein amino acid, has been introduced as a crucial plant growth regulator for alleviating the water deficiency during the growing season (Yong et al., 2017). The use of γ-aminobutyric acid (GABA) is proposed for diminishing the side effects of water stress by modulating the plant signaling (Ji et al., 2018), scavenging the reactive oxygen species, and regulating the osmolytes (Vijayakumari and Puthur, 2016), which improves the plant growth under water scarcity (Rezaei-Chiyaneh et al., 2018); therefore, this study aims to evaluate GABA effects on yield and yield components of quinoa under different levels of drought stress. Furthermore, the interaction between vermicompost and GABA was assessed for finding a suitable strategy in improving water use efficiency.

2 MATERIALS AND METHODS

2.1 SITE DESCRIPTION

The experiment was conducted in two experimental stations located in Birjand [Faculty of Agriculture, University of Birjand (latitude: 32° 53′N, longitude: of 59°13′E, elevation: 1480 m above the sea level)] and Mashhad [Faculty of Agriculture, Ferdowsi University of Mashhad (latitude: 36◦15′N, longitude: 59◦28′E, elevation: 985 m above the sea level)] during the growing seasons 2019 (Fig. 1). Both experimental sites were located in a semi-arid region, Razavi Khorasan and South Khorasan provinces, Iran. Meteorological data during the growing season are given in Table 1.

2.2 EXPERIMENTAL DESIGN

Two experiments were similarly designed as the factorial-split plot with four replicates. The experimental factors were included. Irrigation regimes (50, 75, and 100 % of water requirement) and vermicompost (0, 5 t ha^{-1}) × GABA (Sigma-Aldrich, A2129; $NH_2(CH_2)_3COOH$) levels (0, 5, 10 mg l^{-1}) were considered as the main plot and subplot, respectively (Fig. 2). The materials related to the analyses of vermicompost (produced by Arta Manufacturing Group, Iran) included N (1.45 %), P (1.16 %), K (1.2 %), and organic matter (18.65 %).

2.3 AGRONOMIC PRACTICES

In both Brijand and Mashhad stations, all agronomic operations were relativity similar. Before preparing the seed bed, sampling was done from 0-30 cm depth based on the soil of testing location for determining the physical and chemical properties (Table 2). In the following, vermicompost was applied according to experimental treatments.

After preparing the experimental soils (deeply plowing, twice disking, and leveling), the plots were designed (5 m width and 3 m length). Each block consisted of three main plots with six subplots. The distances among the plots and blocks were considered as 0.5 and 2 m, respectively. Seeds ('Titicaca') were sown on 29th and 26th July 2019 in Mashhad and Birjand, respectively. The final density was determined as 60 plant m^{-2}. Foliar spraying of GABA was performed at 2-4 and 10-12 leaf stages, respectively.

Based on the weather conditions at both sites, the plant water requirement was calculated by using CROPWAT software (FAO 1992). The first irrigation was done immediately after the seed sowing and the second irrigation was performed three days later. Weed management was conducted manually during the growing season. Af-

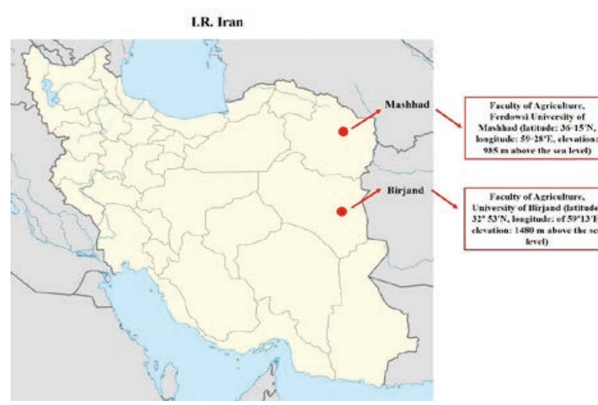

Fig 1: A map graphical presenting the position of the experimental field in Iran. The experiment was conducted in two experimental stations located in Birjand and Mashhad

Table 1: Weather criteria of experimental sites located at Birjand (B) and Mashhad (M), Iran in 2019

	Minimum temperature (°C)		Maximum temperature (°C)		Total rainfall (mm)		Relative humidity (%)	
Month	B	M	B	M	B	M	B	M
July	21.3	22.4	37.6	37.4	0.0	0.5	13.6	23.6
August	18.8	21.0	36.4	35.5	0.0	0.0	15.1	24.0
September	14.7	15.9	34.0	30.7	0.0	0.0	15.3	26.9
October	10.0	11.7	29.7	26.2	0.0	0.1	18.9	38.4
November	3.9	4.2	20.6	15.8	1.0	0.6	31.9	52.3
Average	13.7	15.0	31.7	29.1	-	-	19.0	33.0
Total	-	-	-	-	1.0	1.2	-	-
30 years	13.0	14.2	30.7	28.9	1.8	5.1	24.6	39.1

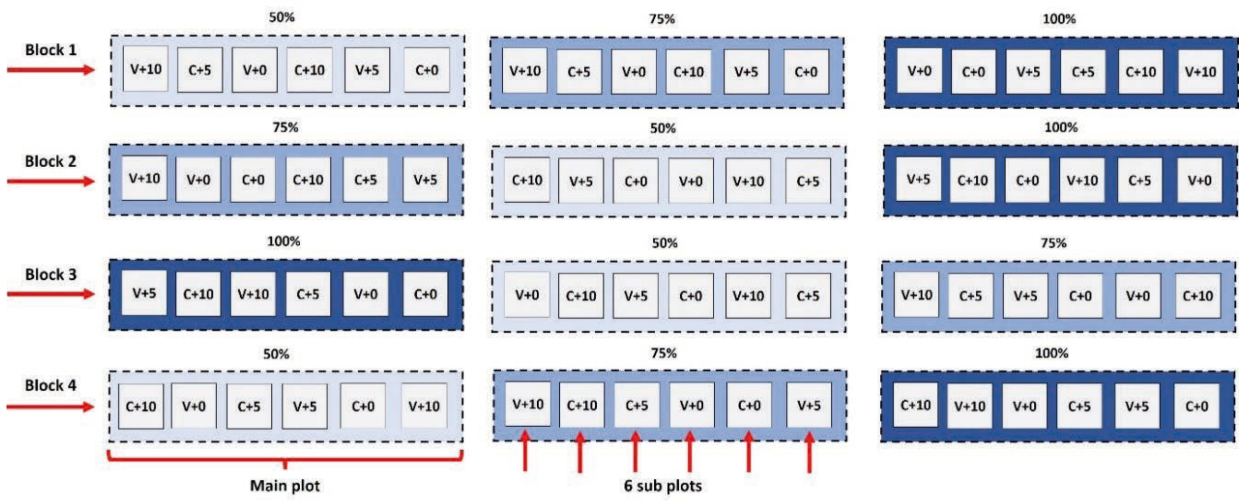

Fig 2: A schematic design of the experiment. Experiments were designed as the factorial-split plot with four replicates. Irrigation regimes (50, 75, and 100 % of water requirement) and vermicompost (0 and 5 t ha^{-1}) × GABA levels (0, 5, 10 mg l^{-1}) were considered as the main plot and subplot, respectively

Table 2: The soil quality characteristics of experimental sites located at Birjand and Mashhad, Iran

	%				mg/kg				
Experimental sites	Clay	Silt	Sand	OC	N	P	K	pH	EC (dS/m)
Birjand	18	12	70	0.16	0.031	11.75	225.3	7.80	4.58
Mashhad	44	32	24	0.48	0.080	6.71	181.5	8.34	1.16
Average	31	22	47	0.32	0.055	9.23	203.4	8.07	2.87

ter establishing the plant, the use of drought stress began for this purpose.

2.4 DATA COLLECTION

Six plant $plot^{-1}$ were randomly selected by considering the marginal effects (25 cm at the first and end of the plots and 2 sides of the rows) at the maturity stage (26th and 21st October 2019, in Birjand and Mashhad, respectively). Then, plant height, seed number/plant, seed mass/plant and 1000 seed mass were determined. Seed and biological yields and harvest index were also measured at the central part of the plots (11.25 m^2). Sugar and proline contents were determined based on the studies of Dubois et al. (1956) and Bates (1973), respectively, after measuring the seed yield. Moreover, P and K concentrations were measured based on the study of Murphy and Riley (1962) and Miller (1998).

After determine yield, water use efficiency (WUE) was calculated as suggested by Dong et al. (2011). To evaluate WUE, total amount of underground water and probable run off were to be ignored (Eq. 1).

WUE (kg. m^{-3}) = (grain yield (kg) / total amount of water used (m^3) x 100

2.5 STATISTICAL ANALYSES

Finally, the measured characteristics were statistically analyzed. After Bartlett test, data analysis (ANOVA) was conducted in split plot factorial design (SPFD) based on the randomized complete block design (RCBD) in two locations (combined analysis). Means were compared by using LSD test at 5 % probability level. The SAS software (Version 9.4) and Excel were used for analyzing the data and drawing the figures.

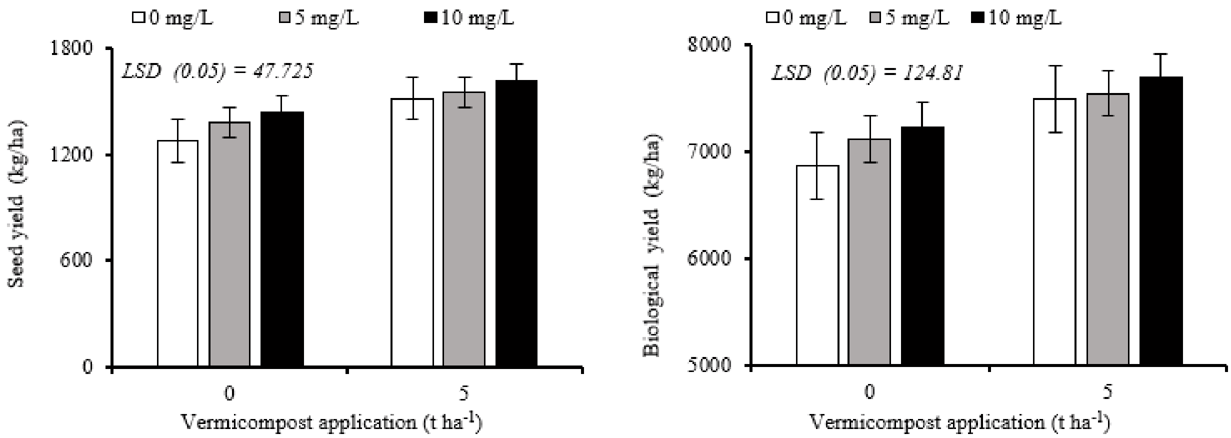

Fig 3: Seed and biological yields in quinoa as affected by interaction between vermicompost application and GABA levels

3 RESULTS

3.1 WEATHER CRITERIA IN BIRJAND AND MASHHAD REGIONS

Table 1 indicates the average minimum and maximum temperatures, as well as total rainfall in Birjand and Mashhad regions. During the growing season, the average of maximum temperature in Birjand region (31.66 °C) was slightly higher than Mashhad region (29.12 °C). Similarly, Mashhad region received the higher relative humidity (33.03 %) compared to Birjand region (19.96 %). Therefore, it seems that quinoa plant was comparatively experienced more heat stress under Birjand climate.

3.2 YIELD AND YIELD COMPONENT

As shown in Table 3, the plant height and 1000 seed mass of quinoa were affected by irrigation regimes, GABA, and vermicompost (V) under Birjand and Mashhad conditions. Irrigation treatments had the significant effects on plant height and 1000 seed mass. High level of drought stress had a significant negative effect on plant height. Thus, the plant height was reduced to 31.8 % after using 50 % of PWR compared to the control (Table 3). Reduction was observed in Birjand than Mashhad region (Table 3). Further, severe drought stress significantly decreased 1000 seed mass in Birjand and Mashhad regions. Although drought stress caused the negative effects on plant height and 1000 seed mass, GABA foliar application had the positive effect in reducing these effects. Therefore, the use of 5 and 10 mg l^{-1} GABA increased the plant height for 6.8 and 10.5 %, respectively, compared to the non-foliar application (Table 3). V played an effective role in decreasing the adverse effects of drought stress, similar to the GABA effect (Table 3).

Based on the results, the interaction between irrigation and V had a significant effect on seed yield, biological yield, and harvest index (Table 5). Thus, the amount of seed yield, biological yield, and harvest index decreased under the drought stress. However, V reduced the negative effects of drought on these indices (Table 5). No significant difference was observed between the interaction of 5 t ha^{-1} of V + 75 % of PWR treatment and 0 t ha^{-1} of V + 100 % of PWR treatment as the seed yield. Moreover, harvest index in 75 % of PWR + 5 t ha^{-1} of V treatment (21.57 %) was significantly higher than 100 % of PWR + 0 t ha^{-1} of V treatment (20.44 %).

GABA significantly improved the seed yield, biological yield, and harvest index, similar to the V use. Under severe drought stress, the maximum seed yield (1292.25 kg ha^{-1}) was obtained by using 10 mg of GABA l^{-1} (Table 6). Similar results were obtained for biological yield (6847.38 kg ha^{-1}) and harvest index (18.82 %) (Table 6).

The maximum seed and biological yields (1724.5

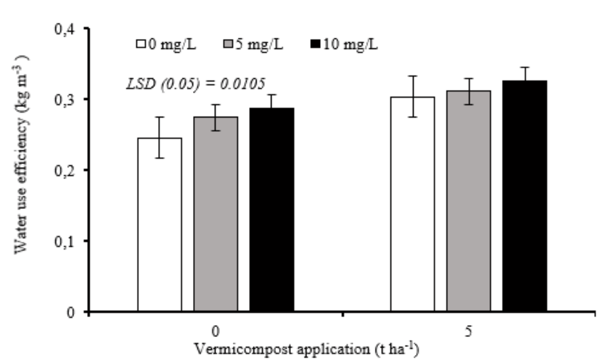

Fig 4: Water use efficiency in quinoa as affected by interaction between vermicompost application and GABA levels

Table 3: Plant height, 1000 seed mass, of seed yield, biological yield, and harvest index of quinoa as affected by irrigation regimes, vermicompost application, and GABA levels in Birjand and Mashhad, Iran

Treatments	Plant height (cm)	1000 seed mass (g)	Seed yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Harvest index (%)
Location					
Birjand	89.81 (12)	1.74	1371.4	7225.2	18.85
Mashhad	104.60	1.82	1554.8	74133.1	20.83
LSD (0.05)	7.179	0.052	251.30	297.49	2.939
Irrigation regimes (%)					
50	76.21	1.60	1190.7	6635.6	17.81
75	103.58	1.80	1508.1	7162.8	20.69
100	111.81	1.93	1690.4	8159.1	21.01
LSD (0.05)	5.254	0.071	33.95	120.35	0.355
Vermicompost application (t ha^{-1})					
0	90.60	1.733	1364.4	7068.1	19.11
5	103.83	1.83	1561.7	7570.3	20.57
LSD (0.05)	2.629	0.031	22.03	53.51	0.356
GABA levels (mg l^{-1})					
0 (control)	91.90	1.75	1394.5	7171.4	19.22
5	98.13	1.77	1466.5	7326.8	19.90
10	101.58	1.82	1528.2	7459.3	20.40
LSD (0.05)	3.220	0.038	26.98	66.53	0.437
Average	97.20	1.78	1463.06	7319.15	19.84
S.O.V					
Location (L)	**	**	NS	NS	**
R (L)	-	-	-	-	-
Irrigation regimes (I)	**	**	-	-	-
L × I	NS	NS	**	**	**
R × I (L)	-	-	-	-	-
GABA levels (G)	**	**	**	**	**
Vermicompost application (V)	**	**	**	**	**
G × V	NS	*	**	**	**
I × G	NS	*	*	**	NS
I × V	*	NS	**	**	**
I × G × V	*	NS	**	**	**
L × G	NS	NS	**	**	**
L × V	NS	NS	NS	NS	NS
L × G × V	NS	NS	NS	NS	NS
L × I × G	NS	NS	*	*	*
L × I × V	NS	NS	NS	NS	NS
L × I × G × V	NS	NS	NS	NS	NS
Bartlett's test for homogeneity	0.51	0.11	0.35	0.84	0.40

**: significant at $p \le 0.01$; *: significant at $0.01 < p \le 0.05$; NS: non-significant ($0.05 < p$)

Table 4: Some quantity and quality traits of quinoa as affected by irrigation regimes, vermicompost application, and GABA levels in Birjand and Mashhad, Iran

Treatments	Sugar (mg 100 g^{-1})	Proline (µmol g^{-1} FM)	P concentration (mg g^{-1})	K concentration (mg g^{-1})	Water use efficiency (kg m^{-3})
Location					
Birjand	124.75	15.47	0.98	1.58	0.2717
Mashhad	114.34	14.77	0.99	1.59	0.3121
LSD (0.05)	6.860	2.160	0.049	0.110	0.0061
Irrigation regimes (%)					
50	125.48	18.36	1.14	1.70	0.3421
75	118.95	15.61	1.03	1.58	0.2894
100	114.20	11.38	0.80	1.47	0.2442
LSD (0.05)	3.580	1.087	0.063	0.089	0.0074
Vermicompost application (t ha^{-1})					
0	118.57	13.59	0.93	1.51	0.2696
5	120.52	16.65	1.05	1.66	0.3142
LSD (0.05)	1.146	0.0347	0.032	0.034	0.0061
GABA levels (mg l^{-1})					
0 (control)	114.84	13.99	0.94	1.53	0.2750
5	119.29	15.10	0.99	1.57	0.2933
10	124.50	16.26	1.04	1.65	0.3037
LSD (0.05)	1.404	0.425	0.039	0.041	0.0074
Average	14.70	15.12	0.99	1.58	0.2919
S.O.V					
Location (L)	**	**	NS	NS	**
R (L)	-	-	-	-	-
Irrigation regimes (I)	**	**	**	**	**
L × I	NS	**	NS	NS	*
R × I (L)	-	-	-	-	-
GABA levels (G)	**	**	**	**	**
Vermicompost application (V)	**	**	**	**	**
G × V	NS	NS	*	NS	**
I × G	**	*	*	*	**
I × V	*	**	*	*	**
I × G × V	NS	NS	NS	NS	**
L × G	*	NS	*	*	NS
L × V	*	NS	NS	NS	NS
L × G × V	NS	NS	NS	NS	NS
L × I × G	NS	NS	NS	NS	NS
L × I × V	NS	NS	NS	NS	**
L × I × G × V	NS	NS	NS	NS	NS
Bartlett's test for homogeneity	0.52	0.07	0.07	0.21	0.51

**: significant at $p \leq 0.01$; *: significant at $0.01 < p \leq 0.05$; NS: non-significant ($0.05 < p$)

Table 5: Plant height, seed yield, biological yield, harvest index, sugar, water use efficiency (WUE), sugar content, proline, P and K concentration of quinoa as affected by interaction between irrigation regimes and vermicompost application

Irrigation regimes (%)	Vermicompost application (t ha^{-1})	Plant height (cm)	Seed yield (kg ha^{-1})	Biological yield (kg ha^{-1})	HI (%)	Sugar (mg 100 g^{-1})	Proline (μmol g^{-1} FM)	P (mg g^{-1})	K (mg g^{-1})	WUE (kg m^{-3})
50	0	70.25 ± 16.61	1043.2 ± 220.6	6309.5 ± 397.7	16.43 ± 2.78	125.26 ± 9.91	16.71 ± 1.69	1.08 ± 0.09	1.63 ± 0.16	0.300 ± 0.066
	5	82.17 ± 10.22	1338.2 ± 178.9	6961.7 ± 266.6	19.19 ± 2.23	125.70 ± 10.42	20.01 ± 2.28	1.19 ± 0.08	1.78 ± 0.14	0.385 ± 0.053
75	0	96.08 ± 8.65	1445.1 ± 161.6	7052.4 ± 206.5	20.46 ± 1.89	118.34 ± 7.82	13.77 ± 2.70	0.97 ± 0.19	1.50 ± 0.16	0.277 ± 0.032
	5	111.08 ± 10.65	1571.1 ± 176.9	7273.2 ± 245.9	21.57 ± 2.01	119.56 ± 7.68	17.45 ± 2.03	1.09 ± 0.08	1.66 ± 0.08	0.302±0.035
100	0	105.38 ± 9.44	1605.1 ± 176.5	7842.3 ± 268.5	20.44 ± 1.91	112.10 ± 7.58	10.29 ± 1.52	0.73 ± 0.08	1.40 ± 0.12	0.232 ± 0.027
	5	118.25 ± 14.61	1775.8 ± 192.1	8475.8 ± 279.1	20.94 ± 2.09	116.30 ± 7.19	12.47 ± 1.46	0.87 ± 0.05	1.54 ± 0.11	0.256 ± 0.029
LSD (0.05)		4.648	37.41	87.95	0.613	1.8277	0.431	0.0533	0.0565	0.0105

Table 6: Sugar content, proline, P and K concentration, water use efficiency (WUE), seed yield, biological yield, and harvest index of quinoa as affected by interaction between irrigation regime and GABA levels

Irrigation regimes (%)	GABA levels (mg l^{-1})	Sugar (mg 100 g^{-1})	Proline (μmol g^{-1} FM)	P (mg g^{-1})	K (mg g^{-1})	Water use efficiency (kg m^{-3})	Seed yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Harvest index (%)
50	0	118.63 ± 8.56	17.14 ± 2.19	1.07 ± 0.08	1.62 ± 0.2	0.305 ± 0.082	1066.1 ± 282.38	6405.4 ± 586.2	16.44 ± 3.17
	5	125.02 ± 7.89	18.41 ± 2.44	1.13 ± 0.09	1.69 ± 0.12	0.349 ± 0.057	1213.7 ± 194.05	6654.1 ± 307.4	18.17 ± 2.29
	10	132.79 ± 8.64	19.54 ± 2.69	1.22 ± 0.08	1.79 ± 0.15	0.372 ± 0.064	1292.3 ± 218.54	6847.4 ± 385.8	18.82 ± 2.66
75	0	114.62 ± 7.34	14.25 ± 2.97	0.98 ± 0.22	1.54 ± 0.2	0.282 ± 0.035	1468.5 ± 171.28	7108.5 ± 238.9	20.62 ± 1.92
	5	118.48 ± 6.51	15.64 ± 2.93	1.03 ± 0.11	1.56 ± 0.11	0.287 ± 0.033	1495.7 ± 170.03	7158.6 ± 204.4	20.86 ± 1.96
	10	123.76 ± 6.66	16.93 ± 2.67	1.07 ± 0.11	1.64 ± 0.11	0.299 ± 0.039	1560.0 ± 194.24	7221.3 ± 302.4	21.56 ± 2.16
100	0	111.27 ± 7.05	10.60 ± 1.63	0.76 ± 0.11	1.42 ± 0.13	0.238 ± 0.031	1648.9 ± 205.58	8000.2 ± 401.4	20.59 ± 2.13
	5	114.36 ± 7.48	11.24 ± 1.67	0.80 ± 0.09	1.46 ± 0.13	0.244 ± 0.030	1690.1 ± 204.12	8167.8 ± 392.1	20.67 ± 2.07
	10	116.96 ± 7.65	12.31 ± 1.88	0.84 ± 0.07	1.53 ± 0.12	0.251 ± 0.029	1732.3 ± 200.09	8309.1 ± 430.4	20.82 ± 1.9
LSD (0.05)		2.238	0.528	0.065	0.069	0.0129	45.82	107.72	0.751

and 7794.6 kg ha^{-1}) belonged to 5 t ha^{-1} of V + 10 mg l^{-1} GABA treatment by considering the interaction between V and GABA (Fig. 3). Moreover, based on the three-way interactions, the highest amount of seed yield, biological yield, and harvest index at 50 % of PWR was observed in 5 t ha^{-1} of V + 10 mg l^{-1} of GABA treatment (Table 7).

In both Mashhad and Brjand regions, the lowest seed and biological yield, as well as harvest index, were observed in the control treatment; while the highest values were obtained when 5 t ha^{-1} V was applied + 10 mg l^{-1} GABA. For instance, under Mashhad region, its co-application compared to non-application caused an increase in seed yield by 28.1 % (Table 8).

3.3 QUALITY TRAITS IN QUINOA SEEDS

Sugar and proline contents, as well as P and K concentrations were significantly affected by irrigation regimes, V, and GABA levels (Table 4). The amounts of sugar and proline contents increased by reducing PWR from 100 to 50 %. In other words, under the high level of drought stress, these parameters increased in quinoa seeds, which were consistent with results of P and K concentrations (Table 4).

The use of V improved the amounts of sugar and proline contents as well as P and K concentrations, regardless of drought stress levels. Under the use of 50 % of PWR, sugar and proline contents increased in the presence of 5 t ha^{-1} of V (Table 5). Similar results were observed after increasing the amount of GABA. In fact, 10 mg l^{-1} of GABA led to the best results when 50 % of PWR was treated (Table 6). This trend was observed in both Mashhad and Birjand regions (Table 9).

3.4 WATER USE EFFICIENCY

According to the results presented in Table 4, the interaction of irrigation and V had a significant effect on WUE. Irrespective of V application, reducing irrigation amount led to an increase in WUE. For example, 50 % of PWR compared to 75 % and 100 % of PWR improved WUE by 7.3 and 27.8 %, respectively (Table 5).

Similar to the effect of irrigation regime and V, the interaction of irrigation and GABA on water use ef-

Table 7: Seed yield, biological yield and harvest index of quinoa as affected by interaction between irrigation regimes, GABA levels, and vermicompost application

Irrigation regimes (%)	GABA levels (mg l^{-1})	Vermicompost application (t ha^{-1})	Plant height (cm)	Seed yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Harvest index (%)	Water use efficiency (kg m^{-3})
50	0 (control)	0	61.76 ± 24.24	836.5 ± 154.55	5916.6 ± 388.5	14.11 ± 2.25	0.239 ± 0.046
		5	73.50 ± 10.72	1295.7 ± 162.74	6894.2 ± 198.3	18.77 ± 2.02	0.318 ± 0.053
	5	0	75.50 ± 8.83	1101.2 ± 172.14	6439.6 ± 211.1	17.05 ± 2.26	0.343 ± 0.050
		5	76.88 ± 8.68	1326.3 ± 148.67	6868.6 ± 229.7	19.29 ± 1.82	0.371 ± 0.049
	10	0	82.38 ± 9.68	1191.9 ± 168.55	6572.5 ± 221.9	18.11 ± 2.32	0.381 ± 0.043
		5	87.25 ± 10.61	1392.6 ± 225.7	7122.3 ± 311.2	19.53 ± 2.94	0.401 ± 0.066
75	0	0	91.25 ± 8.86	1409.3 ± 161.44	6994.2 ± 244.9	20.12 ± 1.88	0.271 ± 0.033
		5	97.00 ± 7.86	1527.8 ± 169.66	7222.8 ± 180	21.12 ± 1.94	0.275 ± 0.033
	5	0	100.00 ± 7.78	1439.7 ± 169.75	7045.7 ± 213.2	20.40 ± 2.01	0.285 ± 0.033
		5	104.63 ± 8.35	1551.7 ± 161.17	7271.4 ± 122.5	21.32 ± 1.92	0.293 ± 0.035
	10	0	112.50 ± 11.12	1486.2 ± 165.71	7117.4 ± 161.1	20.85 ± 1.98	0.299 ± 0.031
		5	116.13 ± 10.02	1633.8 ± 202.35	7325.3 ± 381.1	22.27 ± 2.22	0.314 ± 0.041
100	0	0	101.50 ± 8.64	1574.1 ± 188.58	7663.2 ± 168.8	20.52 ± 2.15	0.228 ± 0.029
		5	105.38 ± 9.24	1723.6 ± 205.52	8337.2 ± 239.0	20.66 ± 2.25	0.233 ± 0.027
	5	0	109.25 ± 9.92	1607.0 ± 179.64	7858.6 ± 196.0	20.43 ± 2.01	0.236 ± 0.027
		5	115.38 ± 9.04	1773.2 ± 203.03	8477.1 ± 269.2	20.91 ± 2.24	0.249 ± 0.031
	10	0	118.00 ± 8.4	1634.1 ± 180.28	8005.0 ± 320.7	20.39 ± 1.81	0.255 ± 0.030
		5	121.38 ± 22.98	1830.6 ± 176.67	8613.2 ± 287.7	21.26 ± 2.00	0.265 ± 0.026
LSD (0.05)			8.050	64.80	152.33	1.062	0.0182

Table 8: Seed yield, biological yield and harvest index of quinoa as affected by interaction between location, irrigation regimes, and GABA levels

Location	GABA levels (mg l^{-1})	Vermicompost application (t ha^{-1})	Seed yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Harvest index (%)
Birjand	0 (control)	0	1200.1 ± 310.93	6800.3 ± 747.8	17.41 ± 3.07
		5	1295.8 ± 262.05	7043.0 ± 685.8	18.26 ± 2.44
	5	0	1347.2 ± 236.42	7117.9 ± 647.1	18.84 ± 2.18
		5	1417.4 ± 220.38	7379.9 ± 671.7	19.15 ± 1.95
	10	0	1454.3 ± 219.7	7431.2 ± 686.2	19.52 ± 1.9
		5	1513.4 ± 255.94	7579.2 ± 791.4	19.92 ± 2.35
Mashhad	0	0	1346.5 ± 405.1	6915.7 ± 845.9	19.10 ± 4.02
		5	1469.4 ± 263.1	7186.2 ± 581	20.33 ± 2.37
	5	0	1527.6 ± 237.2	7345.3 ± 653.3	20.73 ± 2.1
		5	1613.9 ± 243.27	7589.6 ± 662.4	21.21 ± 2.09
	10	0	1646.5 ± 247.1	7646.9 ± 782.2	21.49 ± 1.9
		5	1724.5 ± 241.94	7794.6 ± 711.4	22.12 ± 2.41
LSD (0.05)			52.91	124.38	0.867

Table 9: Sugar content, P and K concentration of quinoa as affected by interaction between location and GABA levels

Location	GABA levels (mg l^{-1})	Sugar (mg 100 g^{-1})	P (mg g^{-1})	K (mg g^{-1})
Birjand	0 (control)	119.98 ± 6.02	0.94 ± 0.16	1.53 ± 0.17
	5	124.72 ± 6.25	0.99 ± 0.16	1.57 ± 0.15
	10	129.54 ± 8.16	1.03 ± 0.17	1.63 ± 0.16
Birjand	0	109.70 ± 6.54	0.94 ± 0.23	1.52 ± 0.21
	5	113.85 ± 6.66	0.99 ± 0.18	1.57 ± 0.15
	10	119.47 ± 9.16	1.06 ± 0.19	1.68 ± 0.17
LSD (0.05)		1.828	0.053	0.057

Table 10: Water use efficiency of quinoa as affected by interaction between location and irrigation regimes

Location	Irrigation regimes (%)	Water use efficiency (kg m^{-3})
Birjand	50	0.316 ± 0.064
	75	0.271 ± 0.029
	100	0.228 ± 0.024
Mashhad	50	0.368 ± 0.074
	75	0.308 ± 0.032
	100	0.260 ± 0.027
LSD (0.05)		0.0105

ficiency was significant (Table 6). By increasing GABA consumption in both levels of V, WUE significantly increased. As an example, 10 mg l^{-1} of GABA under 50 % of PWR compared to no application of GABA led to an increase in WUE to 21.9 %. Under 75 and 100 % of PWR, a positive effect was also observed in terms of GABA application.

By increasing the rate of GABA under both V levels, WUE was observed to be significantly increased, so that the lowest value was observed in the control treatment (no V + no GABA foliar spraying) and the highest value (0.33 kg m^{-3}) was observed at 5 t ha^{-1} of V + 10 mg l^{-1} of GABA (Fig. 4). The results also showed that under severe stress (50 % water requirement), the quinoa plant grown in Mashhad had a higher WUE than Birjand (Table 10).

4 DISCUSSION

Drought stress is an adverse factor associated with the reduced seed yield in crops, which can be imposed by unbalanced irrigation (Yuan et al., 2019). Decreased plant growth, 1000-seed mass, and harvest index are the most important consequences of water stress which were observed in the plants such as barley and canola (Dreccer et al., 2018).

Heat stress, along with drought stress, is another disorder in agriculture leading to the reduced crop yields, especially in arid and semi-arid regions (Plazas et al., 2019). This hypothesis can justify the higher seed and biological yields in Mashhad compared to Birjand. Based on the results of Table 1, quinoa experienced the higher heat stress under Birjand climate compared to Mashhad climate. Furthermore, the low soil organic matter in Birjand compared to the Mashhad region (Table 2) may cause the soil conditions to be less favorable for quinoa growth.

Water deficiency led to a decrease in yield indices and an increase in seed quality traits. Therefore, it seems that accumulated compounds such as proline, protein, and sugar acted as a compromising mechanism for improving the adaptation of quinoa to drought conditions under severe stress. In this regard, Ebrahimian et al. (2019) reported an increase in proline concentration under the drought stress and indicated proline can create an internal resistance in the plant under water shortage, which is consistent with the results of the study of Naeem et al. (2018). These researchers considered the effective role of proline in improving the growth, regulating the water relations along with the antioxidant activity. Ghaffari et al. (2019) indicated the changes in proline concentration under water-deficient conditions and described its changes as a reliable index.

The use of V under drought stress can be effective in various aspects. The plants which have a higher ability to absorb more nutrients such as V from the soil are more tolerant against the drought stress (Demir, 2020; Saba et al., 2019). Based on the results of the study of Lim et al. (2015), the positive effects of V on increasing the seed yield of crops are attributed to the improved soil criteria including physical, chemical and biological parameters. Aboelsoud and Ahmed (2020) indicated that V increased the plant height and 1000-seed mass along with seed yield in wheat. Based on the result of this study, V may proliferate the adaptive responses against the drought stress by inducing the cellular resistance and increasing the water uptake. Furthermore, V caused a significant increase in tomato yield under drought stress conditions (Chanda et al., 2011). Roberts et al. (2007) reported that V increased the number of seeds per plant, plant height, 1000-seed mass, and the seed yield in wheat. Therefore, it seems that V improved the quinoa seed yield by transition the more organic matter to seeds compared to the morphological and physiological indices. Li et al. (2019) demonstrated that the use of GABA improved the plant tolerance and adaptability to stressful conditions by increasing the content of amino acids. Based on the results of this study, the effective role of GABA in rising the values of the mentioned parameters is mainly related to the high adaptation of morphological stages to drought stress, especially during the pollination stage. Therefore, a positive and significant relationship exists between GABA foliar and V application, which improves the mechanism of GABA in the plant by absorbing the nutrients. The use of V and GABA can reduce the consequences of drought stress when quinoa is exposed to moderate or intense drought stress which is unavoidable in semi-arid climates.

As stated before, increasing irrigation rounds led to an increase in WUE. Such behavior is probably achieved through further development of the root system which can improve the plant's ability to absorb more available water. In this regard, Feizabadi et al. (2021) reported an increase in physiological growth, root system development, and yield of rapeseed as a result of V application.

The results of the triple-interaction effects can express a precise concept for the simultaneous application of GABA and V, whether under stress or non-stress conditions (Table 16). Under severe stress, co-application of 5 t ha^{-1} of V + 10 mg l^{-1} of GABA recorded the highest WUE (0.401 kg m^{-3}). As a new result, a synergistic relationship can be observed between the simultaneous application of GABA and V, which is considered useful for dealing with drought stress in quinoa plants.

Under both Birjand and Mashhad regions, a higher grain yield was observed following GABA and V. Their simultaneous application, as mentioned before, is known as the innovation of this research to improve plant resistance against drought stress. Interestingly, simultaneous application of GABA and V in Birjand region had a lower effect on increasing grain yield than Mashhad region. Therefore, it can be concluded that the co-application of these treatments may require more suitable conditions in terms of temperature to record increasing grain yield. Therefore, it can be seen as a crucial relationship between the supply of nutrients in the plant and the external environment.

Along with severe drought to quinoa, the application of V played an effective role in increasing WUE. This increase was also recorded under mild stress (75 % of PWR) and no stress (100 % of PWR). In this regard, similar results have been reported in terms of V application on increasing WUE in wheat (Azimi et al., 2018). Therefore, it can be concluded that the effective role of V in increasing WUE has been achieved through the further development of the root system and aerial parts. In fact, it can be stated that there is a positive and significant relationship between plant growth traits and the improvement of WUE, which can cause an increase in the plant's ability to deal with drought stress. Generally, any factor that can be effective in increasing the plant's ability for water absorption can improve WUE (Hosseinzadeh et al. 2018). As mentioned earlier, GABA spraying can play an effective role in improving growth traits and plant height. According to Hosseinzadeh et al. (2018), there is a positive relationship between increasing plant growth ability and WUE. In fact, under constant water consumption, any factor that led to increasing plant performance can improve WUE. Therefore, increasing plant yield after spraying GABA will be an effective technique for increasing WUE.

According to Eq. (1), there is a positive relationship between plant performance and WUE. Considering the similar irrigation scheme applied in both regions, the higher WUE in Mashhad may be due to increased plant growth and grain yield. In other words, more suitable climatic conditions in the Mashhad region probably provide the possibility of plant performance in a better way, which has subsequently caused more water absorption.

In the present study, no significant difference was observed between 75 % of PWR + 5 t ha^{-1} of V treatment and 100 % of PWR treatment based on the seed yield. V can decrease the negative effect of moderate drought stress by reducing the PWR to 75 %. Thus, the balance between the use of V and PWR can be effective in decreasing the intense of drought stress.

5 CONCLUSION

Based on the results, the foliar application of GABA can improve the plant height and seed yield of quinoa if a relative reduction occurred in PWR. 75 % of PWR can be justified as a practical treatment. The same results were observed for 1000 seed mass, harvest index, sugar, protein, P, and K contents. Moreover, the V treatment caused a similar response for improved WUE, which is consistent with the use of GABA. The co-application of V and GABA was proposed as a new approach in quinoa cultivation, which can be particularly interesting in semi-arid areas so that farmers can implement it under water-limited conditions. Therefore, the use of 10 mg l^{-1} of GABA simultaneous with 5 t ha^{-1} of V can be recommended when the quinoa plant is faced with moderate or severe drought stress. However, further research is yet needed regarding integrated application of GABA with other bio-stimulant inputs in quinoa production.

6 AUTHORS' CONTRIBUTION STATEMENT

M.A. Behdani and S. Parsa proposed the experimental idea and underwent the all stages of the study. M. Jamshideyni conduced the experiment and did the sampling data. S. Khoramdel managed data analysis and supervised the experiment. Finally, the manuscript was proofed by all the authors.

7 DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

8 FUNDING AND/OR CONFLICTS OF INTERESTS/COMPETING INTERESTS

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

9 ACKNOWLEDGEMENTS

This research has been financially supported by University of Birjand (Grant Number: 21833/1398). The authors gratefully acknowledge the research council of University of Birjand.

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doi:10.14720/aas.2023.119.1.2899 Original research article / izvirni znanstveni članek

Endophytic bacteria enhance the growth and salt tolerance of rice under saline conditions

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Received October 31, 2022; accepted March 19, 2023.
Delo je prispelo 31. oktobra 2022, sprejeto 19. marca 2023

Endophytic bacteria enhance the growth and salt tolerance of rice under saline conditions

Abstract: Developing biostimulants from salt-tolerant plant growth-promoting (PGP) bacteria is an emerging strategy for sustainable agriculture in the context of increasing soil salinization. This study aimed to isolate endophytic bacteria (EB) capable of promoting rice seed germination and seedling growth at different NaCl concentrations. Nine salt-tolerant EB strains were isolated and two, ST.6 and ST.8, with the rice seed promoting effect 99.3 and 99.7 %, respectively, were selected and identified as *Pantoea dispersa* and *Burkholderia cenocepacia*, respectively. ST.6 showed a higher value of the activity of phosphatase (617 mg P ml^{-1}), production of indole-3-acetic acid (19.7 **µg** IAA ml^{-1}), the activity of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (13.5 *µmol mg*$^{-1}$ protein h^{-1}), and production of siderophore (76.3 %). Especially, rice seedlings inoculated with strain ST.6 showed a significant improvement in root length (58.95 %), shoot length (16.6 %), dry biomass (7.0 %), the content of chlorophyll (46.2 and 57.1 % for chlorophyll a and b, respectively), carotenoids (22.2%), and proline (19.0 %). A decrease in antioxidant enzyme activities was also observed in the rice seedlings inoculated with either ST.6 or ST.8 strain under salt stress. Furthermore, the salt stress condition enhanced the colonization of roots by both studied endophytic bacteria. More experiments should be done to develop endophytic bacteria ST.6 and ST.8 as efficient bio-inoculants.

Key words: endophytes; salt stress; plant growth-promoting bacteria; antioxidant enzymes; seed germination; rice (*Oryza sativa*)

Endofitske bakterije pospešujejo rast in toleranco na sol riža v razmerah slanosti

Izvleček: Razvoj biostimulantov iz bakterij, ki pospešuejo rast na sol tolerantnih rastlin (PGP) je razvijajoča se strategija za vzdržnostno kmetijstvo v času naraščajočega zasoljevanja tal. Namen raziskave je bil izolirati endofitske bakterije (EB), ki so sposobne pospeševati kalitev semen riža in rast sejank pri različnih koncentracijah NaCl. Izoliranih je bilo devet na sol tolerantnih sevov EB in dva seva, ST.6 in ST.8, ki sta pospeševala kalitev semen riža za 99,3 in 99,7 %. Ta dva seva sta bila izolirana in določena kot vrsti *Pantoea dispersa* in *Burkholderia cenocepacia*. Sev ST.6 je pokazal večjo vrednost v aktivnosti fosfataze (617 mg P ml^{-1}), v produkciji indole-3-ocetne kisline (19,7 µg IAA ml^{-1}), v aktivnosti deaminaze 1-aminociklopropan-1-karboksilne kisline (ACC) (13,5 µmol mg^{-1} protein h^{-1}) in v tvorbi sideroforov (76,3 %). Še posebej so sejanke riža, inokulirane s sevom ST.6, pokazale značilno izboljšanje v dolžini korenin (58,95 %), dolžini poganjkov (16,6 %), suhi biomasi (7,0 %), v vsebnosti klorofila (46,2 in 57,1 % za klorofil a in b), karotenoidov (22,2 %) in prolina (19,0 %). V sejankah riža, inokuliranih s sevom ST.6 ali ST.8, je bil opazen tudi upad aktivnosti antioksidacijskih encimov v razmerah solnega stresa. Nadalje so razmere solnega stresa pospešile kolonizacijo korenin z obema sevoma preučevanih endofitskih bakterij. Še več poskusov bo potrebnih, da bi razvili seva endofitskih bakterij ST.6 in ST.8 kot učinkovita bioinokulanta.

Ključne besede: endofiti; solni stres; bakterije, ki pospešujejo rast rastlin; antioksidacijski encimi; kalitev semen; riž (*Oryza sativa*)

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1 INTRODUCTION

Rice (*Oryza sativa* L.) is an important food crop and is mainly cultivated in developing countries. It is also reported as one of the cultivated crops that required a high amount of water to grow. However, climate change is one of the factors that cause drought, and soil salinity more often. These led to a strong reduction in rice productivity (Kumar et al., 2020). The salt stresses mainly affect plant growth and development through changes in morphology, biochemistry, and physiology such as plants' ability to uptake nutrients and water (Liang et al., 2018; Bistgani et al., 2019; Hao et al., 2021), and/or reduction of photosynthesis efficiency (Wang et al., 2019a). Further, during soil salinization, some plants could potentially be adapted to changing salinity in soil by adjusting their metabolism through the regulation of signal transduction and gene expressions (Daliakopoulos et al., 2016; Hao et al., 2021); or ion-selective absorption and compartmentalization (Liang et al., 2018; Hao et al., 2021); or through the production of antioxidants and inhibition of reactive oxygen species (ROS) generation (Abbas et al., 2019; Hao et al., 2021). The microbial supplement of halotolerant microorganisms to the soil is an emerging method that assists salt stress tolerance in plants (Etesami and Glick, 2020).

The important group of plant growth-promoting bacteria (PGPB) are endophytic bacteria that live inside the plant tissues and do not cause plant disease (Schulz and Boyle, 2007). Under salinity stress conditions, endophytic bacteria could improve plant growth under salinity conditions by producing exopolysaccharides (EPS), and/or enhancing the activity of enzymes like ACC deaminase and antioxidative enzyme and/or accumulating proline in the plant (Wang et al., 2019b). In addition, it was reported that endophytic bacteria could promote plant development through many mechanisms such as the production of indole acetic acid (IAA), siderophores, and phosphate solubilization (Trung et al., 2022). There were several studies that demonstrated the plant growth-promoting functions of endophytic bacteria isolated from wild rice and trade rice (Chu et al., 2021). For example, the endophytic bacteria isolated from wild rice such as *Bacillus, Microbacterium, Pantoea ananatis* D1, *Herbaspirillum, Ideonella, Enterobacter,* and *Azospirillum* were able to produce IAA, which showed roles in promoting plant growth (Lu et al., 2021; Zhang et al., 2021). These results suggest that endophytic bacteria could be developed into biofertilizers used in organic cultivation to promote the growth and development of plants in normal as well as saline conditions.

It is a fact that the farmers used an extensive amount of chemical fertilizers and pesticides in modern agriculture to increase crop productivity causing a change in nutrient supply, a decrease in microbial diversity, limitation of productivity, and deterioration of soil health (Etesami and Glick, 2020). Recently, the organic rice cultivation model produced a positive shift in rice production and has been effectively applied by many farmers in Nam Dinh, Viet Nam. Using natural systems and avoiding the use of synthetic substances increased biodiversity in soil by up to 30 % on organic farms compared to conventional farming (Rundlöf et al., 2016). Hence, exploiting the salt-stress plant growth-promoting bacteria (ST-PGPB) strains as a safe bioinoculant to improve crop productivity in organic agriculture is an alternative strategy. However, there are not many studies to exploit endophytic bacteria isolated from rice grown under organic cultivation as potential biofertilizers in organic agriculture. Therefore, this research aimed to isolate native endophytic bacteria from rice tissue grown in organic farming and investigated their plant growth promotion abilities under salt stress conditions. These endophytic bacteria with multiple plant growth promotion abilities could be used to develop biofertilizers for organic agriculture in Nam Dinh province.

2 MATERIAL AND METHODS

2.1 ISOLATING RICE ENDOPHYTIC BACTERIA

Samples of rice roots were collected from farms in Nam Dinh, Viet Nam. The soil adhered to the root samples was removed under running tap water. The endophytic bacteria were isolated by the method described by Trung et al. (2022). Briefly, the root samples were sterilized by soaking for 2 minutes in 70 % ethanol; then immersed in 3 % NaOCl solution for 5 minutes with the addition of 2-3 drops of Tween 20, and finally washed five times with sterile distilled water. Plating 100 µl of the final rinse on LB (Luria-Bertani) agar plates and incubating them at 28 °C for 72 h was used to verify the effectiveness of surface sterilization. The surface-sterilized roots were dried on sterile filter paper and placed on LB media plates supplemented with 3 % NaCl, adjusted pH = 8. These plates were cultured for 3 days at 28 °C. Single colonies on each plate were observed for their morphology and color before being separately transferred to new plates to purify and finally stored at -80 ˚C in LB broth containing 18 % glycerol.

2.2 SCREENING THE ISOLATED BACTERIA FOR IMPROVING RICE SEED GERMINATION UNDER SALT STRESS

The selected bacterial strains were grown on LB overnight at 28 °C. The rice seeds (*Oryza sativa* L.) variety ST24 were sterilized as the method described by Sarkar et al. (2018). The surface-sterilized rice seeds were bacterized by soaking at room temperature for 24 h in prepared bacterial culture (10^6 CFU ml^{-1}). In the negative control experiment, the sterilized rice seed was soaked in sterile distilled water The experiment was designed randomly with 3 repetitions, each experiment included 30 bacterized rice seeds placed in a plastic container, which had a double layer of sterile filter paper moistened with 100 mM NaCl solution. The plastic containers containing inoculated seeds were incubated in a dark at 28 °C for 7 days. The parameters such as germination rate, shoot, and root length were measured. The bacteria strains with the highest efficiency were selected for further studies.

2.3 MOLECULAR IDENTIFICATION OF ISOLATES

The genomic DNA of selected strains was extracted by using the Rapid Bacteria Genomic DNA Isolation Kit (Biobasic, Canada) as per the kit instructions. The PCR amplification of the 16S rRNA gene fragment (1.5 kb) was done by using universal primers 27 F (5′-AGA GTT TGA TCC TGG CTC AG-3′), and 1492 R (5′-TAC GGT TAC CTT GTT ACG ACT T-3′). The obtained PCR products were cleaned and sequenced by First Base Company (Singapore). The nucleotide sequence was checked for quality before being used to blast on the BLAST server (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to find the closest species. The high-similarity sequences (more than 99 %) were selected and used to regenerate a phylogenetic tree by using MEGA software (v.7.2) (Kumar et al., 2016). The obtained sequences of isolates were deposited on GenBank (accession numbers ON326555 and ON326556).

2.4 ASSAY FOR PLANT GROWTH-PROMOTING ACTIVITIES UNDER IN VITRO CONDITIONS

The plant growth promotion (PGP) traits (production of indole-3-acetic acid (IAA), siderophore, ACC deaminase; and phosphate solubilization) of isolates were investigated under *in vitro* conditions.

IAA production of isolates was measured by the method described by Patten and Glick (2002). Briefly, 20 µl of bacterial culture (OD_{600} = 1) were pipetted and cultured in flash containing LB media supplemented with 1 g l^{-1} L-Tryptophan at 28 °C for 24 h. After incubation, the culture was centrifuged to remove bacteria cells and a 1 ml aliquot of the supernatant was added with 4 ml of Salkowski's reagent (150 ml of 95–98 % H_2SO_4, 75 ml of 0.5 mol l^{-1} $FeCl_3.6H_2O$ and 250 ml of distilled H_2O). The mixture solution was incubated at room temperature and measured the absorbance at 535 nm. Finally, the IAA amount in the mixture was calculated based on the standard curve of IAA.

The siderophore production of isolated strains was measured by the method described by Arora and Verma (2017). A single colony was taken to culture in the Tryptone Soy Agar (TSA) broth media at 28 °C for 3 days. After that, the culture was centrifuged at 8000 rpm for 10 min to get the supernatant. Then, the collected supernatant (1 ml) was mixed with prepared the chrome azurol S (CAS) assay solution (1 ml) and incubated at room temperature for 1 h. For the control, the autoclaved TSA medium was used instead. The absorbance of the sample (As) and the control (Ac) at 630 nm was determined and the amount of siderophore was calculated according to the formula:

$$\% \, siderophore \ units \ = \frac{Ar - As}{Ar} \, \mathrm{x100}$$

The isolates were also investigated for their ability of phosphate solubilization on the NBRIP (National Botanical Research Institute's Phosphate) medium. The bacterium was cultured at 28 °C on the shaker. After 3 days of incubation, the pH of the culture broth was determined by a pH meter and the phosphate amount released in the culture supernatant was measured by the molybdenum blue method (Murphy and Riley, 1962).

The 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase production of the isolates was carried out as the method described by Penrose and Glick (2003).

All of the above experiments were designed in triplicate and were repeated three times.

2.5 EVALUATING THE PLANT GROWTH-PROMOTING CAPACITY OF ISOLATED BACTERIA UNDER SALINE STRESS

Surface-sterilized rice seeds were inoculated by soaking for 1 h in bacterial solution (10^6 CFU/ml) or in sterile water for control. 6 bacterized rice seeds were grown in plastic pots (25 x 30 cm) containing 400 g of non-sterile soil collected from Ca Mau, Vietnam, which has characteristics as follow: clayey soil, pH 7.3, available-P (P_2O_5) 16 mg kg^{-1}, available K 218 mg kg^{-1}, total nitrogen 28 mg kg^{-1}, E.C (0.72 ds m^{-1}), $CaCO_3$ 3.3 %, organic matter 1.1 %. The bacterial solution (10^6 CFU ml-

[1]) or sterile water was added to the base of seedlings 7 days after transplantation, respectively for the treatment and control. After that, the salt solution (30 ml of 150 mM NaCl) was used to water the seedlings at 48 h intervals while the water with a similar volume was applied to the ones with no salinity stress. The experiment was designed randomly with 3 repetitions.

All the pots were kept in the grow chamber for 20 days (light/dark cycle: 16h/8h). At the harvest, plant growth parameters were recorded including shoot and root length, the number of roots; the dry mass of plants after drying at 60 °C for 24 h (mg/plant), and chlorophyll content/fresh mass was determined by the colorimetric method as described by Ben et al. (1980) on spectrophotometers at different wavelengths: chlorophyll a (OD = 663 nm), chlorophyll b (OD = 645 nm) and carotenoids (OD = 440.5 nm).

To explore the response of plan under salt stress, the proline content was also measured as the method described by Bates et al. (1973); the activities of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) were determined by using the suitable kits as the manufacturer's instructions (College of Forestry Biotechnology, Chuong My, Vietnam).

2.6 DETERMINATION OF INOCULATED BACTERIA IN THE PLANTS

The inoculated bacteria were screened for their presence in the inoculated rice plants under normal and salt stress conditions to investigate the effect of abiotic conditions on their colonization ability. The surface sterilization of plant samples was done as the method described by Trung et al. (2022). The sterilized samples were homogenized in 0.9 % NaCl solution and 1 ml of the homogenized solution was plated on the LB agar plates. The inoculated plates were cultured overnight at 37 °C. Then the presence of inoculated bacteria was done by screening 100 isolated colonies using Restriction Fragment Length Polymorphism (RFLP) method, in which the identity of the isolated bacteria was compared with the inoculated strains.

2.7 DATA ANALYSIS

All the experiments were conducted in triplicate. Data analysis was done by applying the SPSS ver. 17 package (SPSS Inc., Chicago, IL), and statistically significant differences were done by using one-way ANOVA followed by Duncan's test ($p < 005$).

3 RESULTS AND DISCUSSION

3.1 ISOLATION AND EVALUATION OF ENDOPHYTIC BACTERIA FROM RICE ROOTS FOR ENHANCING RICE SEED GERMINATION UNDER SALT STRESS

From rice root and shoot samples, a total of nine endophytic strains capable of forming colonies on the salt stress media were isolated and distinguished from others by colony morphological characteristics. All of them were used to evaluate their abilities in improving rice seed germination under salt-stress conditions. The results are shown in Table 1.

As can be seen from Table 1, the results indicated the ability to enhance the seed germination of most iso-

Table 1: Effect of isolated endophytic bacteria on germination rate and growth parameters of rice seedlings after 7 days

No.	Bacterial strains	Germination rate (%)	Shoot length (mm)	Root length (mm)
1	ST.1	93.67 ± 0.5 ^{ab}	81.89 ± 0.7 ^{b}	75.54 ± 0.7 ^{b}
2	ST.2	94.33 ± 0.9 ^{ab}	83.92 ± 0.4 ^{b}	74.32 ± 0.4 ^{b}
3	ST.3	83.13 ± 0.8 ^{c}	63.43 ± 0.5 ^{c}	58.18 ± 0.6 ^{c}
4	ST.4	94.67 ± 1.2 ^{ab}	64.14 ± 0.9 ^{c}	57.69 ± 0.7 ^{c}
5	ST.5	82.31 ± 0.7 ^{c}	74.41 ± 0.3 ^{bc}	66.18 ± 0.9 ^{bc}
6	ST.6	99.33 ± 0.8 ^{a}	99.47 ± 0.6 ^{a}	82.16 ± 0.8 ^{a}
7	ST.7	92.71 ± 0.9 ^{ab}	82.32 ± 0.7 ^{b}	76.31 ± 0.5 ^{b}
8	ST.8	99.67 ± 0.6 ^{a}	98.87 ± 0.6 ^{a}	81.26 ± 0.6 ^{a}
9	ST.9	81.33 ± 0.9 ^{c}	75.34 ± 0.8 ^{bc}	67.19 ± 0.7 ^{bc}
10	No bacteria	81.24 ± 0.9 ^{c}	63.14 ± 0.5 ^{c}	56.43 ± 0.6 ^{c}

The data represent the mean ± standard error (SE) based on three replicates. Values in the same column with the same letter(s) are not significantly different as determined by Duncan's test ($p < 0.05$)

lates. Among those, two bacteria strains, ST.6 and ST.8 produced significant improvements in germination rates, shoot and root length, and statistically significant differences ($p < 0.05$) compared to control tests (no bacteria).

The experimental results showed that two lines of isolated endophytic bacteria, ST.6 and ST.8 at densities of 10^6 CFU ml^{-1}, were highly effective in supporting germination stimulation, helping to support the growth and biomass of rice crops. Hence, the isolates ST.6 and ST.8 were chosen for further studies.

The results of molecular identification indicated the ST.6 and ST.8 strains were close to *Pantoea dispersa* Gavini et al. 1989 (accession number MT275631.1) and *Burkholderia cenocepacia* AU16956 (accession number CP034545.1), respectively. The 16S rDNA sequences of two strains ST.6 and ST.8 were deposited on GenBank with the accession numbers ON326555 and ON326556.

3.2 EVALUATION OF THE *IN VITRO* PLANT GROWTH-PROMOTING ABILITIES OF SELECTED ENDOPHYTIC BACTERIA

Two selected endophytic bacteria were investigated for the plant growth-promoting (PGP) traits. The results were presented in Table 2.

Based on the data shown in Table 2, the endophytic bacteria strain ST6 presented the ability in producing all tested plant growth-promoting traits, while strain ST.8 only presented three of four but not the siderophore production trait under *in vitro* conditions. Notably, a remarkable decrease in pH was observed during the phosphate solubilization process of these two endophytic bacteria strains.

3.3 INOCULATION WITH SELECTED ENDOPHYTIC BACTERIA ENHANCED RICE GROWTH UNDER NORMAL AND SALINE CONDITIONS

The results of greenhouse experiments were carried out to investigate the capacity of isolated endophytic bacteria in improving rice growth under stress conditions. The results were illustrated in Figure 1 and shown in Table 3.

The data indicated a significant decrease in the analyzed growth parameters (shoot and root length, number of roots per plant, and plant biomass) when watered seedlings with 150 mM NaCl solution. Interestingly, the inoculation with both selected strains, ST.6 and ST.8, resulted in a significant improvement in plant growth parameters compared with the control ($p < 0.05$). Presumably, the results showed that the isolates, ST.6 and ST.8, are beneficial for rice seedling growth promotion and improve the salt tolerance of rice seedlings as well (Figure 1).

In Viet Nam, rice is one of the important crops that

Table 2: Characterization of in vitro plant growth-promoting traits of endophytic bacteria isolated from rice root

Bacterial strain	IAA (µg ml^{-1})	ACC Deaminase	Siderophore (%)	Soluble phosphate amount (mg ml^{-1})
ST.6	19.71 ± 1.83	13.52 ± 0.14	76.32	617.35 ± 0.23
ST.8	15.67 ± 1.79	12.72 ± 0.47	-	579.75 ± 0.45

The data represent the mean ± standard error (SE) based on three replicates. The ACC deaminase activity was measured spectrophotometrically at 590 nm and expressed as µmol mg^{-1} protein h^{-1}

Table 3: Enhancement of growth parameters of rice seedlings inoculated with selected endophytic bacteria under normal and saline conditions

Watering solution	Treatment	Root length (cm)	Shoot length (cm)	Average number of roots/plant	Plant dry weight (mg)
Water	No bacteria	3.12 ± 0.4 ^{b}	21.4 ± 1.2 ^{b}	8.12 ± 0.3 ^{a}	40.35 ± 0.3 ^{b}
	Strain ST.6	4.83 ± 0.5 ^{a}	26.8 ± 2.3 ^{a}	7.95 ± 0.5 ^{a}	43.21 ± 0.2 ^{a}
	Strain ST.8	4.21 ± 0.7 ^{a}	25.7 ± 1.8 ^{a}	8.13 ± 0.3 ^{a}	42.57 ± 0.3 ^{a}
150 mM NaCl	No bacteria	2.29 ± 0.5 ^{c}	17.5 ± 1.6 ^{d}	7.1 ± 0.5 ^{b}	36.78 ± 0.5 ^{c}
	Strain ST.6	3.64 ± 0.7 ^{ab}	20.4 ± 1.3 ^{bc}	7.6 ± 0.4 ^{ab}	39.36 ± 0.2 ^{b}
	Strain ST.8	2.78 ± 0.4 ^{bc}	19.1 ± 1.5 ^{c}	7.4 ± 0.7 ^{ab}	38.14 ± 0.3 ^{bc}

The data represent the mean ± standard error (SE) based on three replicates. Values in the same column with the same letter(s) are not significantly different as determined by Duncan's test ($p < 0.05$)

Figure 1. Inoculation of strains ST.6 and ST.8 improves rice growth under normal conditions (A) and in the presence of 150 mmol l-1 NaCl (B). Scale bar: 5 cm

provide food for domestic consumption and international export as well. However, it is a plant sensitive to salination that appeared more often in some areas in Viet Nam including Ca Mau, Vietnam due to climate change. Among methods that have been applied to reduce the effect of salination on crop yield, the one using plant growth-promoting bacteria is emerging as an alternative method that could alleviate biotic and abiotic stresses in plants (Vaishnav et al., 2019). There were several studies have been carried out to explore the endophytic bacterial community colonizing the internal tissues of healthy rice plants (Chu et al., 2021; Zhang et al., 2021). An example is endophytic bacteria, strain Fse28, obtained from the surface-sterilized seeds of Dongxiang wild rice presented a significant improvement in the rice seed germination (86 %) compared with the control (70 %) after 5 days of germination (Zhang et al., 2021). The results of this study showed that two strains ST.6 and ST.8 presented the strongest abilities in enhancing the germination rate of rice seed under salt stress (100 mM NaCl). There were several studies also presented the beneficial effect of endophytes on seed germination. Wang et al. (2019b) reported that the *Epichloë bromicola* Leuchtm. & Schardl endophyte significantly increased root length, coleoptile length, and germination rate of *Hordeum brevisubulatum* (Trin.) Link. seeds at high NaCl concentrations (200 and 300 mM). In another report, Lu et al. (2021) showed that *Pantoea ananatis* D1 endophyte clearly improved rice seed germination percentage in comparison to the negative control at 100 mM NaCl. These findings suggest that the endophyte plays an important role in seed germination under different conditions. In this research, two endophytic strains ST.6 and ST.8 enhanced germination under salt stress, and this may ensure more seedling establishment and enable rice plant associations to withstand saline conditions.

It was reported that the PGPB could enhance the salt tolerance of plants through several direct and indirect mechanisms such as the production of PGP compounds, the modulation of nutrients metabolization, and/or the accumulation of osmolytes (Ilangumaran and Smith, 2017; Otlewska et al., 2020). Otlewska et al. (2020) demonstrated that IAA produced by endophytic bacteria could improve the stress tolerance of plants by increasing root length, promoting nutrient access, and enhancing root exudation. Another PGP compound is ACC that also been proven able in reducing the accumulation of ethylene that was induced by salt stress, hence improving the plant salt resistance (Vaishnav et al., 2019). In this study, two selected endophytic bacterial strains, ST.6 and ST.8, generated two PGP compounds (including IAA and ACC) and promoted rice development under normal and salt stress conditions compared to the control. These might be due to the strain ST.6 and ST.8 still producing IAA in high NaCl concentration (150 mM NaCl) to enhance the rice root development. These results were consistent with other studies, in which the bacteria strains produced IAA at high NaCl concentrations. For example, Kumar et al. (2021) demonstrated that *Bacillus pumilus* Meyer and Gottheil 1901 (Approved Lists 1980) JPVS11 could produce IAA at high NaCl concentrations (up to 1200 mM NaCl) and inoculation with *B. pumilus* JPVS11 significantly improved the agronomic traits of rice at 50 mM NaCl compared to the control. Presumably, the production of IAA and/or ACC may be a strategy of endophytic bacteria to adapt to stress conditions.

Moreover, salinity stress caused a low P availability limiting crop production. Hence, an alternative method to improve crop productivity is the increase of P availability in soil by using salt-tolerant phosphate-solubilizing bacteria (ST-PSB). In this study, endophytic strains ST.6 and ST.8 showed a higher amount of soluble P (617.35 ± 0.23 and 579.75 ± 0.45, respectively) in the supernatant. Hence, inoculation of these two isolates may enhance the solubilization of insoluble phosphate increasing the level of P availability in soil that is easily absorbed by plants (Vaishnav et al., 2019). There were several studies demonstrated that the inoculation with ST-PSB increased the N, P, and K uptake and enhanced the salt tolerance efficiency in different plants such as rice (*Oryza sativa* L.), wheat (*Triticum* sp.), tomato (*Solanum lycopersicum* L.), maize (*Zea mays* L.), and quinoa (*Chenopodium quinoa* Willd.) (Vaishnav et al., 2019; Lu et al., 2021).

Furthermore, it was reported that in iron-limited conditions the PGPB could produce siderophore to chelate iron from the environment transforming insoluble iron into plant-accessible iron-siderophore complexes (Vaishnav et al., 2019). In this study, endophytic bacteria strain ST.6 secreted siderophore into the supernatant, while strain ST.8 did not; and better rice growth was observed when seedlings were inoculated with strain ST.6 but not with strain ST.8. These results could be the ST.6 produced organic acids (with hydroxyl or carboxyl groups), and siderophores, which further interact with different cationic metals of insoluble Pi to generate the bioavailable phosphate and metal-chelating complex that is easily uptaken by plants. These results suggest that the endophytic bacteria strain ST.6 and ST.8 could be developed into biofertilizer to apply in acidic or alkaline soil (Rfaki et al., 2019).

3.4 EFFECT OF INOCULATED ENDOPHYTIC BACTERIA ON THE CONTENT OF CHLOROPHYLL AND PROLINE IN RICE LEAVES EXPOSED TO SALINE CONDITIONS

Chlorophyll plays an important role during plant development. Hence, the chlorophyll composition: chlorophyll a, chlorophyll b, and carotenoids contents in the rice leaves exposed to the saline condition were determined in the interaction with inoculated bacteria strains. The results were shown in Table 4.

There were statistically significant differences between bacterial strains applied (Table 4). The results showed that in all treatments of rice seedlings with bacterial strains under normal and saline conditions, the content of chlorophyll a, chlorophyll b, and carotenoids was higher, statistically significant difference compared to the control without bacterial inoculation. In which, treatment with bacterial strain ST.6 had the highest chlorophyll a, chlorophyll b, and carotenoid contents (0.27; 0.19, and 0.18 mg g^{-1}). The results are in agreement with the results of Sun et al. (2020), in which rice seedlings inoculated with *Pantoea alhagi* NX-11 showed a 32.6 % higher fresh mass, a 30.6 % greater root length, a 26.4 % greater shoot length, and a 42.5 % higher chlorophyll compared with the control under salt stress conditions. These results showed that the bacterial strains were effective in synthesizing chlorophyll a, chlorophyll b, and carotenoids in rice seedlings during the seedling stage under normal or stress conditions.

In addition, proline was reported as an important osmotic adjustment substance, which exists in plant cells. The accumulation of proline was one of the mechanisms to alleviate the salt stress in plants (Liang et al., 2018). Our results showed that the uninoculated and inoculated seedlings treated with 150 mM NaCl solution resulted

Table 4: Effect of bacterial inoculation on chlorophyll content of rice seedlings under salt stress

Watering solution	Treatment	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Carotenoid (mg g^{-1})	Proline (µg g^{-1} FM)
Water	No bacteria	0.15 ± 0.1^{d}	0.16 ± 0.2^{ab}	0.15 ± 0.3^{a}	17.61 ± 0.4^{e}
	Strain ST.6	0.27 ± 0.4^{a}	0.19 ± 0.2^{a}	0.18 ± 0.2^{a}	29.32 ± 0.5^{cd}
	Strain ST.8	0.22 ± 0.3^{b}	0.18 ± 0.3^{b}	0.17 ± 0.3^{ab}	27.15 ± 0.3^{d}
150 mM NaCl	No bacteria	0.13 ± 0.2^{e}	0.07 ± 0.4^{d}	0.09 ± 0.2^{c}	76.83 ± 0.7^{c}
	Strain ST.6	0.19 ± 0.4^{c}	0.11 ± 0.1^{c}	0.11 ± 0.4^{b}	91.51 ± 0.6^{a}
	Strain ST.8	0.17 ± 0.3^{cd}	0.09 ± 0.2^{cd}	0.10 ± 0.3^{bc}	87.95 ± 0.5^{b}

The data represent the mean ± standard error (SE) based on three replicates. Values in the same column with the same letter(s) are not significantly different as determined by Duncan's test ($p < 0.05$)

in a 3 to 5 times higher amount of proline than that of seedlings watered with water only. These results suggest two isolated endophytic bacteria, ST6 and ST.8, could enhance the proline synthesis in rice under normal and salt conditions.

3.5 EFFECT OF INOCULATED ENDOPHYTIC BACTERIA ON THE ACTIVITIES OF ANTIOXIDANT ENZYME IN RICE SEEDLINGS EXPOSED TO SALINE CONDITIONS

As can be seen from Figure 2, the salt treatment caused significant changes in the activities of antioxidant enzymes in rice seedlings. Three tested enzymes (SOP, POD, and CAT) showed a rapid increase in activities under saline conditions and their activities were significantly higher than that in rice grown under normal conditions. The seedlings inoculated with strain ST.6 had higher activities of those enzymes than the ones treated with strain ST.8 under normal conditions, but under salt stress conditions, the opposite results were observed (Figure 2).

Moreover, the results also indicated a significant decrease in SOD, POD, and CAT activities in the rice seedling treated with either strain ST.6 or strain ST.8 compared to the control under salt conditions. These data suggest the endophytic bacteria strain ST.6 and ST.8 isolated from rice roots play roles in alleviating oxidative stress.

It was reported that plant under salt stress produces many changes in physiological and biochemical properties such as a decrease in photosynthetic efficiency, osmotic imbalance, ion toxicity, and generation of ROS (Otlewska et al., 2020). In this study, the increase of chlorophyll and carotenoids amount in seedlings treated with a salt solution was observed (Table 4), meaning the photosynthetic efficiency of seedlings was improved. There were several studies that reported the chlorophyll content of rice seedlings inoculated with PGPB such as *Pantoea alhagi* NX-11 (Sun et al., 2020) and *P. ananatis* D1 (Lu et al., 2020) significantly increased (42.5 and 45 %, respectively) in comparison with control seedlings. In addition, the result of this study also demonstrated that seedlings inoculated with endophytic bacteria also significantly increased the proline content in plants compared to the control. These results were in agreement with the report of Sun et al. (2020), in which rice seedlings inoculated *Pantoea alhagi* NX-11 presented an improved growth after 7 days under salt stress compared to the control with a 37.5 % lower malondialdehyde content, a 133 % higher K^+/Na^+ ratio, and a 52.8 % higher proline content. Later, Lu et al. (2020) reported that a significant accumulation of proline (98.25 μg g^{-1} of fresh mass) in rice seedlings inoculated with *Pantoea ananatis* D1 compared to the uninoculated seedlings grown under saline conditions was observed. These could be proline played as an osmoprotectant to stabilize proteins and cell membranes of plant cells when plants were grown under salt stress (Radhakrishnan and Baek, 2017). Hence, the accumulation of proline by plants aided the balance of the osmotic pressure under salt stress. Further, the generated proline could serve as nitrogen and carbon sources for plants freed from salt stress and could play roles in detoxing the freed radicals from plant cells (Mohammadkhani and Heidari, 2008). Therefore, inoculation of strain ST.6 or ST.8 could promote rice seedlings to adapt to the rapid change of osmotic pressure caused by salt stress through proline accumulation.

In addition, it was documented that plants could alleviate the harmful effects caused by salinization through the production of antioxidant enzymes (Radhakrishnan and Baek, 2017; Lu et al., 2020). The results of this study showed that the seedlings cultivated under salt condi-

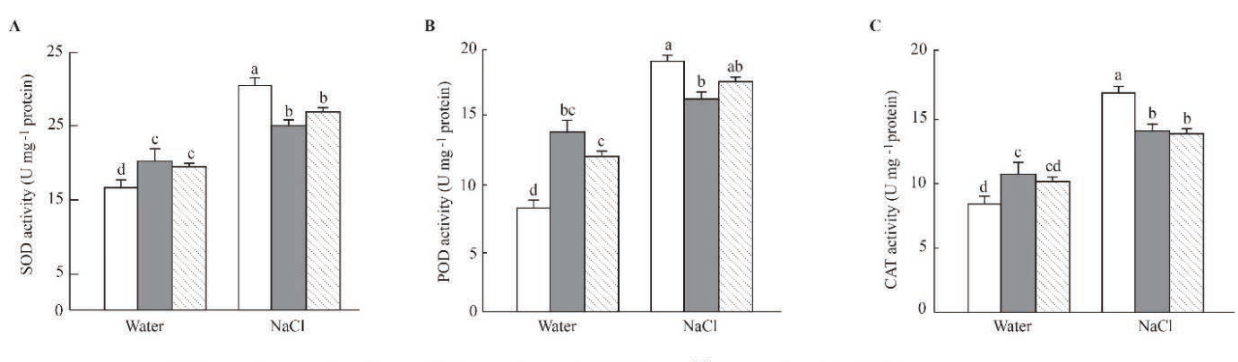

Figure 2: Effect of strain ST.6 and ST.8 inoculation on antioxidant enzyme activities in rice under normal (water) and saline (NaCl) conditions. (A) SOD; (B) POD; (C) CAT. Different letters show statistically significant differences (p < 005)

tions produced a significant increase in the antioxidant enzymes (SOD, POD, and CAT) compared with plants grown in non-saline conditions. These results were supported by the report of Lu et al. (2020), who demonstrated that rice seedlings treated with NaCl resulted in an increase of 92.4 % in SOD activity, 97.2 % in CAT activity, and 139.2 % in POD activity when compared with the water group. Especially, this study also exhibited a reduction in antioxidant enzyme activities observed in seedlings inoculated with both endophytic bacteria strain ST.6 and ST.8 under salt stress, especially, since strain ST.6 presented a better effect than strain ST.8 did. These results were consistent with reports of Sarkar et al. (2018) and Lu et al. (2020), in which salt-stressed plants also presented a decrease in the activity of antioxidant enzymes when they were inoculated with PGPB. Hence, the results suggest both strains, ST.6 and ST.8 could help rice tolerant with salinity.

3.6 DETERMINATION OF THE INOCULATED ENDOPHYTIC BACTERIA IN THE RICE SEEDLINGS

It was reported that plant-bacteria synergism play important role in improving the salt tolerance of plants. In this study, higher numbers of inoculated endophytes were observed in the root, and shoot of the inoculated rice seedlings than in the non-inoculated ones under normal or salt stress conditions (Table 5).

In addition, the screening experiment also showed significantly higher numbers of inoculated bacteria in the root interior than that in the shoot interior. Interestingly, the results also showed a relatively higher number of inoculated bacteria in the plant sample collected from salt treatment than that in the water treatment.

It was reported that the plants produced an optimal microenvironment surrounding their root for the growth of the inoculated and indigenous microbes, subsequently increasing the number of inoculated endophytes (Tara et al., 2019). Our data showed an agreement with this report. Moreover, higher numbers of inoculated bacteria in the root interior than in the shoot interior. This might be because the inoculated bacteria were isolated from rice roots, hence they preferred locating in the root. Especially, the inoculated bacteria in the rice seedling under normal conditions were lower than the ones in rice seedlings grown under salt stress. This might be because the salt conditions could generate a benefit for the development of inoculated bacteria in the root rhizosphere and for their root colonization.

4 CONCLUSIONS

Endophytic bacteria have been exploited as bioinoculant to improve plant growth under abiotic and biotic stress. Two salt-tolerant endophytic bacteria, ST.6 and ST.8, produce the highest value of germination rate (99.33 and 99.67 %, respectively) and were close to *Pantoea dispersa* (accession number MT275631.1) and *Burkholderia cenocepacia* (accession number CP034545.1), respectively. They were capable of dissolving P insoluble, producing IAA, exhibiting ACC deaminase activities, and only ST.6 could produce siderophores. Inoculation of rice seedlings with strains ST.6 and ST.8 promoted rice growth under both normal and salt-stressed conditions. Moreover, ST.6 and ST.8 could have roles in reducing the salt stress on rice plants by penetrating into the plant and then inducing chlorophyll and proline accumulation, decreasing antioxidation activity, and maintaining ionic balance. The results suggest strains ST.6 and ST.8 could be used to develop the biofertilizer for sustainable agriculture in the future.

Table 5: Identification of the inoculated endophytic bacteria in the root interior, and shoot interior of rice seedlings under salt stress

Watering solution	Treatment	Root sample ($x10^3$ CFU g^{-1})	Shoot sample ($x10^3$ CFU g^{-1})
Water	No bacteria	0.82 ± 0.3^{a}	0.21 ± 0.6^{a}
	Strain ST.6	10.21 ± 0.4^{a}	6.4 ± 1.5^{a}
	Strain ST.8	9.25 ± 0.9^{bc}	5.3 ± 1.1^{a}
150 mM NaCl	No bacteria	1.02 ± 0.7^{a}	0.31 ± 0.7^{a}
	Strain ST.6	10.64 ± 0.7^{b}	6.8 ± 1.3^{bc}
	Strain ST.8	9.78 ± 0.4^{c}	6.1 ± 0.7^{c}

The data represent the mean ± standard error (SE) based on three replicates. Values in the same column with the same letter(s) are not significantly different as determined by Duncan's test ($p < 0.05$)

5 ACKNOWLEDGEMENTS

This research has been done under the research project QG.21.60 "Screening of plant endophytic bacteria with ammonium oxidation capabilities for the application in wetland technology" of Vietnam National University Hanoi. The Vietnam National University Hanoi provided funds for collecting samples and buying chemicals for experiments and supported experimental facilities to carry out experiments.

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doi:10.14720/aas.2023.119.1.2739 Review article / pregledni znanstveni članek

Neželene spremembe prsne mišičnine pri pitovnih piščancih

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Delo je prispelo 23. junija 2022, sprejeto 16. marca 2023.
Received June 23, 2022; accepted March 16, 2023.

Neželene spremembe prsne mišičnine pri pitovnih piščancih

Izvleček: V svetovnem merilu perutninsko meso predstavlja cenovno dostopen in kakovosten vir beljakovin, zato se je v zadnjih desetletjih povečala njegova prireja. Slednje smo dosegli z intenzivno selekcijo pitovnih piščancev na hitro rast, učinkovitejše izkoriščanje krme, povečan delež prsne mišičnine in manjši delež trebušne maščobe. Na drugi strani so tako intenzivna selekcija in zahteve po večji prireji, kot tudi nekateri zunanji stresni dejavniki, ki vključujejo neželene spremembe v okolju, prehranskem režimu, tehnologiji reje in nepravilne postopke ravnanja z živalmi pred zakolom, povzročili večjo dovzetnost živali za oksidativni stres, ki poslabšuje senzorične in tehnološke lastnosti perutninskega mesa. Posledice hitrega povečevanja prsne mišičnine pri pitovnih piščancih in intenzivne tehnologije reje se kažejo v naraščajočem trendu pojavnosti različnih neželenih sprememb piščančjega mesa oziroma miopatij. Med najpogostejše miopatije spadajo miopatija globoke prsne mišice, bledo, mehko in vodeno meso, bela progavost prsne mišice, olesenelost prsne mišičnine ter špagetasto meso, ki prizadenejo predvsem veliko prsno mišico in poslabšujejo njene senzorične in tehnološke lastnosti. Prav tako omenjene spremembe prsne mišičnine poslabšujejo kakovost in prehransko vrednost mesa ter vplivajo na sprejemljivost mesa za potrošnike in nedvomno povzročajo ekonomske izgube v živilsko-predelovalni industriji.

Ključne besede: perutnina; pitovni piščanci; prehrana živali, prsno mišično tkivo; kakovost mesa; miopatije

Breast muscle abnormalities in broiler chickens

Abstract: In recent decades, global production of poultry meat has increased due to its affordable prices and good nutritional value. The latter has been achieved by intensive selection of broilers for increased growth rate, feed efficiency, breast yield and reduced abdominal fat deposition. On the other hand, intensive selection and increasing demand for poultry meat, as well as some environmental stressors, such as changes in environmental temperature, feeding regime, breeding technology, and improper handling procedures before slaughter, lead to increased susceptibility of animals to oxidative stress, resulting in poorer sensory and technological characteristics of chicken meat. As a result of intensive broiler production and the increase in breast muscle, various breast muscle abnormalities or myopathies have been observed. The most common ones include deep pectoral myopathy, pale, soft and exudative like meat, white striping, wooden-breast and spaghetti meat, which mainly affect the pectoralis major breast muscle and negatively influence the sensory and technological characteristics of breast meat. The muscle abnormalities have a detrimental effect on quality and nutritional value of meat, affect consumer compromise consumers acceptance, and cause economic losses in the meat processing industry.

Key words: poultry; broilers; animal nutrition; breast muscle; meat quality; myopathies

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1 UVOD

V zadnjih desetletjih se je prireja mesa pitovnih piščancev bistveno povečala, saj v svetovnem merilu perutninsko meso predstavlja cenovno dostopen in kakovosten vir beljakovin, z visoko hranilno vrednostjo in enostavno pripravo (Petracci in sod., 2013). Selekcija vitalnih, hitro rastočih genotipov pitovnih piščancev sledi čedalje večjemu povpraševanju in zahtevam potrošnikov po kakovostnih klavnih trupih in piščančjem mesu ter zahtevam rejcev po boljši gospodarnosti reje (Petracci in sod., 2013; Soglia in sod., 2016b; Zaboli in sod., 2019). Posledice intenzivne selekcije se v reji pitovnih piščancev kažejo v večji dovzetnosti živali za *in vivo* oksidativni stres (Estévez, 2015). Poleg selekcije lahko oksidativni stres sprožijo tudi številni zunanji dejavniki, kot so npr. prehrana, okolje, način reje in postopki ravnanja z živalmi pred zakolom, kar negativno vpliva na zdravstveno stanje in proizvodne lastnosti živali kot tudi na kakovost, prehranske in senzorične lastnosti mesa (Surai in sod., 2019; Xing in sod., 2019). Pri hitrorastočih genotipih piščancev so bile zaznane številne histološke in biokemične spremembe mišičnega tkiva, tako pred zakolom kot po njem, kar se kaže v večji pojavnosti neželenih sprememb oziroma napak v mišičnini, predvsem v prsni mišici (Soglia in sod., 2016b). Med temi napakami sta že opisani miopatija globoke prsne mišice (MGPM) ter bledo, mehko in vodeno (BMV) meso, ki pomembno zmanjšujeta kakovost piščančjega mesa (Cai in sod., 2018). V zadnjih desetih letih pa se je pojavilo večje število novih sprememb v prsni mišici oz. miopatij, med katere spadajo bela progavost prsne mišice (BPM), olesenelost prsne mišičnine (OPM) ter špagetasto meso (ŠM), ki negativno vplivajo predvsem na kakovost velike prsne mišice (*Musculus pectoralis major*) (Petracci in sod., 2019). Čeprav se miopatije prsne mišičnine kažejo v številnih fenotipskih razlikah, so jim skupne podobne histološke spremembe, kot so spremembe v strukturi mišičnega tkiva, mišična degeneracija, razpad mišičnih vlaken, nekroza in liza mišičnega tkiva ter druge. Tako lahko sklepamo, da imajo vse miopatije, čeprav vzroki za njihov nastanek še niso natančno definirani, skupno etiologijo. Ob tem pa se raziskovalci strinjajo, da je najverjetnejši vzrok za nastanek različnih neželenih sprememb oziroma miopatij hipoksija (Baldi in sod., 2020).

Miopatije ne vplivajo zgolj na slabše senzorične lastnosti in zavračanje takšnega mesa s strani potrošnikov, ampak negativno vplivajo tudi na funkcionalne lastnosti in parametre kakovosti mesa, kot so večja izceja, kot posledica slabše sposobnosti mesa za vezanje vode, slabša topnost beljakovin ter spremenjena prehranska vrednost in maščobnokislinska sestava mesa (Soglia in sod., 2016a; Petracci in sod., 2019; Baldi in sod., 2020). Kljub spremembam v mišičnini, ki jo povzročijo različne miopatije, se lahko takšno meso uporabi v živilsko-predelovalni industriji (Petracci in sod., 2019). Tako znanstvena stroka, selekcijski centri kot perutninski sektor in živilska industrija stremijo k iskanju rešitev za zmanjšanje oziroma preprečevanje miopatij oziroma sprememb prsne mišičnine v reji pitovnih piščancev. V prvi vrsti s poznavanjem dejavnikov, odgovornih za nastanek takšnih stanj, in v drugi vrsti z iskanjem alternativ za hitrejše odkrivanje in preprečevanje miopatij v reji pitovnih piščancev, kar bi ugodno vplivalo na dobrobit in prirejo piščancev, zato so na tem področju potrebne nadaljnje raziskave. S preglednim člankom smo tako želeli predstaviti znanstveno literaturo na področju napak prsne mišičnine in predvsem pri novejših miopatijah opredeliti slovensko terminologijo, ki je še nepoznana.

2 NEŽELENE SPREMEMBE PRSNE MIŠIČNINE

Posledica intenzivne selekcije na povečano prirejo pitovnih piščancev so oksidativni procesi v perutninskem mesu, ki vplivajo na večjo dovzetnost živali za razvoj številnih neželenih sprememb mišičnine oziroma miopatij, ki prizadenejo predvsem veliko prsno mišico in negativno vplivajo na kakovost in senzorične lastnosti perutninskega mesa (Trocino in sod., 2015).

Miopatija je definirana kot progresivna degeneracijska živčno-mišična bolezen, pri kateri pride do presnovnih motenj, ki se kažejo v zmanjšanju mišičnega tonusa, poškodbah in atrofiji mišičnih vlaken (Semenova in sod., 2019). Stresni dejavniki v reji živali predstavljajo enega izmed glavnih vzrokov za nastanek miopatij, ki povzročijo povečano tvorbo prostih radikalov in povečano vsebnost intracelularne koncentracije kalcijevih ionov (Ca^{2+}) (Petracci in sod., 2015), zaradi česar pride do povečane obremenitve mišic. Pri povečani koncentraciji Ca^{2+} v mioplazmi pride do povečane proizvodnje toplote zaradi aktivacije fosforilaze in razgradnje adenozin trifosfata (ATP). Pomanjkanje ATP vodi do poškodb miozina in aktina, pojava super kontrakcij miofibrilarnih beljakovin v mišičnem tkivu in togosti mišic. Poleg tega se zmanjša pH krvi, kar lahko povzroči presnovno acidozo (Semenova in sod., 2019). Splošna indikatorja, s katerima lahko potrdimo poškodbe mišic in celic, sta encima kreatin kinaza in laktat dehidrogenaza. Glavna oblika kreatin kinaze, prisotna v plazmi piščancev, izvira iz skeletnih mišic in se uporablja kot indikator različnih miopatij, zato višje vrednosti kreatin kinaze v krvi kažejo na poškodbe mišic. Laktat dehidrogenaza je intracelularni encim, ki katalizira pretvorbo piruvata v laktat v glikolitičnem mišičnem tkivu (npr. prsni mišici) in se sprosti v krvni obtok ob poškodbi celic (Aviagen, 2019).

Najpogostejše nezažele spremembe, ki vplivajo na senzorične lastnosti in kakovost piščančjih prsi in se čedalje pogosteje pojavljajo v intenzivni reji pitovnih piščancev, so MGPM, BPM, OPM in ŠM (Petracci in Cavani, 2012; Petracci in sod., 2019). Omenjene miopatije pomembno zmanjšujejo kakovost mesa, poslabšujejo sposobnost mesa za vezanje vode med toplotno obdelavo in skladiščenjem, kar se kaže v pogostem pojavu pretrganih snopov mišičnih vlaken (Petracci in sod., 2014). Čeprav pojav miopatij pogosto povezujemo s selekcijo na hitro rast živali in povečano prsno mišičnino, so bile ocenjene genetske korelacije med miopatijami, telesno maso in klavnim izplenom prsne mišičnine majhne, kar kaže na to, da nadaljnja selekcija na rast in povečevanje mišičnine ne bi nujno predstavljala večjega tveganja za razvoj miopatij (Bailey in sod., 2015). Z namenom zmanjšanja pojavnosti miopatij v reji pitovnih piščancev je potrebno upoštevati tudi okoljske dejavnike, med katere spadajo vodenje in pogoji reje ter prehrana (Aviagen, 2019). Z namenom preprečevanja miopatij se izvajajo številne raziskave na področju izražanja genov in selekcije, zmanjševanja stresa v reji in pred zakolom, prehrane, metabolomike, proteomike, valilničarstva in tehnologije klanja.

2.1 MIOPATIJA GLOBOKE PRSNE MIŠICE (MGPM)

MGMP je bolezen poznana tudi kot Oregonova bolezen ali bolezen zelene mišice, ki je bila kot degenerativna miopatija pri purah prvič opisana že leta 1968, kasneje pa se je pogosteje pojavljala v rejah pitovnih piščancev, selekcioniranih na povečano prsno mišico (Kijowski in sod., 2014). Bolezen sicer lahko prizadene obe, tako veliko (*Musculus pectoralis major*) kot globoko oziroma malo (*Musculus pectoralis minor*) prsno mišico, vendar je zaradi svojega položaja za to bolezen bolj in tudi v večjem obsegu dovzetna mala prsna mišica (Kijowski in sod., 2014), katere primarna funkcija je dvigovanje peruti. Mišica je obdana z neelastično ovojnico (fascijo) in stisnjena med prsnico in veliko prsno mišico, ki ji ne dovoljujeta, da bi se razširila oziroma nabreknila (Lien in sod., 2012). Med aktivnostjo mišice se zaradi povečanega krvnega pretoka njena masa poveča za približno 20 %, zaradi česar se mišica ukleščí med čvrsto ovojnico in kost (Petracci in Cavani, 2012; Kijowski in sod., 2014). Posledično se pritisk v mišici poveča, zmanjša se dotok krvi, kar povzroči ishemijo (stisnjenje žil) mišice oziroma utesnitveni sindrom, ki povzroči pomanjkanje kisika v prizadeti mišici (Bailey in sod., 2015). Takšno stanje povzroči nekrozo mišičnega tkiva, ki je na začetku oteklo in rdečkaste barve (hiperemija), v fazi nadaljnjih lezij pa se pojavi zelena do sivo-zelena obarvanost. Pri tem so na površini mišice vidne popokane žilice, v kasnejši fazi pa pride do skrčenja mišice (Kijowski in sod., 2014). Pri prizadetih mišicah se pojavijo spremembe v senzoričnih lastnostih, kot so barva, tekstura, sestava in struktura mišičnine. Takšne mišice so bolj trde in gumijaste ter manj elastične kot mišice brez MGPM (Stangierski in sod., 2019).

Miopatijo globoke prsne mišice razvrščamo v štiri faze glede na spremembe v barvi (odtenek in intenzivnost) in teksturi mišičnega tkiva ter glede na velikosti prizadetega območja mišice. V prvi fazi je značilen pojav izliva krvi (ekstravazacije) in strjevanja le-te v krvnih žilah, kar se kaže v živo rdeči barvi male prsne mišice. V drugi fazi je mišica obarvana svetlo rožnato, kot posledica nekroze se pojavijo spremembe v tkivu mišice, ki kasneje postane vlaknasto. V tretji fazi pride do pojava zelene obarvanosti mišičnega tkiva, ki se najprej pojavi v sredini mišice in se nato razširi čez celotno mišico. V četrti fazi pa je mala prsna mišica nekrozna, njena barva pa variira med belo, sivo in zeleno (Kijowski in Kupińska, 2013; Kijowski in sod., 2014).

Najpogostejši vzrok za nastanek MGPM je selekcija pitovnih piščancev na hitro rast in povečano prsno mišičnino, zato se je v zadnjih letih povečala tudi pogostost pojava MGPM (ang. deep pectoral myopathy) (slika 1) (Kijowski in Kupińska, 2012; Petracci in Cavani, 2012).

Slika 1: Miopatija globoke prsne mišice (Foto: Rezar, 2021)
Figure 1: Deep pectoral myopathy (Photo: Rezar, 2021)

Pogostnost in intenzivnost pojavljanja MGMP v rejah je odvisna od številnih dejavnikov, med katere sodijo tehnologija reje, genotip, starost, telesna masa in spol piščancev (Kijowski in sod., 2014). Pojavnost bolezni je povezana tako s starostjo kot tudi končno telesno maso živali (Pajohi-Alamoti in sod., 2016). Predhodne študije so potrdile pozitivno korelacijo med končno telesno maso piščancev in pogostostjo pojava MGMP (Kijowski in Kupińska, 2012; Lien in sod., 2012). Bianchi in sod. (2006) so v študiji primerjali pogostost pojava MGMP pri pitovnih piščancih moškega spola dveh različnih *provenienc* (cobb 500 in ross 508) in ugotovili, da se je MGMP v povprečju pojavila pri 0,84 % piščancev. Pojavnost je bila višja pri piščancih ross 508 (1,27 %) v primerjavi s cobb 500 (0,35 %), tako v začetku kot na koncu rasti. Prav tako so Lien in sod. (2012) ugotovili, da je pojavnost MGMP pri starosti 33 in 36 dni večja pri moških živalih v primerjavi z ženskimi živalmi, medtem ko pri starejših živalih spol ni imel značilnega vpliva. Prav tako MGMP povzroči zmanjšano antioksidativno aktivnost encimov, kar neugodno vpliva na *post mortem* oksidativno stabilnost prsne mišičnine in posledično na kakovost mesa. Yalcin in sod. (2019) so ugotovili, da je pojavnost MGMP (v prvi in drugi fazi) povečala pH, rdečino in izcejo mesa. Pri vseh fazah so izmerili večjo koncentracijo malondialdehida (MDA) kot pokazatelja lipidne peroksidacije ter spremenjeno maščobnokislinsko sestavo velike prsne mišice, pri mišicah, uvrščenih v drugo fazo, pa so izmerili tudi večjo aktivnost encimov SOD in GPx (Yalcin in sod., 2019).

MGPM je miopatija, ki jo lahko z gotovostjo potrdimo šele pri disekciji klavnih trupov, vendar pa lahko MGPM potrdimo tudi *in vivo*, in sicer z merjenjem aktivnosti encima kreatin kinaze v krvni plazmi. Povečana aktivnost omenjenega encima v plazmi je lahko posledica poškodb mišičnih tkiv (predvsem skeletnih), kjer poškodbe celične strukture povzročijo, da pride do sproščanja kreatin kinaze iz mišičnih vlaken. Povečanje aktivnosti encima je povezano s stopnjo poškodb mišic. Krvne analize aktivnosti kreatin kinaze omogočajo razmeroma hitro in učinkovito odkrivanje MGPM. Omenjena metoda se zaradi visokih stroškov in dolžine analize praviloma ne uporablja v komercialnih rejah, vseeno pa lahko predstavlja potencialno osnovo za selekcijo perutnine, odporne na MGPM (Kijowski in sod., 2014). Lien in sod. (2011) so poročali o znatno povišani koncentraciji kreatin kinaze v plazmi pitovnih piščancev 24 ur po sprožitvi MGPM in predlagali uporabo merjenja koncentracije kreatin kinaze kot neinvazivnega označevalca za določanje pojavnosti MGPM. Podatki kažejo, da razširjenost MGPM variira glede na različne pasme, kar pomeni, da lahko genetika igra pomembno vlogo pri pojavu takšnega stanja. Kot neinvazivno metodo za določanje MGPM v celih klavnih trupih po hlajenju in pred klasifikacijo le-teh, so Traffano-Shiffo in sod. (2018) uporabili dielektrično spektroskopijo. Namen študije je bil razviti večfaktorski algoritem in senzorsko napravo, s katero bi globinsko merili dielektrične lastnosti celotnega klavnega trupa s kožo, in ugotoviti, ali je ta dovolj natančen za prepoznavanje in določanje MGPM. Rezultati študije so pokazali, da lahko s pomočjo interakcije s fotoni in disperzije ugotovimo, katere kemijske spojine prevladujejo v mišičnih vlaknih in tako prepoznamo nekrotična tkiva, ki imajo manjšo vsebnost miozina, kolagena in sarkoplazmatskih beljakovin. Dielektrična spektroskopija se je izkazala kot zanesljiva metoda pri odkrivanju MGPM na liniji klanja, zato jo avtorji predlagajo kot možno hitro in zanesljivo metodo za uporabo v klavnicah (Traffano-Shiffo in sod., 2018).

Posledično so začeli selekcijski centri razvijati selekcijske metode, ki bi lahko pripomogle k zmanjšanju pojavnosti MGPM v jatah, pomagala pa bi lahko tudi genomska selekcija z uporabo DNA označevalcev (Petracci in Cavani, 2012). K zmanjšanju pojavnosti miopatije pa lahko preventivno vpliva že rejec sam, tako da poskuša omejiti oziroma preprečiti nepotrebno prhutanje živali s perutmi, npr. z zmanjšanjem hrupa v hlevu in njegovi okolici ter z mirnim in manj pogostejšim rokovanjem z živalmi. Poleg tega je priporočljivo omejiti tudi nekatere aktivnosti rejca v hlevu, kot je npr. število tehtanj živali, rahljanje nastilja, redčenje jat in vstopanje ljudi v hlev.

2.2 BLEDO, MEHKO IN VODENO (BMV) MESO

Bledo, mehko in vodeno (BMV) meso (ang. pale, soft, exudative meat) je bilo prvotno opisano kot neželena sprememba mišičnine, ki se pojavi tako pri surovem piščančjem in prašičjem mesu kot tudi pri tehnoloških lastnostih proizvodov iz takšnega mesa. BMV meso je značilno svetlejše oziroma blede barve, ima spremenjeno, mehko strukturo ter slabo sposobnost vezanja vode. Pri prašičih je BMV meso povezano tudi z genetsko mutacijo rianodinskih receptorjev, ki povzroči presežek sproščanja kalcijevih ionov iz sarkoplazemskega retikuluma skeletnih mišic (Petracci in Cavani, 2012; Dong in sod., 2020). Pri perutnini so spremembo, podobno BMV mesu, opisali že pred desetletji, vendar še vedno ne obstajajo trdni dokazi, ki bi potrdili genetsko mutacijo rianodinskih receptorjev, zato pri perutnini govorimo o BMV podobnem mesu (Petracci in Cavani, 2012).

Pojav BMV mesa je glede na ugotovitve predhodnih študij posledica genetskih in/ali okoljskih dejavnikov, kot so npr. intenzivna selekcija na povečano prsno mišičnino, sezona, vročinski stres ter stresni dejavniki ravnanja z živalmi pred zakolom in po njem, ki vodijo v večjo dovzetnost mišic za oksidacijo maščob in beljakovin (Kuttappan in sod., 2016; Carvalho in sod., 2017; Dong in sod., 2020). Kar se zadeva genetike je bilo dokazano, da selekcija na hitrejšo rast in povečano prsno mišičnino povzroča številne histološke in biokemične spremembe mišičnega tkiva, kar je lahko povezano tudi s pojavnostjo BMV mesa (Petracci in Cavani, 2012).V primeru izpostavitve živali zunanjim stresnim dejavnikom se poveča izločanje adrenalina iz nadledvične žleze, ki vpliva na povečano razgradnjo glikogena v mišičnih celicah, kar pomeni, da se anaerobna glikoliza prične že *in vivo* (Gonzalez-Rivas in sod., 2020). Glikogen se razgradi v glukozo, glukoza pa nato v mlečno kislino, ki povzroči hiter padec pH (Petracci in Cavani, 2012). Takoj po zakolu se anaerobna glikoliza še pospeši zaradi velike količine mlečne kisline in pH hitro pade pod 5,8, medtem ko je temperatura mišičnine še vedno visoka (nad 35 °C) (Carvalho in sod., 2017). Omenjena kombinacija povzroči denaturacijo miofibrilnih in sarkoplazemskih beljakovin, kar zmanjša sposobnost mišičnih vlaken za vezanje vode (Lee in Choi, 2021), poveča se propustnost celične membrane (Petracci in sod., 2015) in več tekočine preide v medcelične prostore, ki se močno povečajo (Barbut in sod., 2005). To vpliva na manjši delež prsne mišičnine in slabšo kakovost mesa ter povzroča ekonomske izgube (Kuttappan in sod., 2016; Dong in sod., 2020). BMV perutninsko meso ima slabše senzorične lastnosti, saj je takšno meso bolj svetlo in rumeno (večji L* in b* vrednosti), ima mehko konsistenco oziroma vodeno teksturo, večjo izcejo, slabšo sposobnost za vezanje vode in manjšo rezno trdoto. Prav tako ima takšno meso slabše tehnološke lastnosti za nadaljnjo predelavo in je v primerjavi z običajnim mesom bolj podvrženo kvarjenju (Tang in sod., 2013; Karunanayaka in sod., 2016).

Med najpomembnejšimi okoljskimi dejavniki, ki vplivajo na pojav BMV mesa pri perutnini, je izpostavljenost živali sezonskemu vročinskemu stresu v zaključni fazi rasti in pred zakolom (Dong in sod., 2020). Hitrorastoči piščanci so bolj dovzetni za neugodne učinke vročinskega stresa, saj pri sodobnih genotipih pitovnih piščancev razvoj mehanizmov termoregulacije ne more slediti hitremu povečevanju mišične mase, kar pri živalih povzroči nezmožnost ohranjanja stalne telesne temperature ob povišani okoljski temperaturi (Zaboli in sod., 2019). Na višjo temperaturo prsne mišičnine pred zakolom lahko vpliva tudi napačno rokovanje z živalmi, ki povzroči vznemirjenost živali ter povečano prhutanje s perutmi, pa tudi večja presnovna aktivnost povečane prsne mišičnine, zato obstaja verjetnost, da se prsne mišice pitovnih piščancev, predvsem pa puranov, po zakolu počasneje hladijo (Petracci in Cavani, 2012).

V predhodnih študijah na BMV mesu so z uporabo proteomskih pristopov ugotovili, da je proces pretvorbe mišic v meso spremenjen v glikolitičnih mišicah, kar povzroči spremembe v funkcionalnosti proteolitičnih encimov in/ali se kaže v denaturaciji mišičnih beljakovin. Prav tako bi spremembe v koncentraciji dveh glikolitičnih encimov (fruktoza-1,6-bifosfat aldolaza in gliceraldehid 3-fosfat dehidrogenaza) pojasnile razlike v hitrosti padca pH pri normalnem in BMV mesu. Proteomske analize bi lahko v prihodnje uporabili za odkrivanje omenjenih sprememb, za katere se domneva, da povzročijo slabšo kakovost mesa in vodijo v BMV meso. V zadnjem času se študije z namenom identifikacije in preprečevanja BMV mesa osredotočajo tudi na nutrigenomiko. Znano je, da dodatek tokoferolov v krmo za piščance ugodno vpliva na zmanjšanje oksidacijskih procesov ter posledično na boljše funkcionalne lastnosti in zmanjšanje pojavnosti BMV mesa. Ugoden vpliv dodatka vitamina E na oksidativno stabilnost lipidov v mišicah, maščobnokislinsko sestavo in kakovost takšnega mesa je verjetno posledica vpliva vitamina E na izražanje genov, povezanih s presnovo lipidov (Petracci in Cavani, 2012).

Ker s pojavom BMV mesa pri perutnini zaenkrat ni bilo ugotovljene povezave z genetskimi označevalci lahko pojavnost BMV mesa v jati zmanjšamo predvsem z vzdrževanjem primernih okoljskih pogojev v hlevu v času reje, kot so temperatura, pretok zraka in gostota naselitve, ter z zmanjšanjem stresnih dejavnikov pred zakolom, kamor spada odvzem krme, rokovanje z živalmi in njihovo nalaganje, transport, način omamljanja in način hlajenja klavnih trupov. Preventivno lahko v krmo za živali dodajamo tudi vitamin E. Olivio in sod. (2001) so ugotovili, da dodatek vitamina E v krmo pitovnih piščancev ugodno vpliva na zmanjšanje denaturacije beljakovin in lahko posledično zmanjša pojavnost BMV mesa ter izboljša funkcionalne lastnosti mesa.

2.3 BELA PROGAVOST PRSNE MIŠICE (BPM)

Bela progavost prsne mišice (BPM) (ang. white striping) je stanje prsne mišice, za katero je značilen pojav belih prog, ki potekajo vzporedno z mišičnimi vlakni, predvsem na površini velike prsne mišice, lahko pa se pojavijo tudi na površini stegenskih mišic pitovnih piščancev (Kuttappan in sod., 2012a). Na površini prsne mišice se bele proge običajno pojavijo v zgornjem oziroma kranialnem delu mišice in se lahko raztezajo vse do kavdalnega dela (Petracci in sod., 2019). Histopatološke spremembe prsne mišice zaradi BPM se kažejo v miopatskih lezijah, povečanem številu degeneriranih in atrofičnih mišičnih vlaken, variaciji v velikosti mišičnih vlaken, lipidozi, fibrozi, blagi mineralizaciji, občasni regeneraciji ter infiltraciji mononuklearnih celic (Petracci in sod., 2015; Bowker in Zhuang, 2016). Poleg tega se na mikroskopskem nivoju v belih progah opazi večja vsebnost maščob in proliferacija vezivnega tkiva (Petracci in sod., 2019).

Čeprav vzroki za nastanek BPM še vedno niso definirani, so lahko le-ti povezani s selekcijo na hitro rast in povečano prsno mišico pri genotipih modernih pitovnih piščancev (Bowker in Zhuang, 2016). Bailey in sod. (2015) so naredili oceno genetskega ozadja nekaterih najpogostejših miopatij in preverili njihovo povezavo z rastjo in klavnim izplenom pri pitovnih piščancih. Heritabiliteta (h^2) za BPM je bila ≤ 0,338, medtem ko sta bili h^2 za MGPM in OPM < 0,1. Med preučevanimi miopatijami se je v reji najpogosteje pojavila BPM. V študiji so ugotovili, da čeprav selekcija na povečano prsno mišičnino igra pomembno vlogo pri nastanku miopatij, imajo okoljski dejavniki večji doprinos. Prav tako so tudi Pampouille in sod. (2018) ugotovili, da ne obstaja določen gen, ki je odgovoren za pojavnost BPM, ampak lahko govorimo o poligenskem dedovanju.

BPM v prvi vrsti neugodno vpliva na senzorične lastnosti mesa, saj daje prsnim filejem marmoriran in neobičajen videz, kar negativno vpliva na percepcijo in izbiro potrošnikov, za katere je videz in način priprave perutninskega mesa izrednega pomena (Kuttappan in sod., 2012c). Bele proge na piščančjih prsih spreminjajo kemijsko sestavo mesa, ki ima tako večjo vsebnost znotrajmišične maščobe, vode in kolagena ter manjšo vsebnost beljakovin in rudninskih snovi, kar se kaže v slabši prehranski vrednosti BPM prsnih filejev v primerjavi z nespremenjenimi fileji (Petracci in sod., 2014; Bowker in Zhuang, 2016). BPM negativno vpliva tudi na različne parametre kakovosti mesa, in sicer povzroča večjo izcejo in izgubo vode pri kuhanju ter spremenjeno maščobnokislinsko sestavo mesa (Adabi in Soncu, 2019). Prav tako zmerna in povečana prisotnost BPM povečuje stopnjo oksidacije v prsni mišici, kar se kaže v večji koncentraciji s tiobarbiturno kislino reagirajočih spojin (TBARS), medtem ko težja oblika BPM zmanjšuje vsebnost aminokislin histidina, arginina in triptofana (Adabi in Soncu, 2019).

Kuttappan in sod. (2012a) so ugotovili, da je bila večja pojavnost BPM zaznana pri težjih in debelejših prsnih filejih. Prav tako sta Lee in Choi (2021) dokazala, da so BPM prsni fileji v primerjavi z običajnimi prsnimi fileji težji in imajo večji premer mišičnih vlaken, kar je posledica mišične hipertrofije. Na večjo pojavnost BPM v reji vpliva tudi krmljenje pitovnih piščancev s krmo z višjo energijsko vsebnostjo in manjšo vsebnostjo beljakovin (Kuttappan in sod., 2012a), starost in spol piščancev ter restriktivno krmljenje. BPM se najpogosteje pojavlja pri samcih z veliko in težko prsno mišičnino, predvsem v zadnjem obdobju pitanja, in sicer med 6. in 8. tednom starosti (Petracci in sod., 2013).

Identifikacija bele progavosti, kot tudi ostalih miopatij, ki prizadenejo prsno mišico, se po zakolu živali izvaja z vizualnim pregledom in/ali palpacijo velike prsne mišice (Petracci in sod., 2019). Gre za vizualno točkovanje, kjer z oceno nič ocenimo mišico brez sprememb, z višjimi ocenami pa spremembe glede na prisotnost in debelino belih prog (Kuttappan in sod., 2012c; Kuttappan in sod., 2016). Čeprav ima BPM podobne karakteristike kot mišična distrofija, povezana z zastrupitvijo z ionofori ali rastlino *Senna ocidentalis* ali s pomanjkanjem selena in vitamina E v prehrani perutnine (Bailey in sod., 2015; Kuttappan in sod., 2014), so Kuttappan in sod. (2012b) ugotovili, da dodatek različnih koncentracij vitamina E v krmo pitovnih piščancev ni vplival na pojavnost BPM pri pitovnih piščancih. Na drugi strani pa so Boerboom in sod. (2018) kot najverjetnejši razlog za nastanek BPM pri pitovnih piščancih navedli lokalno hipoksijo, ki povzroči poškodbe mišičnega tkiva.

Ker se BPM lahko določi šele ob pregledu prsne mišičnine po zakolu, zaenkrat preventivno preprečevanje pojavnosti BPM v jati glede na aktualno literaturo ni možno (Kuttappan in sod., 2016). Kot preventivni ukrep za zmanjšanje pojavnosti BPM poleg uspešnega vodenja reje in spremljanja okoljskih dejavnikov lahko izpostavimo še izbiro počasi rastočih genotipov pitovnih piščancev moškega spola, saj je BPM vezana predvsem na večji prirast in končno telesno maso piščancev ter večjo maso prsne mišičnine. V prihodnje bi bilo potrebno narediti več raziskav z namenom razumevanja vpliva okolja in vodenja reje na rast in razvoj mišičnine pri pitovnih piščancih (Bailey in sod., 2015). Podrobneje bi bilo potrebno tudi raziskati, kateri so ključni geni in mutacije, ki vplivajo na pojavnost BPM (Kong in sod., 2020) ter poiskati učinkovite biooznačevalce, s katerimi bi lahko pojavnost BPM v reji odkrili že pri živih živalih (Kuttappan in sod., 2016).

2.4 OLESENELOST PRSNE MIŠIČNINE (OPM)

Olesenelost prsne mišičnine (OPM) (ang. wooden ali woody breast) je miopatija za katero je značilna trda in toga prsna mišičnina, ki spominja na strukturo lesa in povzroči značilne histološke lezije v veliki in občasno tudi mali prsni mišici (Petracci in sod., 2019). Prvič so OPM pri piščancih opisali leta 2013, ko so poročali o neželeni spremembi mišičnine, podobni MGMP, za katero so značilne trše in blede izbokline na kavdalni in kranialni strani velike prsne mišice (Cai in sod., 2018). V Evropi so kot prvi patologijo OPM bolj podrobno opisali Sihvo in sod. (2014), ki so s pomočjo mikroskopije in imunohistokemijskega barvanja mišičnih tkiv ugotovili fibrozo z mišično degeneracijo in regeneracijo ter kopičenje limfocitov v veliki prsni mišici pitovnih piščancev pred zakolom in po njem. Histološko opisujemo OPM kot zmerno ali hudo mišično degeneracijo, ki jo spremlja nekroza tkiva, akumulacija vnetnih celic in fibroza. Prav tako se poškodbe tkiva kažejo v nenormalni strukturi in obliki celic, npr. kot izguba poligonalne oblike celic, na videz razcepljene celice in internalizacija jeder (Dalle Zotte in sod., 2017). OPM je povezana tudi z degeneracijo in regeneracijo mišičnih vlaken, nekrozo mišičnih vlaken, hialinizacijo, miofibrilarno hipertrofijo, zamenjavo nekrotičnih mišičnih vlaken z vlaknastim vezivnim tkivom, infiltracijo makrofagov in limfocitov v mišična tkiva, zadebelitvijo perimizijskega vezivnega tkiva, zunajceličnim odlaganjem kolagena, spremembami v obliki, velikosti in premerom mišičnih vlaken itd. (Chatterjee in sod., 2016; Dalle Zotte in sod., 2017; Sihvo in sod., 2017).

Glavni dejavnik, ki povzroča OPM, je intenzivna selekcija pitovnih piščancev na hitro rast in na povečano prsno mišičnino. Na pojavnost in resnost OPM tako kot pri BPM vplivajo dejavniki, kot so tehnologija reje, rast in razvoj mišičnega tkiva, genotip piščancev, spol, starost, prehrana ter telesna masa pred zakolom (Caldas-Cueva in Owens, 2020), pri čemer imajo okoljski dejavniki in vodenje reje večji vpliv na pojavnost OPM kot genetika (Bailey in sod., 2015). Čeprav so fiziološki vzroki za nastanek omenjene miopatije še vedno nejasni, je možno, da so glavni vzroki hipoksija, oksidativni stres in povečana vsebnost intracelularne koncentracije kalcijevih ionov (Dalle Zotte in sod., 2017).

Spremembe v prsni mišici v povezavi z omenjeno miopatijo se tako odrazijo tudi na sestavi mesa, ki ima tako kot pri BPM večjo vsebnost maščob, kolagena in vode ter manjšo vsebnost beljakovin in rudninskih snovi (Soglia in sod., 2016a; Geronimo in sod., 2019; Caldas--Cueva in Owens, 2020). Degeneracija mišičnih vlaken in spremembe v sestavi mišičnine se kažejo v slabših funkcionalnih lastnostih takšnih prsnih filejev, kot so npr. slabša sposobnost za vezanje vode, povečana izceja med skladiščenjem in spremenjena tekstura mesa (Petracci in sod., 2019; Caldas-Cueva in Owens, 2020). OPM prsni file je v primerjavi z običajnimi prsnimi fileji težji, debelejši, širši in bolj blede barve (Zhang in sod., 2021b). OPM zmanjšuje prehransko vrednost, vpliva na senzorične lastnosti in slabšo kakovost piščančjega mesa, kar negativno vpliva na percepcijo potrošnikov.

OPM je pogosto povezana tudi s težavami pri hoji oziroma mobilnosti piščancev, kar pomeni, da se lahko morebitna pojavnost OPM odkriva že pri živih živalih. Tako so Norring in sod. (2019) želeli ugotoviti povezavo med pojavnostjo OPM in mobilnostjo pitovnih piščancev. V študiji so se osredotočili na oceno hoje piščancev, ki so jo izvedli petkrat v času poskusa ter na koncu poskusa naredili še oceno poškodb nožnih blazinic ter palpacijo prsne mišičnine. Ugotovili so, da je bila pojavnost OPM povezana z manj pogostim gibanjem živali po prostoru in bolj nepremično držo med ležanjem oziroma mirovanjem. Ob tem so avtorji predvidevali, da bi lahko razvoj OPM povzročal večjo občutljivost prsne mišičnine in bi dodaten premik med mirovanjem lahko živali povzročil nelagodje in bolečino. Poleg omenjenega se je OPM pogosteje pojavljala pri težjih živalih z večjo prsno mišico (Borring in sod., 2019).

Kot zanesljiv označevalec, s katerim lahko predvidimo prisotnost OPM že pri živih živalih, Kong in sod. (2021) navajajo povečano aktivnost encima kreatin kinaze v krvnem serumu pitovnih piščancev, medtem ko je kompresijski test najbolj zanesljiva metoda za določanje OPM *post mortem*. Čeprav se aktivnost kreatin kinaze s starostjo piščancev povečuje, so v študiji izmerili značilne razlike med normalnimi prsnimi fileji in zmerno obliko OPM. Prav tako so potrdili pozitivno korelacijo med aktivnostjo kreatin kinaze v prsni mišičnini in kompresijsko silo, s katero lahko določimo teksturo mesa (Kong in sod., 2021).

Spremembe v makroskopskih lastnostih prsne mišice oziroma OPM na liniji klanja s senzorično oceno in palpacijo razvrščamo v štiri faze, in sicer blago obliko (na sredini prsne mišice opazne manjše zatrdline), zmerno obliko (na sredini prsne mišice opazne trše zatrdline), težjo obliko (več kot 75 % prsne mišičnine je izredno trde in so trše zatrdline opazne po celotni prsni mišici), ter težko obliko (celotna prsna mišica je otrdela in blede barve) (Petracci in sod., 2019). Za identifikacijo in karakterizacijo OPM se poleg senzorične ocene prsne mišičnine uporabljajo tudi instrumentalne metode za analizo teksture prsnih filejev z merjenjem strižnih sil oziroma rezne trdote mesa (Chatterjee in sod., 2016; Zhang in sod., 2021a). Prav tako Caldas-Cueva in sod. (2021) kot enostavno za uporabo, hitro, zanesljivo in neinvazivno metodo za odkrivanje OPM opisujejo uporabo slikovne analize klavnih trupov. Slikovna analiza v predelovalni industriji sicer ni novost, vendar so z omenjeno študijo želeli avtorji predstaviti metodo, ki bi ponudila potrditev OPM že na začetku procesa predelave in tako omogočila identifikacijo in razvrščanje klavnih trupov glede na normalno kakovost in OPM. Ugotovili so, da lahko uporaba najbolj ustreznega validiranega modela pri slikovni analizi doseže 84-% natančnost pri zaznavanju OPM (normalna mišičnina, zmerna in težja oblika OPM), medtem ko so Geronimo in sod. (2019) poročali celo o 91,8-% natančnosti pri identifikaciji OPM (normalna mišičnina in OPM) z uporabo kombinacije bližnje infrardeče spektroskopije in slikovne analize. Omenjena analiza bi tako pripomogla k lažjemu predvidevanju oziroma klasifikaciji prsne mišičnine glede na kakovost. Prav tako Kong in sod. (2021) predlagajo uporabo kompresijskega testa kot kvantitativnega indikatorja, s katerim bi lahko bolj zanesljivo potrdili prisotnost OPM (in BPM) ter prsne fileje razvrstili v različne razrede glede na obliko OPM kot je definirana s palpacijo.

Možna alternativa uporabe OPM piščančjih prsi v humani prehrani je tako nadaljnja predelava in uporaba le-teh v predelanih živilih. Med predelavo je možno spremeniti kemijsko sestavo mesa, kar vpliva na spremenjene lastnosti končnega živila. S tem bi lahko zmanjšali neželene učinke OPM in izboljšali kakovost končnih proizvodov, kar bi bilo ugodno tako za perutninsko oziroma predelovalno industrijo kot tudi za potrošnika (Caldas-Cueva in Owens, 2020).

Za zmanjšanje pojavnosti OPM v jati se lahko rejci že med vsakodnevnimi rutinskimi opravili in pregledom jate osredotočijo na težave živali s hojo, palpacijo prsne mišice in s spremljanjem dvigovanja peruti piščancev z namenom ocenjevanja gibljivosti peruti (Baldi in sod., 2021). Prav tako je možno pojavnost OPM omiliti z dodatkom antioksidantov v krmo piščancev. V študiji selekcijskega centra Aviagen (2019) so namreč ugotovili, da je bila pojavnost OPM za 28 % nižja ob dodatku 200 ppm vitamina C in 180 IU vitamina E/kg krmne mešanice v primerjavi s skupino, ki ni dobivala antioksidantov. Vseeno pa je na področju *in vivo* zaznavanja pojavnosti OPM še veliko neznank in bodo v prihodnje potrebne nadaljnje študije za natančneje razumevanje poteka pojavnosti OPM in možne preventivne ukrepe.

2.5 ŠPAGETASTO MESO (ŠM)

Miopatijo, ki povzroči, da je meso na videz podobno špagetom (ang. spaghetti meat, stringy-spongy, mushy breast), so prvič opisali leta 2015 in jo poimenovali „kašaste prsi", kjer so avtorji kot vzrok za pojav miopatije navedli izgubo skladnosti mišic v veliki prsni mišici pri hitrorastočih genotipih pitovnih piščancev (Baldi in sod., 2021). Kasneje so stanje poimenovali kot špagetasto meso (ŠM) ali „špagetaste prsi", saj se stanje fenotipsko odrazi v slabi veznosti med mišičnimi vlakni oziroma odstopanju mišičnih snopičev, kar se kaže v dolgem, tankem, trdnem in valjastem videzu vlaken, ki na videz spominja na špagete (Petracci in sod., 2019; Baldi in sod., 2021). Pri ŠM pride v osnovi do poškodb vezivnega tkiva znotraj perimizijskih frakcij, pri čemer nastanejo velike znotrajcelične praznine, kar privede do ločevanja snopov mišičnih vlaken, ki sestavljajo mišično tkivo (Baldi in sod., 2018). Posledice so vidne v mehki konsistenci mišičnine, običajno v ventro-kranialnem delu prsnega fileja, vendar je možno, da se pojavi tudi v kavdalnem delu in občasno celo v stegenskih mišicah (Tasoniero in sod., 2020; Baldi in sod., 2021). Prav tako so s ŠM povezane histološke spremembe podobne kot pri BPM in OPM in se kažejo v obsežni mišični degeneraciji skupaj z regeneracijo, spremenjeni strukturi mišičnega tkiva, nekrozi in razpadu mišičnih vlaken, infiltraciji maščobnega tkiva in vnetnih celic ter edemu (Tasoniero in sod., 2020; Baldi in sod., 2021). Po drugi strani pa je posebnost pri ŠM, ki je ni mogoče zaznati pri drugih miopatijah, progresivno redčenje endomizija in perimizija, ki vodi v slabšo vezavo med mišičnimi vlakni. Po nekaterih raziskavah bi lahko bila slaba skladnost oziroma celovitost mišice posledica akumulacije nezrelega, na novo naloženega kolagena, katerega primarne funkcije še niso povsem razvite (Tasoniero in sod., 2020).

Vzroki za nastanek ŠM so tako kot pri BPM in OPM prsne mišice neznani, vendar se predvideva, da imajo vse miopatije skupno etiologijo. Glede na aktualno literaturo se miopatiji BPM in OPM pojavljata predvsem pri moških živalih, kar bi lahko sklepali tudi za ŠM, vendar pa so Pascual in sod. (2020) v študiji, kjer so preučevali vpliv dodatka natrijevega butirata na prirejo in kakovost mesa, ugotovili, da je bila značilno večja pojavnost ŠM pri ženskih v primerjavi z moškimi živalmi. Kljub temu pa avtorji vzroka za tovrsten pojav niso mogli določiti, zato so na to temo potrebne dodatne raziskave. Tudi Che in sod. (2022) so preiskovali pogostost pojavljanja ŠM v jatah pitovnih piščancev v Kanadi in ugotovili, da se je ŠM pojavilo pri tretjini preučevanih prsnih filejev ter da se pojavnost ŠM povečuje z naraščajočo telesno maso in je pogostejša med poletnimi meseci v primerjavi z zimskimi meseci. Prav tako naj bi imeli na pojavnost ŠM velik vpliv izvedeni postopki pri zakolu, kot so npr. odstranjevanje perja, čas in način izkoščevanja trupov ter ohlajanje trupov, pri čemer je bila na liniji klanja pojavnost ŠM večja za 50 % pri klavnih trupih, ki so bili počasi ohlajeni, v primerjavi s tistimi, ki so bili hitro ohlajeni (Baldi in sod., 2021).

ŠM negativno vpliva tudi na parametre kakovosti mesa in prehransko vrednost mesa. Baldi in sod. (2018) ter Tasoniero in sod. (2020) poročajo, da imajo piščančji fileji s ŠM v primerjavi z normalnimi fileji na površini manjšo vsebnost beljakovin, skupnega in topnega kolagena ter večjo vsebnost vode in maščob. Miopatija vpliva tudi na maščobnokislinsko sestavo prsnih filejev, ki imajo v primerjavi z normalnimi fileji manjšo vsebnost eikozapentaenojske (EPA) in dokozaheksaenojske (DHA) kisline (Baldi in sod., 2021). Poleg omenjenega imajo piščančji fileji s ŠM slabše funkcionalne lastnosti, kot so višji pH, meso je bolj rumene barve, poveča se izceja, poslabša se sposobnost za vezanje vode ter mehkoba po kuhanju (Petracci in sod., 2019; Baldi in sod., 2021). Pri ŠM do zmanjšane sposobnosti za vezanje vode verjetno pride zaradi zmanjšane topnosti beljakovin, ki je posledica neprestanih procesov razgradnje beljakovin, kar dokazuje višji indeks miofiblilarne fragmentacije ŠM ter večja koncentracija prostih aminokislin (Baldi in sod., 2021). Za določanje resnosti stanja ŠM na liniji klanja so Sirri in sod. (2016) predlagali klasifikacijo s tritočkovno lestvico. Predlagajo, da se prsni file, ki ima normalno konsistenco, brez vidnih lezij mišičnega tkiva, oceni z nič točkami. Prsni file z zmerno obliko ŠM, kjer se ne kažejo površinske laceracije, ampak so strukture mehke in ohlapne, in jih je možno zaznati s stiskanjem zgornjega oziroma kranialnega površinskega dela mišice, se oceni z eno točko. File s težjo obliko ŠM pa ima obsežne površinske laceracije, tako na kranialni kot kavdalni površini velike prsne mišice, z vidno ločbo snopov mišičnih vlaken, ki spominjajo na videz špagetov, in se oceni z dvema točkama (Sirri in sod., 2016; Baldi in sod., 2021). Za predelovalno industrijo je pojav ŠM izredno neugoden, saj ŠM zaznamo šele pri sekciji klavnih trupov na prsne fileje. Ker ŠM negativno vpliva na senzorične lastnosti mesa, se prsnih filejev s ŠM na trgu ne prodaja kot sveže, zato se količina zavrženega mesa poveča (Tasoniero in sod., 2020). Za prepoznavanje in razvrščanje ŠM že na liniji klanja so v predhodnih študijah predlagali uporabo bioelektrične impedančne analize ali meritev barve mesa (Baldi in sod., 2021).

Omeniti je potrebno, da ŠM v primerjavi z OPM ne moremo določiti pri živih živalih, prav tako pa glede na aktualne raziskave ni poznane učinkovite strategije z vidika prehrane in oskrbe živali, ki bi jih lahko uporabili kot preventivo za zmanjšanje pojavnosti ŠM. Trenutno se kot najbolj praktična rešitev za zmanjšanje ŠM uporablja fizično ločevanje površinskih in globinskih delov prsnih filejev na liniji klanja. Ker ŠM prizadene predvsem površino mišice, se površinski deli uporabijo za nadaljnjo predelavo, globinski pa se prodajo naprej kot sveži fileji (Baldi in sod., 2021). Fileje s slabšo kakovostjo bi lahko tako vključili v pripravo mletih mesnih izdelkov, kjer bi lahko z dodatki različnih sestavin (npr. škroba, fosfatov, hidrokolidov …) prekrili slabše tehnološke lastnosti takšnega mesa (Petracci in sod., 2019). Prav tako je možno ŠM zamrzniti, saj se s skladiščenjem kakovost mesa dodatno ne poslabšuje in se lahko kasneje uporabi v predelavi (Baldi in sod., 2021).

2.5.1 Razbrazdanost male prsne mišice (RMPM)

Poleg omenjenih miopatij v zadnjem času na liniji klanja ugotavljajo novo napako mišičnine, ki prizadene malo prsno mišico in so jo prvič opisali Soglia in sod. (2019). Napaka, opisana kot razbrazdanost (ang. gaping defect) male prsne mišice (RMPM), je podobna že opisani napaki pri ribjih filejih, kjer pride do raztrganin intramuskularnega vezivnega tkiva med potekom mrtvaške otrplosti, kar se kaže v ločitvi mišičnih snopičev na površini fileja, ki so opazne kot manjše ločitve v obliki razpok oziroma brazd na površini mišičnine ali popolne, globoke ločitve vse do kože. Prav tako pri RMPM pride do vidnega ločevanja mišičnih snopičev.

Na pojavnost RPMP lahko vpliva sistem hlajenja trupov, čas, pretečen od zakola do izkoščevanja in tudi drugi vzroki, kar predstavlja težavo pri primerjanju pojavnosti RPMP med različnimi predelovalnimi obrati (Soglia in sod., 2019). Poleg omenjenega tudi selekcija pitovnih piščancev na hitro povečevanje prsne mišičnine (predvsem velike prsne mišice) vpliva na to, da gredo piščanci v zakol čedalje mlajši in pri tem akumuliran kolagen še ne gre čez posttranslacijske modifikacije, ki so potrebne za njegovo zorenje. Tako lahko redčenje perimizijskega vezivnega tkiva in nezrelost vezivnega tkiva pri hitrorastočih pitovnih piščancih povzroči, da je mala prsna mišica bolj dovzetna za nastanek brazd pri postopkih po zakolu (Soglia in sod., 2019).

Zaradi slabših senzoričnih lastnosti, ki jih povzroči razbrazdanost, je takšno meso slabše kakovosti, kar povzroča ekonomske izgube. RPMP je na videz analogna ŠM, miopatiji značilni za veliko prsno mišico, vendar so Soglia in sod. (2019) ugotovili, da so imele razbrazdane prsne mišice bistveno nižji pH, nespremenjeno vsebnost makrohranil in so bile bolj svetle (višje L* vrednosti), medtem ko so velike prsne mišice s ŠP imele višjo pH vrednost, spremenjeno kemično sestavo (večja vsebnost vlage ter manjši delež surovih beljakovin in surovega pepela) in bile bolj rumene (višje b* vrednosti). Glede na drobljenje rezultate avtorji predvidevajo, da razbrazdanost ne vpliva na histološke spremembe v takšni meri, kot je to značilno za ŠM, ampak da je RPMP verjetno posledica biokemičnih procesov, ki potečejo med posmrtno pretvorbo mišice v meso. RPMP tako poslabšuje kakovost mesa, saj imajo takšni fileji slabšo sposobnost za vezanje vode, kar skupaj z nizkim pH in bledo barvo filejev spominja na BMV meso (Soglia in sod., 2019).

Za lažjo oceno resnosti stanja RPMP so Soglia in sod. (2019) predlagali klasifikacijo, kjer pri normalnih prsnih filejih ni zaznati nobenih ločb mišičnih snopičev, pri zmerni obliki lahko določimo do dve mesti ločbe mišičnih snopičev na površini mišice (zgornji ali sredinski del mišice), krajše od 1,5 cm, pri težji obliki pa lahko določimo več kot dve mesti ločbe mišičnih snopičev na površini mišice (tako zgornji kot sredinski del), daljše od 1,5 cm. V raziskavi so ugotovili, da so bile razlike med zmerno in težjo obliko RPMP majhne, kar bi lahko kazalo tudi, da RPMP ni posledica drugačne predispozicije mišičnine, temveč različne sile, uporabljene pri izkoščevanju trupov.

Kljub ugotovitvam, da lahko na pojavnost in obliko RPMP vplivajo tako postopki pred zakolom kot po njem, so potrebne nadaljnje raziskave na področju etiologije RPMP in njenega vpliva na kakovost mesa ter potrebnih preventivnih in/ali kurativnih ukrepov, s katerimi bi zmanjšali pojavnost RPMP.

3 SKLEPI

Vzrokov za nastanek neželenih sprememb oz. miopatij prsne mišičnine pri pitovnih piščancih je več in so povezane predvsem z intenzivno rejo in selekcijo na povečano prsno mišico. Neželene spremembe prsne mišičnine poslabšujejo kakovost in prehransko vrednost mesa ter perutninskemu sektorju povzročajo ekonomske izgube. Glede na to, da do danes še ni razvite učinkovite strategije za zmanjšanje omenjenih miopatij, se zdi trenutno najbolj praktična rešitev ločevanje površinskih in globinskih delov prsnih mišic na liniji klanja, kjer bi površinske dele filejev uporabili v nadaljnjem postopku predelave, globinske dele pa bi lahko sveže distribuirali v prehransko verigo (Baldi in sod., 2021). Prav tako je možno, da meso, ki je bilo predhodno razvrščeno v kategorijo z neželenimi spremembami, vključimo v predelana živila, kjer lahko dodatek funkcionalnih sestavin (npr. škroba, fosfatov, hidrokoloidov itd.) izboljša slabše tehnološke lastnosti takšnega mesa (Petracci in sod., 2019), ki pa sicer nima škodljivih vplivov na zdravje potrošnikov. V prihodnje je potrebno poiskati rešitve za zmanjšanje pojavnosti neželenih sprememb v prsni mišičnini pitovnih piščancev. To bi lahko dosegli s prilagojeno selekcijo in spremembami na področju tehnologije reje piščancev, vezane predvsem na skrb za dobrobit in zmanjšanje izpostavljenosti živali stresnim dejavnikom. Po drugi strani pa je potrebno zaradi čedalje večjega povpraševanja po perutninskem mesu in posledično težnje industrije po večji prireji in zaslužku, najti načine in možnosti uporabe takšnega mesa v živilsko predelovalni industriji.

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doi:10.14720/aas.2023.119.1.2900 Review article / pregledni znanstveni članek

Increased occurrence of five Noctuidae species in Slovenia in the period 2019-2022: presentation of the species and preliminary results of their occurrence and damage

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Received November 02, 2022; accepted December 13, 2022.
Delo je prispelo 2. novembra 2022, sprejeto 13. decembra 2022

Increased occurrence of five Noctuidae species in Slovenia in the period 2019-2022: presentation of the species and preliminary results of their occurrence and damage

Abstract: In this paper, we present five species of owlet moths (Noctuidae), whose greater abundance was found in Slovenia in the period 2019-2022 as part of research and expert work in the field of plant protection. The greatest economic importance is attributed to the tomato looper (*Chrysodeixis chalcites*), which two years after its first strong appearance on the Slovenian coastal area is already causing major problems for tomato growers. We did not confirm the harmfulness of the caterpillars on cultivated plants for the silver-Y moth (*Autographa gamma*), the adult males of which are caught in large numbers using pheromone traps in central Slovenia, and the less numerous beet armyworm (*Spodoptera exigua*). We can confirm the same for the shark moth (*Cucullia umbratica*), whose adult males are caught in pheromone traps of the EU quarantine pest *Spodoptera frugiperda* and the copper underwing (*Amphipyra pyramidea*), for which we suggest the Slovenian name 'bakreni podkrilec', which appeared in, on or in the immediate vicinity of the pheromone traps of the spongy moth (*Lymantria dispar*) in the oak-hornbeam forest in Prekmurje.

Key words: Noctuidae, *Chrysodeixis chalcites*, *Autographa gamma*, *Spodoptera exigua*, *Cucullia umbratica*, *Amphipyra pyramidea*, Slovenia

Številčnejši pojav petih vrst sovk (Noctuidae) v Sloveniji v obdobju 2019-2022: predstavitev vrst in preliminarni rezultati njihovega pojavljanja in škodljivosti

Izvleček: V prispevku predstavljamo pet vrst sovk (Noctuidae), katerih večjo številčnost smo v Sloveniji ugotovili v obdobju 2019-2022 v okviru raziskovalnega in strokovnega dela na področju varstva rastlin. Največji gospodarski pomen pripisujemo paradižnikovi sovki (*Chrysodeixis chalcites*), ki dve leti po prvem močnejšem pojavu v Slovenski Istri že povzroča večje težave pridelovalcem paradižnika. Za glagolko (*Autographa gamma*), katere samci se v feromonske vabe v osrednji Sloveniji lovijo v velikih številih in manj številčno pesno sovko (*Spodoptera exigua*), nismo potrdili škodljivosti gosenic na gojenih rastlinah. Podobno lahko potrdimo tudi za škrbinkinega meniha (*Cucullia umbratica*), katerega odrasli samci se lovijo v feromonske vabe karantenske sovke *Spodoptera frugiperda* in sovko *Amphipyra pyramidea*, za katero predlagamo slovensko ime ‚bakreni podkrilec', ki se je pojavljal v, na ali v neposredni bližini feromonskih pasti gobarja (*Lymantria dispar*) v hrastovo-gabrovem gozdu v Prekmurju.

Ključne besede: Noctuidae; *Chrysodeixis chalcites; Autographa gamma*, *Spodoptera exigua*, *Cucullia umbratica*, *Amphipyra pyramidea*, Slovenija

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1 INTRODUCTION

Noctuidae is the second largest family in the superfamily Noctuoidea, with about 1,089 genera and 11,772 species (Zhang, 2011; Simonović et al., 2020). In Slovenia only some Noctuidae species are occasionally important pests of cultivated plants, for example three species from the genus *Agrotis*, which are known as soil pests, and *Mamestra brassicae* (L., 1758), *Lacanobia oleracea* (L., 1758) and *Helicoverpa armigera* (Hübner, [1808]), which caterpillars feed with the aboveground parts of the plants (Devetak et al., 2010). More than 300 Noctuidae species occur in Slovenia (Database of invertebrate pictures, 2022), which means that about 2 % of them are pests of cultivated plants.

In our research and expert work over the past three years, we have become aware of the more massive occurrence of five Noctuidae species, which could become economically important in the future due to climate change, the extension of agricultural production as well as the fact that most noctuids are polyphagous and at a certain moment they can find certain plant species as more suitable host and attack it more strongly than other host species.

In the paper we present *Spodoptera exigua* (Hübner, [1808]), *Chrysodeixis chalcites* (Esper, 1789), *Autographa gamma* (L., 1758), *Amphipyra pyramidea* (L., 1758) and *Cuculia umbratica* (L., 1758), whose massive occurrence has been recorded in the Slovenian agricultural or forest environment since 2020, but have never been described in details until now.

2 DESCRIPTION OF FIVE SPECIES OF Noctuidae

2.1 BEET ARMYWORM / SMALL MOTTLED WILLOW MOTH (*Spodoptera exigua* [Hübner])

Spodoptera exigua (Hübner) is a tropical insect native to Southeast Asia (Capinera, 2008), and presently found in all continents, except on Antarctica (Falsafi et al., 2022). It was named (sugar) beet armyworm, because it colonized sugar beet plantations in America around the late 1800s and early 1900s (Wilson, 1932). Nowadays this species is present in most countries in Asia, Africa, Europe, North America, and Oceania (CABI, 2022a). Particularly in Europe, *S. exigua* has been reported to feed in some important crops from the family Solanaceae and Cucurbitaceae, and also in some other plants such as asparagus (*Asparagus officinalis* L.) and cotton (*Gossypium* spp.) (EPPO, 2022). Larvae of this insect feed on young leaves and fruits and might skeletonize foliage. They like to form a colony while feeding and as they mature, they become more solitary and cause large, irregular holes in foliage (Capinera, 2008).

The adult of *S. exigua* (Figure 1.1) is a moth of moderate size, with a wingspan measuring 25–30 mm. The forewings are colored grayish brown while the hind wings are usually gray or white color with a black line at the margins. The eggs are deposited in a clump of 50–250 eggs, colored white or greenish white, and covered with whitish scales that has a cotton-like appearance. The individual egg looks circular from above, but the top looks a little pointy from the side. Normally, there are five stages of instar. The larvae are pale green to yellow during the first two stages, and they obtain strips on the dorsal as they mature. In the last (fifth) stage larvae have variety in their appearance, with the most common ones being green dorsally with pink or yellow color ventrally and white stripe laterally. Pupation occurs in the soil at

Figure 1.1: Adult of *Spodoptera exigua* (photo: Dejan V. Stojanović)

Figure 1.2: First male adults of *Spodoptera exigua* caught in the pheromone traps in Ljubljana in 2022 (photo: Stanislav Trdan)

around 1 cm from the soil surface. The pupa color is light brown and 15–20 mm in length (Capinera, 2008).

Life cycle of *S. exigua* can be completed in 24 days, and six completed cycles can be achieved in a warm location such as Florida. The eggs hatch in 2–3 days in warm summer and took longer when it is cold, with the development threshold around 12.4 °C. The duration for each instar stage varies between 1 to 3 days, depending on the temperature, with the threshold around 13.6 °C. On summer days the duration of the pupal stage is 5–7 days (Capinera, 2008). Maharjan et al. (2022) reported that the total time for each stage of development decreased between the temperature of 15–35 °C, and that the eggs failed to hatch in the temperature above that range. As for the adaptation to the colder temperature, Zheng et al. (2011) concluded that *S. exigua* can either migrate to the warmer place or overwinter the season with hibernation.

So far, not enough data are available for its bionomics in Slovenia, therefore in 2022 we have started the monitoring of this insect at the Laboratory Field of Biotechnical Faculty in Ljubljana. At the second half of April, we have placed three pheromone traps (producer: CSalomon, Budapest, Hungary). The first adults (Figure 1.2) were captured in the traps in the middle of June, and in the beginning of October last adults were found in the traps. During this period, 40 males were caught in the traps, of which the maximum was in the first two weeks of July (0.4 males/trap/day). Since 2015 *Spodoptera exigua* is being mentioned also in Technological guidelines for integrated vegetable production (2015). Its occurrence in Slovenia has been also reported at collection at Slovenian Museum of Natural History (Database of invertebrate pictures, 2022). In 2015 Jež et al. reported the occurrence of *S. exigua* in Goriška Brda and Pohorje, while in 2018, Gomboc and Zakšek (2018) reported the occurrence of *Spodoptera exigua* in Ljubljana. In Slovenia, we have not yet recorded information about the greater harmfulness of this noctuid in agricultural production, however, we must pay attention to this species in the future, as increasingly pronounced climate changes and the reduction in the number of insecticides for its suppression could be the causes of its greater economic importance.

2.2 TOMATO LOOPER / GOLDEN TWIN-SPOT MOTH (*Chrysodeixis chalcites* [Esper])

Chrysodeixis chalcites (Esper) is a native species in the area between 45 °N and 35 °S, which is including southern Europe, the Mediterranean, the Middle East, and southern Africa, and now its distribution has been reaching some countries in Oceania, North America, and South America. This species is present in northern Europe, but winter mortality prevents its establishment outside this area. However, it has managed to extend its distribution area in southern Europe by establishing greenhouse areas. Nowadays, with global trading, the risk of its distribution is also increasing with the international transport of its host plants (CABI, 2022b). Larvae mostly fed on leaves and sometimes also on flowers, and the main host are including vegetables like tomatoes (*Solanum lycopersicum* L.), cultivated plants of *Brassica oleracea* L., pepper (*Capsicum annuum* L.), and sweet potatoes (*Ipomoea batatas* [L.] Lam.), and ornamental plants like pelargonium, amaryllis, and hosta (Simonović et al.,2020). Early larvae feed on the bottom surface of leaves and leave upper leaf cuticles as a whole, while later larvae eat all parts of leaves (Munippan, 2012).

The adult moth (Figure 2.1) has a moderate size, with a wingspan measuring 32-37 mm and a body length of around 20 mm. The forewings are golden brown, with two oval silver freckles, approximately the same size, in the central part. The hindwings are brownish-gray, darker toward the outer edge, with distinctive dark gray veins. The male has two tufts of black hair at the end of the abdomen, while in the female moth this characteristic is missing (Simonović et al., 2020). The larvae have twelve legs, and their color is pale green and darker on the back, with some white lines and dots along the body. The pupa is enclosed between two dead leaves on or near the ground and surrounded by a cocoon of white silk (Hudson, 2022).

Eggs are laid singly onsite of leaves and hatch in 3–4 days, and each female can lay up to 200 eggs. There

Figure 2.1: Adult of *Chrysodeixis chalcites* (photo: Dejan V. Stojanović)

are five larval instars with around 13 days total larval period. The adult moth takes 7 days to emerge from the cocoon (Muniappan et al., 2012). In nature with favourable conditions, nine generations per year were recorded (Harakly and Farag, 1975). The temperature thresholds for the development of eggs, larvae, and pupae are 4.8, 2.7, and 4.6 °C, respectively. The length of one life cycle, starting from the incubation period of eggs until the end of pupation, is recorded at 60.4 days at 20 °C, and 37.4 days at 30 °C (Abd Allah, 2013).

We first recorded adults of *C. chalcites* in 2020 within surveillance of *Spodoptera frugiperda*, an EU A1 quarantine pest. Greater injuries caused by feeding of caterpillars and caterpillars itself were detected in 2021 on tomato leaves in greenhouses in the Slovenian Coast (wider area of Koper, Western Slovenia, Figure 2.2) in late summer. In Slovenia, the species was recorded in 2001 for the first time (Lesar, 2001), but then for nearly twenty years there were no reports of its harmfulness. In the past two years, the pest has been reported to occur in tomato greenhouses almost all over the country. Therefore we have started with its monitoring in 2022 at two different areas, Western Slovenia (Dekani) and Central Slovenia (Laboratory Field of Biotechnical Faculty, Ljubljana). 3 pheromone traps (producer: Russel IPM Ltd, Flintshire, United Kingdom) were placed at each of selected areas. In Dekani the first males were recorded in the traps in the end of May, and the peak was established in August with almost 10 males/trap/day. In Ljubljana, the first males were caught in the traps in July 20 and at the time this article was submitted for publication (end of October 2022), an average of two males were caught in the traps per day. This data represents one of the two peaks of occurrence of the pest in Ljubljana, where the first peak with the same number of males (2/trap/day) was reached in mid-August.

Figure 2.2: Strong attack of *Chrysodeixis chalcoides* caterpillars in the tomato leaves in Dekani (Slovenian Coast) in September 02 2021 (photo: Stanislav Trdan)

2.3 SILVER-Y MOTH (*Autographa gamma* [L.])

Autographa gamma (L.) is an important pest widespread throughout Europe, Asia, northern Africa, and some countries in north and south America. It has around 300 host plants known with some of them being important crops. It mainly spreads through population migration and international transport of plant materials (CABI, 2022c). Its main hosts are sugar beets, lettuces, cabbages, tomatoes, potatoes, beans, and peas. The larvae feed on leaves and can skeletonize the leaves, and as they mature, the older caterpillars can eat the whole leaves. When there is a population explosion of *A. gamma,* the whole crops can be easily destroyed (Hill, 1987).

Female moths lay eggs at the bottom of the leaf surface of low-growing plants, either singularly or in a lump of a few eggs. The eggs are round, ribbed, and white. The larvae are 'semi looper', with two pairs of abdominal legs. The larvae are usually colored green with a darker color in the back, dorsal white line, and yellow lateral line. On maturity, larvae reach the size of 20–30 mm, then started pupation. The pupas are black and wrapped in a silk-like cocoon. The adult moths (Figure 3.1) are grey with some darker patterns and distinctive silvery gamma (**γ)** shaped in the middle of both forewings. The wingspan measures 35–48 mm (Hill, 1987).

Depending on the location, this moth can make up to four generations in one year. The moth is known as a good migrant, and it favours warm and moist conditions. The optimum temperature for larvae is 23–30 °C, around 25 °C for pupae, and 20–25 °C for the moth, and the optimum humidity is 80–95 % (Chumakov and Kuznetova, 2022). One female moth can lay 500–1000 eggs in the

Figure 3.1: Adult of *Autographa gamma* (photo: Dejan V. Stojanović)

Figure 3.2: Numerous catch of *Autographa gamma* males in pheromone trap in Ljubljana in 2019 (photo: Stanislav Trdan)

Figure 4.1: Adult of *Amphipyra pyramidea* (photo: Dejan V. Stojanović)

whole season. After 10–12 days, the eggs hatch into larvae. Larvae develop for around 3–4 weeks before dropping into the soil and starting the pupation period, which lasts 10–14 days (Hill, 1987).

While the larvae are mostly feeding during the night, the adult moths are mostly active at dusk and feed on the nectar in the flowers of many wild-growing and cultivated plants. Under favourable conditions, breeding continues, but most adults reared in northern Europe migrate south to a warmer area (Alford, 2012).

In Ljubljana, this moth completed three generations in 2019 (April–October) and 2020 (April–November), with the peak of population happening when plants were in full growth. It was also proven that population dynamics are influenced by temperature, humidity, rainfall, and solar radiation (Gornik, 2021). This was the first study known for Slovenia, and it was based on pheromone dispensors and pheromone traps (VARL+) produced by CSALOMON® (Budapest, Hungary). Despite the large number of males (2019: a total of 418 or maximum daily catch/trap of 3.4 males in the middle of July; 2020: a total of 594 or max daily catch/trap of 3.3 males in the end of April) caught in the traps (Figure 3.2), we did not find any major injuries on its hosts in the immediate vicinity of the traps.

2.4 COPPER UNDERWING (*Amphipyra pyramidea* [L.])

Amphipyra pyramidea (L.) (Figure 4.1) is a common moth recorded from up to 50 species of deciduous trees and bushes and is very commonly attacking pedunculate oak *(Quercus robur* L.*)*. The species hibernate as an egg in winter, and then hatches in spring and is fed on foliage (Roslin and Salminen, 2009). Some of its alternative hosts are ash tree (*Fraxinus* spp.), privet (*Ligustrum* spp.), honeysuckle (*Lonicera* spp.), apple (*Malus* spp.), *Rhododendron* spp, and roses (GBIF, 2022a). According to CABI (2022d) this moth is present in some countries in Asia and Europe.

The fully grown larvae are plump with green or whitish green colored with white or pale yellow dots. The body consisted of eight abdominal parts that measure up to 45 mm long. It has three incomplete lines, one along the back and one along each left and right side. Adults are longer than larvae (45–55 mm) and have brownish forewings with black or pale yellow marks, while the hind wings are coppery red (Alford, 2012).

It is a univoltine species, meaning that it produces only one brood in the season. Eggs started to hatch in June and can be as late as October (GBIF, 2022a). The adult moths are attracted to sugar and light, and from August until October they are rearing around and inhabit woodland, parkland, and hedgerow (Skinner and Wilson, 2009).

Amphipyra pyramidea has been detected in Slovenia in 1993 for the first time (Titovšek, 1993), and so far, no major damage caused by the larvae has been recorded in the country. The copper underwing is considered as an interesting nocturnal butterfly, as mentioned by Jež et al. (2015). In 2022, we have detected massive occurrence of *A. pyramidea* in the pheromone traps for monitoring the males of spongy moth (*Lymantria dispar* L.) within the LIFE eGYMER research project (funded by EU in the period 2021-2024). We have placed different types of pheromone traps containing pheromone dispensor, produced by CSalomon (Budapest, Hungary), into the forest Ginjevec near Slovenian border with Hungary. The dominant tree species in the forest Ginjevec are oak (*Quercus* spp.) and European hornbeam (*Carpinus betulus* L.). The first adult males of copper underwing in or on (Figure 4.2) the traps or their immediate vicinity was recorded in last 10-day period of June, and they also appeared during the

Figure 4.2: Adult male of *Amphipyra pyramidea* on the pheromone trap for the monitoring of *Lymantria dispar* in the forest Ginjevec (NE Slovenia), August 17 2022 (photo: Stanislav Trdan)

last inspection of the traps (September 01 2022). Males were caught on pheromone traps in the highest numbers from the beginning of July to the beginning of August, when we found more than 1 male/trap/day in five consecutive 7-day intervals. We did not notice injuries from caterpillars on the deciduous trees caused by the caterpillars of *A. pyramidea*, but it is true that our attention was mainly focused on monitoring of the spongy moth.

2.5 SHARK MOTH (*Cuculia umbratica* [L.])

Cuculia umbratica (L.) is present in some countries in Asia, Europe, and North America (CABI, 2022e). The caterpillars, which emerge from the eggs laid on the leaves of lettuce *(Lactuca* spp.) and sowthistle (*Sonchus* spp.), devour the leaves of these species and can cause great injury to the plants (Newman, 1869).

The moth (Figure 5.1) is fairly big, with a wingspan measuring 52–59 mm (Skinner and Wilson, 2013). The forewings color is smokey-gray with a slender but very distinctive black line from the middle of the base to the middle of the wing. The wing rays are also black but the fringe is lighter. The hindwing's color is also smokey-gray, with the wing rays darker and the base paler. The head is smokey black while the thorax and body are smokey-gray (Newman, 1869).

The moth is known as single brooded and not migrating. It overwinters as a pupa and starts emerging every year around June to July, inhabiting waste ground, sandhills, shingle beaches, marshy places, and gardens. Larvae cause a problem around late July to September. They feed at night and hide during the day under the leaves of the bottom plant canopy. Comes regularly to light and is attracted to various flowers, for example

Figure 5.1: Adult of *Cucullia umbratica* (photo: Dejan V. Stojanović)

honeysuckle (*Lonicera* spp.), sweet-william (*Dianthus barbatus* L.), valerians (*Valeriana officinalis* L.), and thistles (group of flowering plants characterised by leaves with sharp prickles on the margins, mostly in the family Asteraceae) (Skinner and Wilson, 2013). Until now, it is not presented as important agricultural insect pest.

The results of the occurrence of *Cucullia umbratica* in Slovenia in the period 2011-2013 are given by Jež et al. (2015). They found that the butterfly occurs in different areas of Slovenia, from Goriška Brda to Slovenske Gorice and Pohorje. In Slovenian coastal region, massive occurrence of *Cuculia umbratica* was confirmed in 2020 for the first time. Massive occurrence was recorded with pheromone traps (Figure 5.2) for surveillance of *Spodoptera frugiperda*. Pheromone dispensors and pheromone traps (funnel trap [green lid/green funnel/transparent bucket]) were produced by Pherobank (Belgium). Within survey of *S. frugiperda* in 2022, in Slovenian Coastal area and central Slovenia the first males of *C. umbratica* was re-

Figure 5.2: Four adult males of *Cucullia umbratica* in the pheromone trap for the monitoring of *Spodoptera frugiperda*, Sečovlje (Slovenian coast), July 17 2020 (photo: Stanislav Trdan)

corded in pheromone traps since the first half of May. By the end of August, a total of 150 males were caught in three baits in Ljubljana, with two peaks (the last week of May and the beginning of August), when on average almost 1 male/bait/day was caught in the baits. Despite the large number of males caught in pheromone traps, we did not find any damage due to the feeding of caterpillars on the cultivated plants.

3 CONCLUSIONS

In this article, we present five species of owlet moths that we have been paying attention to since 2020, when in our research and expert work we found some cases of increased occurrence of butterflies or injuries due to feeding caterpillars. In connection with the latter statement and as potentially the most important pest among the five species, we highlight the tomato looper (*Chrysodeixis chalcites*), which occur in Slovenia for at least 20 years, but we found a greater extent of damage on tomato leaves, which the caterpillars feed on, in greenhouses in the Slovenian coastal area in 2021. In 2022, the occurrence of this pest on tomatoes in greenhouses was also reported from other areas of Slovenia, even from Prekmurje (NE Slovenia). Currently, the search for suitable solutions to limit the spread and harmfulness of this greenhouse pest is among the priorities of phytomedical experts and tomato growers. In some European countries, the pest is already effectively controlled with egg parasitoids from the genus *Trichogramma* (Polaszek et al., 2012) and *C. chalcites* nucleopolyhedrovirus (Bernal et al., 2018) and these or other biological control agents should be considered for implementation in our country for reducing the economic importance of this pest.

In the period 2019-2020, silver-Y moth (*Autographa gamma*) was caught on a very large scale using pheromone traps in central Slovenia, but interestingly, we did not notice any major damage to the cultivated plants, which these extremely polyphagous caterpillars normally feed on. We did not find any records on the bionomics and harmfulness of beet armyworm (*Spodoptera exigua*) in Slovenia, so in 2022 we decided to study its seasonal dynamics in central Slovenia. Males were caught in traps from mid-June 2022 onwards, but in relatively small numbers, and even for this species we have not yet confirmed the harmfulness of caterpillars on cultivated plants.

Since 2020, we have identified a significant number of captured adult males of the shark moth (*Cucullia umbratica*) in pheromone traps for fall armyworm (*Spodoptera frugiperda*) on the Slovenian coast, but we have not found damage caused by caterpillars on cultivated plants, for example on lettuce. The reason for this may be the fact that we have set up pheromone traps right next to greenhouses with monoculture tomato production, near which there is otherwise a fairly widespread sowthistle (*Sonchus* spp.), the leaves of which the caterpillars like to feed on. We were surprised by the continuous appearance of copper underwing (*Amphipyra pyramidea*) in the oak-hornbeam forest in Prekmurje, namely in, on or in the immediate vicinity of pheromone traps for monitoring spongy moth (*Lymantria dispar*). They are species from different families, as the spongy moth belongs to the Erebidae family. And perhaps the results of our research indicate a greater relationship between the species *Amphipyra pyramidea* and *Lymantria dispar* than their current taxonomic classification otherwise indicates.

4 ACKNOWLEDGEMENT

This work was carried out within Horticulture (P4-0013-0481), a program funded by the Slovenian Research Agency, and within Expert Tasks from the Field of Plant Protection, a program funded by the Ministry of Agriculture, Forestry, and Food - Administration for Food Safety, Veterinary Sector and Plant Protection.

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doi:10.14720/aas.2023.119.1.2953 Review article / pregledni znanstveni članek

Vplivi salicilne kisline in njenih derivatov na rastline, škodljive in koristne organizme in njihove interakcije v okolju

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Received December 07, 2022; accepted January 09, 2023.
Delo je prispelo 7. decembra 2022, sprejeto 9. januarja 2023

Effects of salicylic acid and its derivatives on plants, harmful and beneficial organisms and their interactions in the environment

Abstract: Global food production is forced to search for new approaches to protect plants from harmful organisms and environmental factors. One of the alternatives could be the use of salicylic acid (SA) and its derivatives. Overall, the effects of SA at the primary ecosystem level are encouraging, contributing to improved productivity and quality of many plants and improving tolerance to many stressors. The secondary level of effects of SA in the environment represents the effects on harmful organisms due to direct action and also the indirect effects of SA that occur due to morphological and physiological changes when the plant adapts to stressors. In many cases, SA has the effect of reducing infections, and it also acts as a deterrent to some pests. After being attacked by a pest, plants release volatile compounds into the environment, mainly SA derivatives such as methylated SA (MeSA). This attracts the natural enemies of pests, which could be used to protect plants from pests, as MeSA has been found to act as an attractant in many species. Salicylates have a very wide spectrum of action, which trigger various effects in the environment, which intertwine with each other and consequently affect several levels in the exosystem. In this article, we divided the effects of salicylates according to different levels in the environment, which gave us a broader insight into the potential use of salicylates in agriculture.

Key words: salicylic acid; methyl salicylic acid; acetyl salicylic acid; biosinthesis; induced systemic resistance; plant protection

Vplivi salicilne kisline in njenih derivatov na rastline, škodljive in koristne organizme in njihove interakcije v okolju

Izvleček: Svetovna proizvodnja hrane je prisiljena v iskanje novih pristopov za zaščito rastlin pred škodljivimi organizmi in okoljskimi dejavniki. Ena izmed alternativ, bi lahko bila uporaba salicilne kisline (SA) in njenih derivatov. Na splošno so učinki SA na primarni ravni ekosistema, vzpodbudni, saj pripomorejo k izboljšani produktivnosti in kakovosti številnih rastlin ter izboljšujejo toleranco na številne stresorje. Sekundarna raven učinkov SA v okolju predstavlja učinke na škodljive organizme zaradi direktnega delovanja in tudi posrednih učinkov SA, ki nastanejo zaradi morfoloških in fizioloških sprememb, ko se rastlina prilagaja stresorjem. SA v veliko primerih vpliva na zmanjšanje okužb, na nekatere škodljivce pa deluje tudi odvračalno. Rastline po napadu škodljivega organizma sproščajo v okolje hlapne spojine, predvsem derivate SA kot je metilirana SA (MeSA). Ta privablja naravne sovražnike škodljivcev, kar bi se lahko uvedlo v varovanje rastlin pred škodljivimi organizmi, saj je bilo ugotovljeno pri številnih vrstah, da MeSA nanje deluje kot atraktant. Salicilati imajo zelo širok spekter delovanja, ki v okolju sprožajo različne vplive, ki se med seboj prepletajo in posledično vplivajo na več ravni v eksosistemu. V tem članku smo učinke salicilatov razdelili glede na različne nivoje v okolju, kar nam je dalo širši vpogled na potencialno uporabo salicilatov v kmetijstvu.

Ključne besede: salicilna kislina; metil salicilna kislina; acetil salicilna kislina; biosinteza; varstvo rastlin; sistemska inducirana odpornost

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1 UVOD

Salicilna kislina (SA), znana tudi kot *orto*-hidroksibenzojska kislina in 2-hidroksibenzojska kislina, je rastlinski hormon, ki spada v raznoliko skupino fenolnih spojin in ima pomembno vlogo pri obrambi rastlin na različne patogene organizme in rastlinojede (Raskin, 1992; Vlot in sod., 2009; Dempsey in Klessig, 2012; Klessig in sod., 2018). Poznamo jo tudi kot signalno molekulo, ki pripomore k sistemski inducirani odpornosti (systemic inducing resistence; SAR) na biotični in abiotični stres (Raskin, 1992).

Poleg omenjenih osnovnih lastnosti lahko uporaba SA in njenih derivatov vpliva tudi na izboljšanje nekaterih parametrov kakovosti sadja in zelenjave (Zhang in sod., 2013; Dieryckx in sod., 2015; Giménez in sod., 2017). Med drugim tretiranje sadnih vrst s SA poveča maso in trdoto plodov ter vsebnost vitamina C v hruškah (Tareen in sod., 2012), izboljša obarvanje in zakasnitev zorenja plodov jagod (Gačnik in sod., 2021a) in povzroča počasnejšo degradacijo karotenoidov v pomarančah (Huang in sod., 2008). S povečanjem aktivnosti nekaterih ključnih encimov v fenil-propanoidni poti, kot je fenilalanin amonijak-liaza (PAL) (Gačnik in sod., 2021b; Gačnik in sod., 2021c), vpliva SA tudi na povečanje nekaterih bioaktivnih komponent (Huang in sod., 2008; Giménez in sod., 2017; Blanch in sod., 2020; Gačnik in sod., 2021a; Gačnik in sod. 2021c). SA pozitivno vpliva tudi na skupni pridelek nekaterih gojenih rastlin, naprimer endivije (Trdan in sod., 2008).

Svetovno kmetijstvo se sooča z različnimi izzivi, ki jih povzročajo podnebne spremembe, odpornost škodljivih organizmov na fitofarmacevtska sredstva in želje potrošnikov po varni hrani, ki je pridelana s čim manjšo porabo fitofarmacevtskih sredstev, zaradi njihovih negativnih vplivov na zdravje ljudi in okolje. To vodi v iskanje novih pristopov varstva rastlin pred škodljivimi organizmi in okoljskimi dejavniki. Med temi so tudi alternativna, okolju bolj prijazna sredstva za varstvo rastlin. Ena od alternativ bi lahko bila eksogena uporaba SA in njenih derivatov (Babalar in sod., 2007), ki imajo kot rastlinski hormoni pomembno vlogo pri izražanju inducibilnih lastnosti pri aktivaciji obrambe rastlin ob napadu škodljivcev ali okužbi patogenih organizmov (Stella de Freitas in sod., 2019). Uporaba SA se je izkazala za učinkovito pri direktnem zatiranju povzročitelja plodove monilije (*Monilinia fructicola* [G. Winter] Honey (Yao in Tian, 2005), glive *Botrytis cinerea* var. *sclerotiophila* (Klotzsch) Sacc. na jagodah (Babalar in sod., 2007) glive *Erysiphe cichoracearum* DC. (Trdan in sod., 2004) ter pri zatiranju jablanovega škrlupa (*Venturia inaequalis* var. *inaequalis* (Cooke) G. Winter*)* in jablanove rjave listne pegavosti (*Botryosphaeria obtusa* (Schwein.) Shoemaker) (Abbasi in sod., 2019). Uporaba SA kot vzpodbujevalca (elicitorja) lahko v rastlinah sproži obrambne mehanizme, ki vodijo v odpornost na škodljive organizme, kar se je pokazalo v študiji Stella de Freitas in sod. (2019), kjer so dokazali odpornost riža, tretiranega s 16 mmol l^{-1} SA na stenico *Oebalus pugnax* (Fabricius, 1775).

Uporaba SA in njenih derivatov bi bila lahko uspešna alternativa sintetičnim sredstvom za zaščito rastlin, ki poleg tega lahko izboljša produktivnost rastlinske pridelave in hkrati ohranja kakovost pridelka ali jo celo pri nekaterih gojenih vrstah izboljša. Vendar pa je še vedno nekaj odprtih vprašanj, na katera je treba odgovoriti, preden se lahko salicilate na odgovoren način priporoči za splošno uporabo, zlasti na polju oziroma v nasadih. Potrebno je poznati učinke tretiranj s salicilati in njihov vpliv na okolje. Namen članka je prikazati čim širšo sliko vplivov SA in njenih derivatov na različnih ravneh ekosistema in na interakcije med njimi.

2 SALICILNA KISLINA IN NJENI DERIVATI

Salicilna kislina (SA) ali orto-hidroksibenzojska kislina in 2-hidroksibenzojska kislina je rastlinski hormon, ki spada med fenolne spojine, natančneje med monohidroksibenzojske fenolne kisline, saj je sestavljena iz aromatskega obroča, na katerega je vezana hidroksilna skupina. SA je brezbarvna kristalinična organska kislina v obliki prahu, zmerno topna v vodi in fluorescira pri 412 nm, kar se lahko izkorišča za njeno detekcijo v rastlinah. Ime je dobila po latinskem imenu za vrbo (*Salix*), saj so že ameriški Indijanci in stari Grki ugotovili, da listi in predvsem lubje vrbe zaradi velike vsebnosti salicilne kisline pomagajo proti bolečinam in zmanjševanju telesne temperature (Raskin, 1992). Nahaja se tudi v drugih rastlinskih vrstah, kar so potrdili s študijo 36 kmetijsko pomembnih vrst (Hayat in sod., 2010). Ima vlogo rastlinskega hormona in signalne molekule v lokalnem odzivu rastlin na napad škodljivcev ali okužbo s patogeni ter v sistemsko pridobljeni odpornosti (SAR) (Raskin, 1992; Durner in sod., 1997). SA ima pomembno vlogo tudi pri uravnavanju nekaterih fizioloških procesov v rastlini, kot so fotosinteza, stomatalna prevodnost, glikoliza in transpiracija ter pri razvoju rastlin (npr. cvetenje, kalitev semen) (Klessig in Malamy, 1994; Durner in sod., 1997). Fizikalne lastnosti SA so prilagojene za prenos na dolge razdalje po floemu, s čimer se hitro prenese z mesta aplikacije ali sinteze v oddaljena tkiva, kar je zelo pomembno pri signalizaciji rastline ob napadu škodljivcev ali okužbi s škodljivimi organizmi (Raskin, 1992). V rastlinah se večinoma nahaja v obliki neaktivnih derivatov, sintetiziranih z glukozilacijo, konjugacijo aminokislin, hidroksilacijo in sulfoniranjem (Kumar, 2014).

Salicilna kislina predstavlja pomemben aktivni presnovek njenega bolj znanega derivata - acetilsalicilne kisline (ASA), ki je bolj znana kot aspirin. Uporaba ASA v komercialne namene se je začela leta 1898 v Nemčiji v farmacevtski družbi Bayer in se hitro razširila zaradi njene učinkovitosti ter manjše razdraženosti prebavnega sistema pri ljudeh. ASA je v ljudeh prisotna endogeno, nekaj pa je dobimo s hrano (Raskin, 1992; Paterson in sod., 2008). Za razliko od SA, ASA kot naravne substance v rastlinah niso identificirali. ASA se od SA razlikuje po acetilni skupini, katera se s hidrolizo v vodnih raztopinah odcepi do nastanka SA. Znano je, da to pretvorbo v krvi katalizirajo arilesteraze, katere najdemo v vseh živih tkivih in znanstveniki predvidevajo, da se podobno zgodi tudi ob eksogenih aplikacijah ASA na rastline, kar je podprto z raziskavami učinkov ASA, ki so navadno zelo podobni učinkom SA v primerjavi z drugimi salicilati (Raskin, 1992).

Pogosto se v raziskavah preverja tudi vpliv znanega derivata SA – metil-salicilne kisline (MeSA) (Wang in Li, 2008; Martínez-Esplá in sod., 2017; Martínez-Esplá in sod., 2018; Gačnik in sod., 2019; Gačnik in sod., 2021a; Gačnik in sod., 2021b; Gačnik in sod., 2021c) in metil estra SA - metil salicilata, ki je neaktiven prekurzor SA in ima ključno vlogo pri signalizaciji na dolge razdalje od okuženega do neokuženega tkiva skozi floem (Shulaev in sod., 1997; Hayat in sod., 2010; Valverde in sod., 2015). Gre za hlapno molekulo, ki lahko hitro prehaja membrane in ima pomembno vlogo pri razvoju, rasti in vzpostavitvi obrambnega sistema rastlin in njihovi sposobnosti prilagoditve na abiotični stres (Hayat in Ahmad, 2007). Ravnovesje med SA in MeSA v rastlinah nadzorujeta encima SABP2 (SA vezani protein 2), ki pretvarja neaktivno obliko MeSA v aktivno obliko SA (Forouhar in sod., 2005) in SAMT1 (SA metiltransferaza 1), ki katalizira nastanek MeSA iz SA (Park in sod., 2007).

Med derivate SA štejemo tudi njene druge metil estre in glukozide, ki se zlahka pretvorijo v SA in katere so že v 19. stoletju izolirali iz številnih rastlinskih vrst (Raskin, 1992). Za glikozide SA se predvideva, da so glavna skladiščna oblika SA, ki se po potrebi lahko pretvarja z encimom SA-β-glukozidazo (Kumar, 2014).

3 BIOSINTEZA SALICILNE KISLINE

Danes je znano, da lahko biosinteza SA v rastlinah poteka po dveh različnih poteh iz cimetne kisline in ne le prek sinteze v fenilpropanopidni poti, kot se je sprva predvidevalo. Druga možna pot, ki je bolj značilna za mikroorganizme, je izohorizmatna pot (Shah, 2003), katera dokazano poteka tudi v kloroplastih rastlin in v večini rastlin omogoča biosintezo SA v stresnih situacijah (Kumar, 2014). To so ugotovili Wildermuth in sod. (2001) v svoji raziskavi, kjer je prišlo do sinteze SA, kljub inhibiciji fenilpropanoidne poti.

Večina SA, ki jo najdemo v rastlinah, se sintetizira v fenilpropanoidni poti v citosolu, prek pretvorbe fenilalanina v trans-cimetno kislino s pomočjo encima fenilalanin amonijak liaza (PAL). Cimetna kislina se lahko nato pretvori po dveh možnih poteh; prva je tako imenovana β-oksidativna pot, kjer gre za dekarboksilacijo cimetne kisline do benzojske kisline in nato 2-hidroksilacija benzojske kisline do SA, kar so prvič opisali v raziskavi na tobaku (Yalpani in sod., 1993). Druga je oksidativna pot, kjer se cimetna kislina hidroksilira v *o*-kumarno kislino, kateri sledi dekarboksilacija do SA. Obe omenjeni poti lahko delujeta neodvisno ena od druge v isti rastlini (Raskin, 1992; Zhang in Li, 2019; Lefevere in sod., 2020).

Izokorizmatna pot poteka v kloroplastu z dvostopenjsko reakcijo, in sicer iz korizmata, katerega rastline dobijo iz šikimatske biosintezne poti, prek izokorizmata in jo pogojujeta dva encima – izokorizmat sintaza (ICS) in izokorizmat piruvat liaza (IPL) (Wildermuth in sod., 2001; Dempsey in sod., 2011). SA, sintetizirana po tej poti, ima pomembno vlogo pri obrambi rastline pred patogeni, predvsem pri tako imenovani lokalno pridobljeni odpornosti (LAR) in SAR (Wildermuth in sod., 2001).

Aktivacija sintezne poti SA je navadno sprožena z napadom škodljivcev ali okužbo s patogeni, možno pa jo je spodbuditi tudi z eksogeno uporabo elicitorjev in prav ta ima pomembno vlogo pri interakcijah SA s patogeni in škodljivci ter njihovim potencialnim zatiranjem (Filgueiras in sod., 2019) ter prilagoditvami rastlin na stresne dejavnike. Kar nekaj elicitorjev je bilo uporabljenih za uspešno povečanje vsebnosti SA v rastlini, med drugimi sama SA in njen derivat MeSA (Gačnik in sod., 2021a; Gačnik in sod., 2021b), benzotiadiazol (BTH) in S-metilmetionin salicilat (Bektas in Eulgem, 2015).

4 PRIMARNI, SEKUNDARNI IN TERCIARNI UČINKI SA V OKOLJU

Znano je, da je SA ena ključnih molekul za obrambni sistem rastlin, predvsem za hitro prilagoditev rastlin na abiotične in biotične stresne dejavnike (Klessig in Malamy, 1994) ter da vpliva na širok spekter fizioloških, metabolnih in morfoloških lastnosti rastlin, kar bi v prihodnosti lahko privedlo do res široke splošne uporabe salicilatov v kmetijstvu za doseg boljše tolerance na stresne dejavnike, za doseg boljše kakovosti pridelkov in večje produktivnosti pridelave ter tudi uporabo v varstvu rastlin pred škodljivimi organizmi in za privabljanje naravnih sovražnikov. Preden pa se lahko salicilate na odgovoren način priporoči za praktično uporabo, je potrebno da poznamo natančne vplive SA na rastline, širše okolje in interakcije v okolju (Janda in sod., 2020). Lahko jih razdelimo na primarne, sekundarne in terciarne. Biosinteza SA ima primarne učinke na samo rastlino, kar povzroči morfološke in fiziološke spremembe, ko se rastlina prilagaja stresnim dejavnikom ali pa se le te pojavijo, če salicilate apliciramo eksogeno. Posledično imajo te spremembe lahko vplive na škodljive organizme, ki napadajo rastlino (sekundarni učinek SA). Vzpostavitev obrambe rastline s pomočjo SA, vpliva tudi na odziv naravnih sovražnikov, v interakcijah s škodljivci, kar opredeljujemo kot terciarni učinek SA (Filgueiras in sod., 2019).

4.1 PRIMARNI UČINKI SA

SA ima v rastlini več pomembnih vlog in ključno vpliva na procese, kot so rast in razvoj rastline ter njene prilagoditve na stresne dejavnike (Klessig in Malamy, 1994). V rastlinah redko zasledimo SA v prosti obliki, pogosteje se nahajajo v vezani obliki, ki je za rastline bolj uporabna. Najpogosteje je glukozilirana (neaktivna oblika SA, ki je primerna za shranjevanje v celičnih vakuolah), metilirana (mobilna oblika SA, ki lahko hitro prehaja med celičnimi membranami) in konjugirana z aminokislinami (povezava z razgradnjo SA), kar rastlini omogoča širok spekter uporabe (Filgueiras in sod., 2019). Kot že zgoraj omenjeno, se SA sintetizira prek dveh sinteznih poti: fenilpropanopidne in izokorizmatne poti (Shah, 2003). Za slednjo velja, da se aktivira predvsem v stresnih situacijah (Kumar, 2014). Raziskave kažejo, da sta lahko obe sintezni poti inducirani z abiotičnimi in biotičnimi stresorji (Dempsey in sod., 2011) ter z elicitorji (Filgueiras in sod., 2019).

Endogeno ali eksogeno prisotna SA ima na rastlino primarne učinke, kar se lahko vidi po morfoloških in fizioloških spremembah, ki se pojavijo, ko se rastlina prilagaja stresnim dejavnikom (Filgueiras in sod., 2019), med drugim SA vpliva na termogenezo rastlin, rast celic, fotosintezo, stomatalno prevodnost, glikolizo in transpiracijo ter na razvoj rastlin (npr. vegetativo rast, cvetenje, kalitev semen) (Klessig in Malamy, 1994; Durner in sod., 1997). Poleg omenjenih osnovnih lastnosti lahko uporaba SA in njenih derivatov pomembno vpliva tudi na izboljšanje nekaterih kakovostnih parametrov pomembnejših kulturnih rastlin. Med drugim tretiranje sadnih vrst s SA poveča maso in trdoto plodov ter vsebnost vitamina C v hruškah (Tareen in sod., 2012), izboljša obarvanje in zakasnitev zorenja plodov jagod (Gačnik in sod., 2021a) in povzroča počasnejšo degradacijo karotenoidov v pomarančah (Huang in sod., 2008). S povečanjem aktivnosti nekaterih ključnih encimov v fenil-propanoidni poti, kot je fenilalanin amonijak-liaza (PAL) (Gačnik in sod., 2021b; Gačnik in sod., 2021c), vpliva SA tudi na povečanje nekaterih bioaktivnih komponent, kot so flavonoli, flavanoli, hidroksicimetne kisline in antocianini (Huang in sod., 2008; Giménez in sod., 2017; Blanch in sod., 2020; Gačnik in sod., 2021a; Gačnik in sod. 2021c). So pa na drugi strani različni avtorji poročali tudi o negativnem vplivu SA na nekatere kakovostne parametre, kot je zmanjšanje obarvanja jabolk sorte ‚Topaz' (zmanjša se delež rdeče barve na plodu) in posledično zmanjšanje količine pridelka prve kakovosti (Gačnik in sod., 2021b) ter o negativnem učinku na vsebnost nekaterih bioaktivnih snovi, kot so karotenoidi v marelicah (Wang in sod., 2015) in paradižniku (Nirupama in sod., 2010) ter rutina in apigenina v ingverju (Ghasemzadeh in sod., 2012).

SA že dolgo povezujejo s posredovanjem odziva rastlin na abiotske stresne dejavnike, predvsem toleranco za težke kovine, slanostjo, ozonom, odpornostjo na sušo, poškodbami zaradi zmrzali, toplotnim oksidativnim in sušnim stresom ter UV-B sevanjem (Strobel in Kuc, 1995; Clarke in sod., 2010; Liu in sod., 2013; Jayakannan in sod., 2013; Siboza in sod., 2014; Martel in Qaderi, 2016; Zanelli in sod., 2022). Težke kovine so ene izmed bolj proučevanih stresnih dejavnikov rastlin, kjer je bila dokazana zaščitna vloga SA, med drugim je tretiranje s SA povzročilo toleranco proti toksičnosti bakra v kumarah in tobaku (Strobel in Kuc, 1995), zmanjšalo škodljive učinke svinca in živega srebra v rižu (Mishra in Choudhuri, 1999) ter zmanjšalo toksičnost kadmija v kalčkih ječmena (Metwally in sod., 2003), ki je eden od najbolj problematičnih onesnaževal tal in lahko povzroči motnje v rastlinskih fizioloških procesih, preko hrane pa ga vnesemo v svoje telo tudi ljudje. SA je odgovorna tudi za zmanjševanje sušnega stresa, kar so v svoji raziskavi potrdili tudi Zanelli in sod. (2022). V odziv na abiotske dejavnike navadno rastlina prek sintezne poti SA poveča učinkovitost antioksidantnega sistema in niža raven kisikovih reaktivnih spojin (ROS), ki nastajajo v stresnih razmerah. Te povzročajo poškodbe lipidov, beljakovin in nukleinskih kislin. (Hayat in Ahmad, 2007). Tudi Strobel in Kuc (1995) sta poročala o vplivu eksogeno dodane SA na ublažitev oksidativnega stresa, ki nastane ob uporabi herbicida parakvata. Ugotovljeno je bilo tudi, da je prisotnost SA začasno zmanjšala aktivnost encima katalaze in povečala raven vodikovega peroksida, ki ima pomembno vlogo pri zagotavljanju SAR in odzivu rastline proti oksidativnem stresu (Janda in sod., 2007). SA izboljša tudi aktivnost nekaterih drugih encimov, ki so pomembni v fenilpropanoidni poti, sintezni poti fenolov, kot sta PAL, halkon sintaza/halkon izomeraza (CHS/CHI) ter poveča vsebnosti nekaterih flavonoidov, ki delujejo kot čistilci ROS (van Lith in Ameer, 2016; Gačnik in sod., 2021b). Povečana toleranca na oksidativni stres rastlin vpliva tudi na interakcije z drugimi organizmi, saj lahko SA povzroči lokalne in sistemske obrambne mehanizme rastline (Filgueiras in sod., 2019).

Na biotski stres, ki ga povzročajo škodljivci in patogeni, se rastline odzovejo z različnimi obrambnimi mehanizmi, ki se prav tako odražajo v različnih genetskih in fizioloških spremembah v ali na rastlini. Nekatere rastline se odzovejo na okužbo s patogeni s hipersenzitivnim odzivom (HR) oziroma preventivno nadzorovano celično smrtjo, kar lahko vodi do pridobljene odpornosti ob poznejši okužbi. Pridobljena odpornost je lahko lokalna (LAR) in jo zaznamo v bližini lezij ali sistemična (SAR), katero zaznamo v neokuženih delih rastline (Raskin, 1992). Raziskave so pokazale, da ima kopičenje SA ključno vlogo pri vzpostavljanju SAR v rastlini in povečanju proteinov, ki so povezani s patogenezo (PR proteini) (Schlösser 1999, Shah in Zeier, 2013). V zagotavljanju SAR pa ni pomembna samo SA, temveč tudi njen derivat MeSA, ki predstavlja eno glavnih signalnih molekul SAR in lahko zaradi svoje hlapne narave hitro posreduje drugim, oddaljenim tkivom signale za vzpostavitev obrambnih mehanizmov v oddaljenih tkivih, ki niso okužena. Aktivacija SAR zahteva kopičenje ustrezne ravni SA in MeSA, ki jo uravnavata SA metiltransferaza in MeSA esteraza ter optimalno komunikacijo med listi, ki razvijejo LAR in listi, ki so neokuženi. (Chen in sod., 2019).

4.2 SEKUNDARNI UČINKI SA

Indukcija sintezne poti SA imam za rastlino lahko več učinkov, ki se odražajo v različnih morfoloških in fizioloških spremembah rastline, ki lahko posredno vplivajo na škodljive organizme. SA pa ima pri aplikaciji salicilatov na rastline lahko tudi direktni vpliv na nekatere povzročitelje bolezni. Oboje opredeljujemo v sekundarni učinek SA (Filgueras in sod., 2019).

SA je ena izmed najpomembnejših fenolnih spojin, saj ključno vpliva na vzpostavitev obrambnih mehanizmov v rastlini pred patogeni in škodljivci (Raskin, 1992; Vlot in sod., 2009; Klessig in sod., 2018). Znanstveniki zaradi te lastnosti že dlje raziskujejo možnosti uporabe SA v varstvu rastlin, in sicer najbolj raziskujejo sledeča področja: (1) eksogena uporaba različnih elicitorjev, ki inducirajo sintezno pot SA in vodijo v povečanje vsebnosti SA v rastlini ter zagotavljajo posredno varstvo rastlin z vzpostavitvijo SAR; (2) eksogena uporaba SA in njenih derivatov za direktno zatiranje škodljivih organizmov, (3) genski inženiring s spreminjanjem izražanja genov za obrambo rastlin, ki selektivno aktivirajo obrambne poti SA; (4) eksogena uporaba salicilatov (SA, MeSA, ASA,…) za privabljanje naravnih sovražnikov (Filgueiras in sod., 2019).

Prisotnost SA v rastlini ima neposredno vlogo pri posredovanju interakcij med rastlino, patogeni, in škodljivci. Indukcija biosintezne poti SA lahko sproži obrambne mehanizme, kar vodi v sintezo proteinov PR in vzpostavitev SAR, kar ima lahko negativne posledice za druge organizme, ne glede na način indukcije biosintezne poti SA (Dempsey in sod., 2011; Klessig in sod., 2018; Filgueiras in sod., 2019). Ta je lahko inducirana z abiotskim in biotskim stresom ali pa je posledica eksogeno dodane SA. Dandanes je na voljo že kar nekaj študij, ki preučujejo vplive eksogeno dodane SA in njenih derivatov na zatiranje škodljivih organizmih. Predvsem se veliko preučuje vplive SA in njenih derivatov na zatiranje bolezni na kmetijsko pomembnih rastlinah (Joyce in sod., 2001; Yao in Tian, 2005; Babalar in sod., 2007; Halim in sod., 2007; Abbasi in sod., 2019; Gačnik in sod., 2019), manj pa je raziskav, ki bi preučevale vpliv bodisi naravno prisotne SA v rastlinah, bodisi eksogeno dodane SA na zatiranje škodljivcev.

Med drugim so ugotovili, da tretiranje s SA proti krompirjevemu virusu X (PVX) sproži metabolni odziv, ki poveča odpornost na okužbo na paradižniku in okužbo za nekaj časa zakasni (Falcioni in sod., 2014), direktno zatira rast micelija glive plodove monilije (*Monilinia fructicola*) in kalitev spor in vitro kar znatno zmanjša premer lezij na plodovih češenj (Yao in Tian, 2005). Izkazalo pa se je, da je učinkovitost induciranja odpornosti pri plodovih češenj, ki so bili tretirani s SA pred obiranjem boljša kot pri plodovih tretiranih po obiranju. SA zavira tudi antraknozo, ki jo povzroča gliva *Colletotrichum gleosporioides* Penz. na mangu (Joyce in sod., 2001), kjer so ugotovili, da koncentracija 2000 mg SA l^{-1} znatno zmanjša pojav bolezni. Ugotovljeno je bilo tudi, da je SA pomembna spojina, ki je potrebna za osnovno zaščito krompirja pred krompirjevo plesnijo (*Phytophthora infestans* var. *phaseoli* (Thaxt.) Leonian), saj se je pokazala drastično povečana rast glive v transgenem krompirju, ki ne more kopičiti salicilne kisline (Halim in sod., 2007). Gačnik in sod. (2019) so *in vitro* preučevali vpliv SA in MeSA na rast micelija različnih gliv (*Monilinia fructicola, Monilinia laxa* (Aderh. & Ruhland) Honey, *Gnomoniopsis smithogilvyi* L.A. Shuttlew., E.C.Y. Liew & D.I. Guest) in ugotovili, da sta obe raztopini zaustavili njihovo rast. Poleg tega so preučevali *in vivo* vpliv SA in MeSA na okužbo jabolk z glivo *Monilinia laxa* in ugotovili, da je imela MeSA najboljši učinek pri zmanjšanju nekroz. Na drugi strani so nekateri poročali o neučinkovitosti SA pri preventivnem zatiranju sive plesni (*Botrytis cinerea*) na plodovih breskev 5 dni po inokulaciji, kjer je bila uporabljena le 0,5 mM raztopina SA. Z dodatkom antagonista kvasovk *Rhodotorula glutinis* (Fresen.) F.C. Harrison jim je uspelo znatno zmanjšati intenzivnost okužbe (Zhang in sod., 2008). Da Rocha Neto in sod. (2016) so primerjali preventivni, kurativni in eradikativni način 2-minutnega tretiranja z 2,5 mM SA, kjer je eradikativno dodana SA 100 % inhibirala modro plesen (*Penicillium expansum* Link) , medtem ko 2-minutno potapljanje v raztopino SA ni imelo preventivnega in kurativnega učinka po 4 in 10 dneh po inokulaciji. Wang in sod. (2011) po drugi strani poročajo o preventivni uporabi SA, ki je zavirala okužbo in zmanjšala premer lezije *B. cinerea* v plodovih paradižnika, ki so bili 15 minut tretirani s 5 mM raztopino SA.

SA vpliva tudi na škodljivce, kot je kapusov belin (*Pieris brassicae* [L., 1758]), kjer so opazili, da SA regulira odziv listov repnjakovca *Arabidopsis thalian*a (L.) Heyhn. na odlaganje jajčec in na poznejše prehranjevanje gosenic (Lortzing in sod., 2019). Njen derivat MeSA pa naj bi z eksogenim tretiranjem repnjakovca odvrnil kapusovega belina od odlaganja jajčec (Groux in sod., 2014). Z eksogeno uporabo SA je mogoče učinkovito zatreti južno plodovrtko (*Helicoverpa armigera* Hubner, [1808]) na arašidih (War in sod., 2015), paradižnikovega molja (*Tuta absoluta* [Meyrick, 1817]) in navadno pršico (*Tetranychus urticae* Koch, 1836) na paradižniku (Pulga in sod., 2020).

SA lahko poleg neposrednega zatiranja okužbe s patogeni povzroči tudi njen posredni sekundarni učinek na druge organizme (Filgueiras in sod., 2019). S tem vpliva na posredne interakcije med patogeni in škodljivci. Vpliv SA na interakcije lahko poteka v obe smeri: patogen-škodljivec in škodljivec-patogen. Eden od zgledov takšne interakcije je hranjenje gosenic vrste *Helicoverpa stombleri* (Okumura and Bauer, 1969) na listih paradižnika, ki inducira sintezno pot SA, kar sproži obrambno reakcijo in vpliva na zmanjšanje okužbe z bakterijo *Pseudomonas syringae* van Hall (Stout in sod., 1999).

4.3 TERCIARNI UČINKI SA

Vpliv SA na privabljanje in vedenje naravnih sovražnikov škodljivih organizmov lahko opredelimo kot terciarni vpliv SA v ekosistemu. Naravni sovražniki so obsežna skupina organizmov, ki se uporabljajo v biotičnem varstvu rastlin za zatiranje škodljivih organizmov na številnih rastlinskih vrstah. Med naravne sovražnike na splošno štejemo plenilce, parazitoide in entomopatogene ogorčice, glive, bakterije in viruse (Hajek in Eilenberg, 2018; Trdan in sod., 2020). V Sloveniji je uporaba predstavnikov prvih treh skupin pravno določena s Pravilnikom o biotičnem varstvu rastlin (Uradni list RS, št. 45/06) in zajema 35 domorodnih ter 78 tujerodnih vrst.

Rastlinski obrambni sistemi lahko delujejo neposredno proti herbivorom in patogenom in s sproščanjem toksinov odvračajo škodljive organizme od njihovega prehranjevanja. Te obrambne reakcije pa lahko posredno povzročijo sproščanje hlapnih komponent, ki privabljajo naravne sovražnike rastlinskih škodljivih organizmov (van Poecke in Dicke, 2002; De Boer in Dicke, 2004). Te, tako imenovane tritrofne interakcije med rastlinami, herbivori in njihovimi naravnimi sovražniki, so pogojene s stimulacijo obrambnih poti, ki so lahko izzvane zaradi rastlinojedstva, komunikacije med rastlinami ali uporabe kemičnih sredstev, ki delujejo kot elicitorji obrambnega odziva rastline (Filgueiras in sod., 2016). Ena od pomembnih spojin pri teh tritrofičnih interakcijah je SA, ki pomembno vpliva na obrambni sistem rastlin, znano pa je tudi, da deluje kot atraktant za naravne sovražnike (van Poecke in Dicke, 2002).

Naravni sovražniki se odzivajo na različne hlapne spojine, ki jih rastline sproščajo v okolje ob napadu herbivorov ter tako locirajo svoje gostitelje. Vloga SA oziroma njenega hlapnega derivata MeSA se že dolgo povezuje z naravnimi sovražniki, ki plenijo ali zajedajo škodljive organizme nad tlemi (De Moraes in sod.,. 1998; Filgueiras in sod., 2019). Številne rastlinske vrste sproščajo MeSA kot sestavino hlapnih mešanic ob napadu herbivorov ali ob okužbi. MeSA med drugimi rastlinami sproščajo fižol, kumare in paradižnik ob napadu navadne pršice (*Tetranychus urticae*) (Dicke in sod., 1990; Dicke in sod., 1998), zelje ob napadu kapusovega belina (*Pieris brassicae*) (Geervliet in sod., 1997) in hmelj ob napadu hmeljeve uši (*Phorodon humuli* [Schrank, 1801]) (Campbell in sod., 1993). V kar nekaj študijah se je izkazalo, da MeSA deluje kot atraktant za številne naravne sovražnike, predvsem na plenilce in parazitoide. Z zaznavanjem MeSA najdejo svojega gostitelja med drugimi tančičarica *Chrysopa nigricornis* Burmeister, 1839 (Mallinger in sod., 2011), plenilska pršica *Phytoseiulus persimilis* Athias-Henriot, 1957 (De Boer in Dicke, 2004) in entomopatogena ogorčica *Steinernema diaprepesi* Nguyen in Duncan, 2002 (Filgueiras in sod., 2016). Vse več se raziskuje vpliv MeSA na naravne sovražnike škodljivih organizmov tudi v koreninah oz. talnih organih rastlin, saj le-ta še ni dobro raziskan. Znano je, da je stimulacija obrambe nadzemskih delov rastlin lahko povezana s podzemnimi deli, kar so dokazali v študiji Filgueiras in sod. (2016), kjer so z eksogeno uporabo MeSA v nadzemnih delih citrusov privabili entomopatogene ogorčice v podzemne dele. Dodatno je uporaba MeSA povzročila izločanje še ene hlapne spojine – limonena, ki prav tako privablja entomopatogene ogorčice.

Uporaba SA in njenih derivatov za privabljanje naravnih sovražnikov za zatiranje škodljivih organizmov, je eden od perspektivnih, okolju prijaznih načinov varstva rastlin, ki je mogoč predvsem z uporabo vab, ki sproščajo hlapne snovi, kot je MeSA (Filgueiras in sod., 2019). Vseeno pa privabljanje naravnih sovražnikov s hlapnimi spojinami, kot način varstva rastlin pred škodljivci, potrebuje še več raziskav. Vsesplošna in časovno neomejena uporaba takšnega načina varstva rastlin, bi namreč lahko povzročila več škode, kot koristi. Potrebno je upoštevati čas uporabe oziroma fenologijo ter relativno gostoto populacije škodljivcev, saj lahko naravni sovražniki na dolgoročno sproščanje privabilnih signalov odreagirajo negativno, če jim niso zagotovljene ustrezne razmere za prehranjevanje, saj se le ti lahko naučijo odreagirati na za njih zavajajoče signale (Hajek in Eilenberg, 2018; Filgueiras in sod., 2019).

5 ZAKLJUČEK

Dandanes se kmetijstvo sooča s pomembnim izzivom, kako pridelati dovolj kakovostne hrane za hitro rastočo svetovno prebivalstvo ob vseh spreminjajočih se okoljskih dejavnikih. Ključna naloga je izboljšanje odpornosti gojenih rastlin na stres, izboljšanje produktivnosti in kakovosti pridelka. Zaradi negativnih vplivov sintetičnih sredstev za varstvo rastlin na okolje, je nujno, da se jih poskuša zamenjati z alternativnimi načini varovanja rastlin, kot je uporaba naravnih rastlinskih spojin. Uporaba SA in njenih derivatov lahko izboljša produktivnost rastlinske pridelave in hkrati ohranja kakovost pridelka ali jo celo pri nekaterih gojenih vrstah izboljša. Vendar pa je še vedno nekaj odprtih vprašanj, na katera je treba odgovoriti, preden se lahko salicilate na odgovoren način priporoči za splošno uporabo, zlasti na polju oziroma v nasadih. Potrebno je poznati učinke obravnavanj s salicilati in kakšen je njihov vpliv na okolje. SA in MeSA sta eni ključnih molekul za obrambni sistem rastlin, predvsem za hitro prilagoditev rastlin na abiotične in biotične stresne dejavnike, kar povzroča različne spremembe v rastlinah, okolju in interakcijah v okolju. V tem članku smo predstavili pomembnejše derivate SA in opisali ter razdelili učinke salicilatov v okolju na primarne, sekundarne in terciarne, kar nam je dalo širši vpogled na potencialno uporabo SA in njenih derivatov v pridelavi hrane in varstvu rastlin pred škodljivimi organizmi in pri prilagajanju različnim okoljskim dejavnikom. Na splošno so učinki SA na primarni ravni, predvsem učinki eksogeno dodane SA vzpodbudni in bi lahko pomenili dobro iztočnico v prihodnosti za zamenjavo okolju škodljivih sintetičnih sredstev, saj salicilati največkrat pripomorejo k povečani odpornosti na različne stresne dejavnike, prav tako pa pripomorejo k izboljšani kakovosti in produktivnosti pomembnejših gojenih rastlin. Sekundarna raven učinkov SA v okolju predstavlja učinke na škodljive organizme zaradi direktnega delovanja SA in posrednih učinkov SA, ki nastanejo zaradi morfološkihin fizioloških sprememb, ko se rastlina prilagaja stresnim dejavnikom. Tudi tu se v večini primerov kažejo vzpodbudni rezultati. Rastline posredno po napadu škodljivega organizma sproščajo hlapne snovi, ki privabljajo naravne sovražnike rastlinskih škodljivcev, predvsem kot hlapni derivati SA - MeSA. Narejenih je bilo že kar nekaj raziskav, kjer so proučevali na katere organizme MeSA deluje kot atraktant. Potrebno pa bi se bilo osredotočiti še bolj specifično na ustrezen čas aplikacije s salicilati, saj je pomembno vedeti, kdaj bi bila uporaba MeSA kot atraktanta smotrna in pri kakšni številčnosti škodljivcev, saj lahko naravni sovražniki na dolgoročno sproščanje signalov odreagirajo negativno, če jim niso zagotovljene ustrezne razmere za prehranjevanje.

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doi:10.14720/aas.2023.119.1.2914 Review article / pregledni znanstveni članek

Inertni prahovi: alternativni pristop v varstvu rastlin pred koloradskim hroščem (*Leptinotarsa decemlineata* [Say, 1824], Coleoptera, Chrysomelidae)

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Received November 15, 2022; accepted February 21, 2023.
Delo je prispelo 15. novembra 2022, sprejeto 21. februarja 2023

Inert dusts: an alternative approach of plant protection against the Colorado potato beetle (*Leptinotarsa decemlineata* [Say, 1824], Coleoptera, Chrysomelidae)

Abstract: In this article, we focused on an alternative approach of plant protection against the Colorado potato beetle (*Leptinotarsa decemlineata*), which is considered the most important pest of potatoes (*Solanum tuberosum*) in the world and also here in Slovenia. We decided to present the use of different inert dusts as a way of controlling the aforementioned pest. We focused on the categorization of powders, the presentation of individual groups of inert dusts and the description of one or more substances belonging to a specific group. In addition to a general presentation and the mode of action of specific inert dusts, we also provided practical examples that show whether the agent/substance has already been used against the Colorado potato beetle. With the aforementioned, we also tried to summarize whether the described substance has the potential to be used in practice or not. We would also like to point out that wood ash and diatomaceous earth are the most promising inert dusts against *L. decemlineata*. Wood ash is effective against larvae and to a lesser extent also against adults. Diatomaceous earth is also effective in reducing the number of pests on potato plants. Other inert dusts mentioned in scientific literature are probably less effective or have not even been tested or used against this pest yet. Further research, both under laboratory conditions and outdoors, are necessary to definitively determine the effectiveness of this selected inert dusts.

Key words: Colorado potato beetle; *Leptinotarsa decemlineata*; inert dusts; diatomaceous earth; zeolite wood ash; plant based dusts; quartz; alternative approaches of plant protection

Inertni prahovi: alternativni pristop v varstvu rastlin pred koloradskim hroščem (*Leptinotarsa decemlineata* [Say, 1824], Coleoptera, Chrysomelidae)

Izvleček: V preglednem članku se osredotočamo na alternativni pristop varstva krompirja (*Solanum tuberosum*) pred koloradskim hroščem (*Leptinotarsa decemlineata*), ki pri nas in v svetu velja za najpomembnejšega škodljivca te pomembne poljščine. V prispevku predstavljamo uporabo različnih inertnih prahov za zatiranje tega škodljivca. Osredotočili smo se na kategorizacijo prahov, predstavitev posameznih skupin inertnih prahov in opis ene ali več snovi, ki pripadajo specifični skupini. Poleg splošne predstavitve načinov delovanja posameznih prašiv navajamo tudi praktične zglede uporabe prašiv proti koloradskemu hrošču, s čimer želimo predstaviti potencial različnih prašiv za uporabo v praksi. Ugotavljamo, da veljata lesni pepel in diatomejska zemlja za bolj perspektivna inertna prahova pri zatiranju škodljivih žuželk. Lesni pepel učinkovito deluje proti ličinkam ter v manjši meri tudi na odrasle osebke, diatomejska zemlja pa prav tako vpliva na zmanjšanje številčnosti koloradskega hrošča na krompirju. Ostala inertna prašiva so v strokovni literaturi izpostavljena kot manj učinkovita oz. sploh še niso bila preizkušena ali uporabljena proti temu škodljivcu. Za dokončno določitev učinkovitosti prašiv bodo potrebne nadaljnje raziskave, tako v laboratorijskih razmerah kot na prostem.

Ključne besede: koloradski hrošč; *Leptinotarsa decemlineata*; inertni prahovi; diatomejska zemlja; zeolit; lesni pepel; rastlinski prahovi; kremen; alternativni pristopi v varstvu rastlin

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1 UVOD

V današnjem času smo priča mnogim spremembam in uvajanju nove zakonodaje na področju kmetijstva v Evropski uniji (EU). Vedno bolj se uveljavlja ekološko kmetovanje ter omejitve pri uporabi fitofarmacevtskih sredstev v državah, članicah EU. Namen vseh držav članic je zmanjšati tveganja in ostale negativne vplive prekomerne rabe fitofarmacevtskih sredstev na zdravje ljudi in okolje. Eno od pomembnejših načel je tudi spodbujanje uporabe alternativnih pristopov v varstvu rastlin. Omenjeni vključujejo različne nekemične načine zatiranja škodljivih organizmov ter morajo biti skladni z načeli integriranega varstva rastlin (IVR) (Sustainable use of pesticides, 2022).

2 KOLORADSKI HROŠČ (*Leptinotarsa decemlineata* [Say, 1824])

Koloradski hrošč (KH) (*Leptinotarsa decemlineata* [Say], Coleoptera: Chrysomelidae) izvira iz območja osrednje Mehike in je domoroden na območju Severne Amerike. Primarno se je škodljivec prehranjeval le z nekaterimi samoniklimi rastlinskimi vrstami in ni predstavljal grožnje takratnim kmetom (CABI, 2022). V Severni Ameriki se je prvič pojavil in nato razširil le vzhodno od Skalnega gorovja. Pozneje, leta 1874, se je s pojavom krompirja (*Solanum tuberosum* L.) kot pomembne poljščine, koloradski hrošč razširil tudi na celoten vzhod celine in posledično postal najpomembnejši škodljivec krompirja v Severni Ameriki. Čeprav je krompir najpomembnejši vir hrane KH, se žuželka prehranjuje tudi na drugih kmetijskih rastlinah, kot so zelje (*Brassica oleracea* L.), poprovec (*Piper nigrum* L.), tobakovec (*Nicotiana tabacum* L.), jajčevec (*Solanum melongena* L.) in paradižnik (*Solanum lycopersicum* L.); (Luckmann in Metcalf, 1994). Škodo povzroča tudi na nekaterih samoniklih rastlinskih vrstah, kot so navadni osat (*Cirsium vulgare* [Savi] Ten.), volčja češnja (*Atropa belladonna* L.) in mnoge druge (Gökçe in sod., 2007). Iz ZDA se je koloradski hrošč razširil na nekatera druga območja sveta. Tako se je v Evropi pojavil leta 1922 v bližini Bordeauxa v Franciji. KH se je po Evropi razširil zelo hitro, kljub različnim intenzivnim načinom zatiranja, ki pa niso bili učinkoviti. V Sloveniji so ga prvič našli leta 1946 na Krškem polju, kamor naj bi bil zanesen s krompirjem leta 1944 (FITO-INFO, 2022). Danes ga lahko najdemo že skoraj na celotni stari celini, škodljivec pa se še vedno širi in bi se lahko potencialno razširil tudi na druga območja z zmernejšim podnebjem, kot so Avstralija, Nova Zelandija, Afrika, Južna Amerika in Indija.

Odrasli osebki KH prezimijo do nekaj 10 cm globoko v tleh ali v odpadli listni gmoti v bližini njiv, kjer so pridelovali krompir. Spomladi začnejo z iskanjem rastlin krompirja, kjer se pripravijo na razmnoževanje in odlaganje jajčec. Samice odlagajo jajčeca v leglih (praviloma do 25 jajčec na eno leglo) na spodnji strani listov. Posamezne samice lahko odložijo do 600 jajčec. Glavnino poškodb povzročajo ličinke, ki se hranijo z listi. Če koloradskega hrošča ustrezno ne zatremo, lahko povzroči do 100 % defoliacijo več tednov pred pobiranjem krompirja ter tudi do več kot 50 % zmanjšanje pridelka krompirja. V hladnejših območjih lahko hrošči dokončajo do en rod na leto, v toplejših območjih pa imajo hrošči neprekinjen cikel razvoja iz roda v rod. Zaradi omenjenih lastnosti uvrščamo KH med najpomembnejše škodljivce krompirja (Sablon in sod., 2013). Na Kitajskem poročajo, da je samo v letu 2012 KH povzročil za 3,2 milijona ameriških dolarjev škode. Izpostavljajo tudi, da, ko bo škodljivec zastopan v vseh delih države, bo škoda zaradi njegovega delovanja na krompirju znašala okrog 235 milijonov dolarjev letno (Liuin sod., 2012).

Krompir uvrščamo med pomembne gojene rastlinske vrste. Po letu 2005 je bil delež svetovne pridelave krompirja v razvijajočih se državah (52 %) prvič večji od proizvodnje krompirja v razvitem svetu, predvsem zaradi velike rasti pridelave omenjene poljščine na Kitajskem in v Indiji (Wijesinha-Bettoni in Mouillé, 2019). Gre namreč za rastlinsko vrsto, ki jo lahko pridelujemo na območjih z omejenimi razmerami za pridelavo, je hitro rastoča in zelo prilagodljiva rastlinska vrsta ter ima zelo velik donos. Skupaj z rižem, pšenico in koruzo, predstavlja krompir prek 50 % svetovnih potreb po energiji, ki jo pridobimo iz hrane (Wijesinha-Bettoni in Mouillé, 2019).

Uporaba sintetičnih insekticidov je že vrsto let najpomembnejši način zatiranja KH, predvsem zaradi hitrega in učinkovitega delovanja. Kljub temu pa je prekomerna raba sredstev s podobnimi aktivnimi snovmi vplivala na razvoj odpornosti KH proti tem snovem. Posledično je razvoj odpornosti KH spodbudil nastanek novih kemičnih insekticidov, ki niso ne ekološko in niti trajnostno naravnani. Posledice uporabe takšnih sredstev s širokim spektrom delovanja so tudi nezaželene, saj zatirajo tudi koristne organizme oz. naravne sovražnike škodljivca ter povzročijo kopičenje ostankov teh aktivnih snovi v vodi in tleh, kar zmoti obstoječe ekološko ravnovesje in negativno vpliva na zdravje ljudi in živali (Göldel in sod., 2020).

Metode zatiranja koloradskega hrošča bi morale biti prilagojene in razdeljene na tiste, ki se uporabljajo posredno oz. preventivno kot osnovni tehnološki ukrepi pri pridelavi krompirja. Neposredne metode pa so tiste, ki so usmerjene v preprečevanje izbruha škodljivega organizma z neposrednim zatiranjem.

Med alternativne metode zatiranja med drugim spadajo: uporaba okolju prijaznih bioinsekticidov na podlagi eteričnih olj ter drugih rastlinskih izvlečkov, ter uporaba inertnih prahov, kateri dokazano delujejo proti številnim skladiščnim škodljivcem in se lahko uporabljajo kot alternativa kemičnim pripravkom (Stanković in sod., 2020).

3 INERTNI PRAHOVI

Po Subramanyamu in Roesli (2000) so inertni prahovi razvrščeni v pet skupin, ki jih je mogoče razlikovati glede na njihovo kemično sestavo ali stopnjo njihove aktivnosti.

Prva vključuje prahove, ki ne vsebujejo kremena. Ta skupina vključuje katelsous (fosfat in mleto žveplo), apno (kalcijev hidroksid), apnenec (kalcijev karbonat) in kuhinjsko sol (natrijev klorid). Kot primer lahko izpostavimo uporabo apna na Filipinih in v Hondurasu kot sredstva za varstvo žita in koruznega zrnja pred škodljivci (Golob in Webley, 1980).

V drugo skupino uvrščajo pesek, kaolinit, pepel, lesni pepel in glino, ki skupaj sestavljajo skupino materialov, ki se pogosto uporabljajo v državah v razvoju kot sredstva za varstvo žita pred skladiščnimi škodljivci. Za omenjene snovi je značilno, da so za učinkovito delovanje potrebne velike količine (uporaba do 5 % mase pridelka) (Golob in Webley, 1980).

Tretja skupina vključuje inertne prahove, ki vsebujejo naravni silicijev dioksid, kot sta diatomejska zemlja in zeolit (do 90 % SiO_2).

V četrto skupino uvrščamo inertne prahove, ki vsebujejo sintetični silicijev dioksid (zelo kakovostni, oborjeni silicijevi dioksidi z nad 98 % vsebnostjo SiO_2). Uporabljajo se v industriji in tudi kot sredstva proti strjevanju in prostemu pretoku.

V peto skupino uvrščajo silicijeve agrogele, ki se proizvajajo s sušenjem vodnih raztopin natrijevega silikata. So zelo lahki, hidrofobni praški, ki so učinkoviti pri manjših koncentracijah kot diatomejska zemlja. Problematika silicijevih agrogelov je njihova majhna gostota in volatilnost (nevarnost vdihavanja), kar je v preteklosti preprečilo njihovo široko uporabo (Golob, 1997).

Različne vrste inertnih prahov so za namene varstva skladiščnega zrnja začela uporabljati že ljudstva v daljni preteklosti. Tako so npr. Azteki skladiščili zrnje koruze premešano z apnom, da bi ohranili kakovost zrnja in s tem tudi preprečili pojav in razmnožitev skladiščnih škodljivcev. O načrtni komercializaciji in poskusih za uporabo takšnih prahov v sodobni tehnologiji varstva zrnja ter v nekaterih zgledih tudi rastlin lahko govorimo šele v zadnjih sedemdesetih letih (Golob, 1997).

Prahovi delujejo na žuželke fizikalno in zato na splošno delujejo počasneje, torej prek neposrednega stika, za razliko od standardnih insekticidov, ki lahko delujejo želodčno, kontaktno, sistemično, ipd. Vendar, tudi, če ne delujejo kemično (ne učinkujejo na presnovo žuželk, ipd.), so lahko kemično aktivni pod določenimi okoliščinami. Smrt žuželk primarno nastopi predvsem kot posledica izsušitve/desikacije: izguba vode je posledica uničenja kutikule žuželke. Silicijevi agrogeli delujejo tako, da absorbirajo delce zaščitnega voska iz površja povrhnjice/kutikule žuželke. Diatomejska zemlja pa na primer deluje bolj abrazivno, ker vsebuje delce SiO_2. Deluje tudi nekoliko absorbcijsko do voska, ki je na povrhnjici/kutikuli žuželke, saj se ta veže na prej omenjene delce SiO_2 (Maceljski in Korunić, 1972). Maceljski in Korunić (1971) izpostavljata, da so silicijevi agrogeli učinkovitejši od diatomejske zemlje oz. ostalih inertnih prahov, ker ohranjajo svojo aktivnost tudi pri povečani relativni zračni vlagi. Pomembno je tudi izpostaviti, da inertni prahovi ne delujejo na presnovne poti žuželk in s tem lahko predvidevamo, da tarčni organizmi ne bodo razvili odpornosti proti omenjenim sredstvom. Kljub temu pa lahko predpostavljamo, da bi lahko žuželke razvile vedenjski odziv na prisotnost specifičnega inertnega prašiva in se izognile stiku s slednjim (Golob, 1997).

Ena izmed prednosti inertnih prahov je tudi majhna toksičnost za sesalce. V ZDA so tako na primer vse vrste diatomejske zemlje priznane kot varne s strani ameriškega oddelka za varno hrano in zdravila. Registrirane so kot dodatki za živila (Banks in Fields, 1995).

3.1 DIATOMEJSKA ZEMLJA

Diatomejska zemlja je organogena sedimentna kamnina, sestavljena v glavnem iz skeletnih ostankov enoceličnih mikroalg - diatomej (Bacillariophyceae). Nastaja z nakopičenjem skeletnih ostankov alg tako v morjih kot v jezerih. Skelete diatomej sestavlja hidratizirane amorfna kremenica - opal ($SiO_2 \cdot nH_2O$). Velike količine diatomejske zemlje so nastajale predvsem v obdobju miocena (23 do 5,5 milijonov let) (Korunić, 1998; Rojht in sod., 2012).

Diatomejsko zemljo pridobivamo z izkopavanjem in jih nato drobimo in meljemo. Lahek prah, ki nastane, vsebuje porozne delce z določenimi abrazivnimi lastnostmi in sposobnostjo absorbiranja lipidov. Vsak delec lahko absorbira do približno tri in večkratno maso samega delca (Korunić, 1998). Vsaka vrsta diatomejske zemlje z visoko sposobnostjo absorpcije olja/lipidov je potencialni insekticid. Poleg absorpcijske sposobnosti na insekticidno učinkovitost snovi vplivajo tudi velikost delcev, enotnost in oblika delcev, pH in čistost formulacije (Korunić, 1998). Insekticidno delujoča diatomejska zemlja mora biti v obliki zelo čistega amorfnega silicijevega dioksida, z delci podobnega premera (< 10 mm), in pH < 8,5. Takšna diatomejska zemlja mora vsebovati tudi najmanjše možno število delcev gline in manj kot 1 % kristalnega silicijevega dioksida. Delce takšne diatomejske zemlje lahko zlahka poberejo grobe žuželke. Diatomejska zemlja na žuželko učinkuje tako, da tej delci poškodujejo povrhnjico z absorpcijo ogljikovodikov in abrazijo, zaradi česar se povrhnjica poškoduje in je prepustna za vodo, ki hitro zapusti telo žuželke in povzroči smrt zaradi izsušitve/desikacije (Korunić, 1998; Rojht in sod., 2012).

Uporaba diatomejske zemlje kot insekticida je v praksi najbolj učinkovita proti skladiščnim škodljivcem iz rodov *Cryptolestes* in *Sitophilus*, manj dovzetne pa naj bi bile vrste iz rodov *Oryzaephilus*, *Rhyzopertha* in *Tribolium* (Maceljski in Korunić, 1972). Pomembno je izpostaviti, da so mnogi preizkušali uporabo diatomejske zemlje tudi na prostem ter tudi proti drugim vrstam škodljivcev (mravlje, polži, termiti, junijski hrošč, koloradski hrošč, itd.,). Kljub spodbudni ideji, pa so bili njihovi rezultati pogosto zelo različni in večkrat popolnoma nasprotni (Korunić, 1998). Avtorji raziskav dostikrat diatomejsko zemljo ne priporočajo kot glavnega pripravka za zatiranje škodljivcev, ampak jo omenjajo kot nosilca/dodatek drugim aktivnim snovem, kot so eterična olja in entomopatogene glive (Zeni in sod., 2021)

3.1.1 Zgled uporabe diatomejske zemlje

Becker (2007) v njenem raziskovalnem delu opisuje možnost uporabe različnih oz. alternativnih metod zatiranja koloradskega hrošča na različnih sortah krompirja. Eno izmed obravnavanj opisuje možnost uporabe diatomejske zemlje kot morebitnega učinkovitega sredstva proti KH. Gre za edino tovrstno raziskavo, ki preučuje uporabo diatomejske zemlje proti koloradskemu hrošču na prostem. Diatomejsko zemljo so nanašali v obliki vodne zmesi in posipanja. Skupno so jo v sezoni nanesli 3-krat. Končni rezultati pa niso bili navdušujoči, saj so nekatera od ostalih obravnavanj dosegala enak ali celo večji končni pridelek krompirja (npr. obravnavanje s privabilnim posevkom jajčevca, ipd.). Diatomejska zemlja je v primerjavi s kontrolo pri sorti ‚Norkotah' imela za 0,2 lbs manj pridelka krompirja. Podobno se je izkazalo tudi pri sortah ‚Pontiac' in ‚Kennebec' (od 0,25 do 1,5 lbs manj pridelka v primerjavi s kontrolo). Le pri sorti ‚Yukon' je bil pridelek krompirja večji pri obravnavanju z diatomejsko zemljo kot pridelek v kontroli.

3.2 ZEOLIT

Zeoliti so kristalni hidratizirani alumosilikati alkalijskih in zemeljsko alkalijskih kationov. V veliki meri se uporabljajo predvsem v kmetijstvu, kot sredstvo za omejevanje smradu, inertni prah za zatiranje skladiščnih škodljivcev, krmni dodatek v prehrani živali, gnojilo za izboljšanje kakovosti tal ter kot sredstvo, ki omejuje mikotoksine, itd. Po klasifikaciji IARC (Mednarodne agencije za raziskave o raku) spada zeolit med sredstva, ki ne spodbujajo nastanka raka in je varen za uporabo v prehrani ljudi (Eroglu in sod., 2017).

Na učinkovitost uporabe zeolita kot sredstva, namenjenega zatiranju škodljivcev, vplivajo okoljske razmere (temperatura, zračna vlaga, ipd.), vrsta žuželke (dlakavost, razvojni stadij, debelina voska na kutikuli, ipd.) in struktura prahu ter njegove fizikalne in kemijske lastnosti (molekularna struktura, vsebnost SiO_2, oblika in velikost delcev, razmerje Al/Si, sorpcijska sposobnost in geografsko poreklo, itd.) (Eroglu in sod., 2017).

Tudi zeolit deluje na podoben način kot diatomejska zemlja, in sicer tako, da žuželki omeji dihalne poti, zaradi česar se žuželka zaduši. Povzroči lahko odrgnino na povrhnjici/kutikuli, kar privede do tega, da se žuželka izsuši. Škodljivec lahko prašne delce zaužije oz. je preprosto prekrit z njimi, kar povzroči absorpcijo epikutikularnih lipidov, kar posledično vodi do izsušitve živali. Glavni način delovanja zeolita je torej desikacija ali izsušitev (Eroglu in sod., 2017).

3.2.1 Zgledi uporabe zeolita

Zeolit se kot sredstvo za zatiranje škodljivih organizmov v praksi uporablja predvsem proti skladiščnim škodljivcem. Uporablja se tako v naravni obliki kot tudi v molekularni obliki posamezne komponente, ki tvori zeolit kot tak (npr. kristali aluminijevega silikata, ipd.). Uporaba naravnega zeolita se je izkazala kot učinkovita metoda zatiranja koruznega žužka (*Sitophilus zeamais* Motschulsky, 1855, Coleoptera: Curculionidae) na zrnju koruze, vendar pri koncentracijah prašiva večjih od 50 g > 1 kg koruze (Haryadi in sod., 1994; Eroglu in sod., 2017). V raziskavi Kljajić in sod. (2010) opisujejo pozitivne rezultate učinkovitosti naravnega tipa zeolita, izkopanega v Srbiji, pri uporabi na zrnju pšenice za zatiranje riževega žužka (*Sitophilus oryzae* [L., 1763], Coleoptera: Curculionidae), žitnega kutarja (*Rhyzopertha dominica* [F., 1792], Coleoptera: Bostrychidae) in riževega mokarja (*Tribolium castaneum* [Herbst, 1797], Coleoptera: Tenebrionidae), Bohinc in sod. (2020a) pa poročajo o zadovoljivem delovanju naravnega zeolita proti koruznemu žužku. Vsi našteti predstavljajo pomembne skladiščne škodljivce žita.

Pomembno je dodati, da omenjeno inertno prašivo načeloma ne uporabljajo za zatiranje gospodarsko pomembnih škodljivcev na prostem. Prav tako ni podatka o uporabi zeolita za preprečevanje škodljivosti koloradskega hrošča.

3.3 LESNI PEPEL

Fizikalni in mehanski načini zatiranja škodljivcev spadajo med najstarejše načine zatiranja škodljivih žuželk. Ti vključujejo neposredne in/ali posredne ukrepe, ki so lahko preventivni ali kurativni. Takšni ukrepi so pogosto okolju prijaznejši, cenejši ter združljivi z drugimi metodami zatiranja škodljivcev. Zaradi teh lastnosti se lahko bolje vključujejo v postopke IVR, čeprav ne povzročijo takojšnjega ali drastičnega zmanjšanja populacije določenega škodljivca (New Zealand Digital Library, 2022).

Eden od teh načinov vključuje rabo lesnega pepela kot sredstva za zatiranje škodljivcev na prostem ter tudi kot sredstva za tretiranje žita proti skladiščnim škodljivcem. V mnogih delih sveta je še danes običajna praksa posipanje lesnega pepela po raznih zelenjadnicah in vrtninah. S tem poskušajo preventivno preprečiti škodo, ki bi jo sicer povzročil masovni pojav škodljivcev ter hkrati izboljšujejo kakovost tal (gnojijo s pepelom, večji delež hranil v tleh) (New Zealand Digital Library, 2022).

Lesni pepel deluje na škodljivce podobno kot ostali inertni prahovi. Načeloma mehansko, torej kot prepreka za žuželke ter fizično ob stiku. Ob stiku žuželke z lesnim pepelom, ta poškoduje epikutikularno povrhnjico in vosek na povrhnjici. Deluje tudi higrofilno, posledično žuželka izgublja vodo in se izsuši, torej pride do desikacije insekta (Boiteau in sod., 2012). Zaradi izrazitega vonja deluje lesni pepel tudi na olfaktorne receptorje škodljivcev in prikrije kemične signale/vonj gostiteljske rastline. S tem škodljivec težje locira gostiteljsko rastlino. Težava uporabe lesnega pepela na prostem je v tem, da zahteva redno posipanje zaradi rose, dežja, vetra in ostalih okoljskih dejavnikov, ki odnašajo lesni pepel z listja (New Zealand Digital Library, 2022).

V praksi zatiranja škodljivcev se lesni pepel primarno uporablja proti skladiščnim škodljivcem, njegova uporaba pa je vezana predvsem na države tretjega sveta (Jean in sod., 2015), kjer v večji meri kot v razvitem svetu izkoriščajo lokalno dostopna inertna prašiva. V raziskavi Demissie in sod., (2008) lahko preberemo, kako zatirati koruznega žužka (*Sitophilus zeamais*) z lesnim pepelom in dvema drugima inertnima prašivoma. Pomembno je dodati, da so po njihovih ocenah vsa prašiva zmanjšala izleganje novih škodljivcev, zmanjšala se je tudi izguba mase zrnja in poškodbe na zrnju. Izpostavijo tudi to, da je uporaba lesnega pepela pri vseh razvojnih stadijih škodljivca povzročila relativno majhno smrtnost žužkov 3 dni po vzpostavitvi poskusa. Po 15 dneh izpostavljenosti pa je bila smrtnost v istem obravnavanju zelo vilika, in sicer med 97 in 100 % (Demissie in sod., 2008). Zelo dobro insekticidno delovanje lesnih pepelov na koruznega žužka je bila nedavno dokazana tudi v domači raziskavi (Bohinc in sod., 2018), po največji učinkovitosti pa je izstopal lesni pepel navadne smreke.

3.3.1 Zgledi uporabe lesnega pepela

Kljub manjšemu poznavanju in relativno novi tematiki zatiranja škodljivcev z lesnim pepelom na prostem je bilo na to temo nekaj že preučenega. Za nas je predvsem pomembno zatiranje koloradskega hrošča z alternativnimi metodami oz. alternativnimi sredstvi. Boiteau in sod. (2012) so preučili insekticidno učinkovitost lesnega pepela proti koloradskemu hrošču. Z laboratorijskimi raziskavami so dokazali, da lesni pepel deluje insekticidno na odrasle osebke in ličinke koloradskega hrošča. Smrtnost odraslih osebkov in ličink je v njihovem poskusu dosegla 100 % po 10 dneh neprekinjene izpostavljenosti lesnemu pepelu. V poskusu enkratnega nanosa lesnega pepela na osebke pa je po 10 dneh smrtnost dosegla le 22 % vseh preučevanih žuželk.

Boiteu in sod. (2012) izpostavljajo tudi to, da je nanos lesnega pepela zmanjšal intenziteto hranjenja vseh preučevanih razvojnih stadijev škodljivca vsaj na samem začetku poskusa. Navedeni raziskovalci ugotavljajo tudi, da njihovi dobri rezultati v laboratorijskih razmerah niso bili potrjeni tudi na prostem. Kot glavno težavo omenjajo vlago in ostale okoljske dejavnike, ki lesni pepel inaktivirajo in sperejo z rastlin. Omenjajo, da bi bila za učinkovitejšo uporabo lesnega pepela na prostem potrebna hidrofobna formulacija pripravkov.

3.4 RASTLINSKI PRAHOVI

Insekticidi na osnovi rastlinskih izvlečkov postajajo vse pomembnejši v komercialni rabi in predstavljajo pomembno komponento prihodnje rabe sredstev za varstvo rastlin, saj lahko svetovna proizvodnja hrane strmo pade zaradi upada razpoložljivih sintetičnih fitofarmacevtskih sredstev. Študij o uporabnosti in učinkovitosti različnih vrst rastlin, tako domorodnih kot invazivnih tujerodnih rastlin, kot sredstev za zatiranje škodljivcev je precej, a je poročil o njihovem učinkovitem zatiranju škodljivih organizmov relativno malo. Rastlinska vrsta, iz katere so doslej pridobili največ insekticidov, je neem (*Azadirachta indica* A. Juss). Poleg te so med ostalimi rastlinskimi vrstami najpogosteje omenjene še vrste *Chrysanthemum cinerariifolium* Sch. Bip., *Rosmarinus officinalis* L., *Nicotiana* sp. itd. (Isman, 2017).

Bioaktivne snovi in rastlinski insekticidi lahko delujejo na več načinov; repelentno ali odvračalno, na zmanjšanje odlaganja jajčec in tudi na zmanjšanje hranjenja ali manjšo ješčnost škodljivcev. Povzročajo lahko tudi motnje v razvoju žuželk in akutno smrtnost (Isman, 2017). Gre za splošni opis načina delovanja rastlinskih snovi in ekstraktov, ker je malo podatkov, ki opisujejo način delovanja specifične snovi ali ekstrakta.

Izpostavili bi lahko tudi uporabo prahov oz. izvlečkov iz invazivnih rastlin. Uporaba slednjih bi lahko razbremenila okolje in omejila njihovo širjenje (Bohinc in sod., 2020b).

3.4.1 Zgledi uporabe rastlinskih prahov in rastlinskih izvlečkov

Obstaja kar nekaj raziskav, ki opisujejo uporabo rastlin kot sredstev za zatiranje skladiščnih škodljivcev. Raziskav glede uporabe rastlinskih prahov proti koloradskemu hrošču je zelo malo, nekoliko več pa iz področja uporabe rastlinskih izvlečkov, ki jih zato izjemoma omenjamo tudi v tem poglavju. Tudi iz tega stališča lahko dodamo, da je delo pri ugotavljanju novih metod in snovi, ki bi lahko delovale zatiralno proti koloradskemu hrošču ključno.

Zaradi znanega dejstva, da populacije koloradskega hrošča, ki so odporne proti insekticidom, najdemo na različnih območjih sveta (Azija, Evropa, Severna Amerika) (Crossley in sod., 2022), se raziskave že dlje usmerjajo v preučevanje rastlinskih izvlečkov ali prahov, pridobljenih iz različnih rastlinskih organov.

Pri preučevanju delovanja rastlinskih izvlečkov in rastlinskih prahov se raziskovalci osredotočajo na ugotavljanje različnih načinov delovanja rastlinske vrste na tega pomembnega škodljivca. Przybylski (2002) ugotavlja repelentno delovanje navadnega vratiča (*Tanacetum vulgare* L.) na vse razvojne stadije koloradskega hrošča. Navadni vratič so uporabili kot prašivo in v obliki izvlečkov. Da zmleti plodovi čilija niso učinkovit insekticid za zatiranje koloradskega hrošča, dokazujejo Mircea in sod. (2015). Med potencialnimi rastlinskimi izvlečki, ki bi lahko zavirali razvoj ličink in odraslih osebkov koloradskega hrošča Scott in sod. (2003) navajajo izvlečke iz rastlinskih vrst iz družine Piperaceae. Rastlinski izvlečki vrst *Angelica archangelica* L., *Grindelia camporum* Greene, *Inula auriculata* Boiss. & Balansa kažejo zelo veliko sposobnost zaviranja hranjenja ličink koloradskega hrošča (Pavela, 2010). Podobno izpostavijo tudi Alkanin sod. (2015), ki navajajo, da izvlečki navadnega hmelja zavirajo hranjenje ličink koloradskega hrošča.

Bohinc in sod. (2020) so preučevali uporabo prahov iz listov sedmih različnih vrst invazivnih rastlin kot sredstva za zatiranje riževega žužka (*Sitophilus oryzae*) na uskladiščnem žitu. Ugotovili so, da nobeden izmed uporabljenih prahov ni imel zadovoljivega učinka na izbranega škodljivca.

Za nas zanimivejšo rastlinsko vrsto, veliki pajesen (*Ailanthus altissima* Mill.), ki je med drugim tudi invazivna na območju Slovenije, lahko preberemo, da nudi določeno insekticidno delovanje proti različnim vrstam skladiščnih škodljivcev (*Sitophilus oryzae*, *Oryzaephilus surinamensis* (L., 1758) idr.). Lu in Wu (2010) sta testirala kontaktno in fumigantno delovanje ekstrakta pripravljenega iz lubja velikega pajesena. V študiji zaključita, da ekstrakt deluje zatiralno tako v kontaktni obliki nanosa kot tudi v obliki fumiganta. S tem, da je umrljivost žužkov pri kontaktni aplikaciji manjša in potrebuje več časa (72 ur za 70 % smrtnost) kot v obliki fumiganta, ki deluje bolje in hitreje (24 ur za 80 % smrtnost).

3.5 KREMEN

Kremen je trd, kristalinični mineral, sestavljen iz silicijevega dioksida. Gre za drugi najpogostejši mineral, ki ga najdemo v zemeljski skorji. Za namene varstva rastlin ga uvrščamo med inertne prahove, ki niso toksični za sesalce. Pozitivna lastnost uporabe kremena je ta, da je lahko dostopen in ne bi predstavljal velikega stroška za končnega uporabnika (Rojht in sod., 2010).

Kljub številnim raziskavam glede uporabe prahov iz različnih mineralov in posledično kremena kot insekticida, njihov način delovanja ni popolnoma ugotovljen. Predlagane so bile različne teorije, ki so bile na splošno naklonjene domnevi ‚desikacije' (izsušitve), ki predvideva, da prah deluje s spodbujanjem izgube vode pri žuželkah (Alexander in sod., 1944a). Ugotovili so tudi, da prašiva s tršo mineralno sestavo ni mogoče zdrobiti na zelo majhne delce, posledično ti ne vplivajo na respiratorne poti žuželk in delujejo predvsem fizično ob stiku (Alexander in sod., 1944a). Dodali bi lahko tudi, da je učinkovitost močno odvisna od relativne zračne vlage v okolju, v katerem se žuželke nahajajo (Alexander in sod., 1944b).

3.5.1 Zgled uporabe kremena

Raziskav o uporabi kremena proti koloradskemu hrošču nismo zasledili. Vendar smo ob brskanju po literaturi ugotovili, da je bila uporaba inertnih prahov ter posledično tudi kremena razširjena že v starem Egiptu, ko so z mešanjem kremenovega peska in žita poskušali omejiti pojavnost različnih skladiščnih škodljivcev (Alexander in sod., 1944b). Danes se v državah v razvoju poslužujejo podobnih praks, vendar so te vseeno bolj izpopolnjene. Zamisel o uporabi kremena kot insekticida je torej več kot živa.

Rojht in sod. (2010) so preučevali insekticidno učinkovitost 5 različnih tipov kremenovega peska, pridobljenega na različnih lokacijah po Sloveniji. Vzorci so se razlikovali tudi po sestavi in obdobju izvora. Delež SiO_2 je bil v vseh vzorcih nad 90 % (od 91,52 % do 99,24%). Za namene raziskave so omenjene vzorce kremenovega peska zmešali z žitom in jim dodali odrasle osebke riževega žužka. Insekticidno delovanje so preučevali po 7, 14 in 21 dneh pri 4 različnih temperaturah (20, 25, 30 in 35 °C) in dveh različnih vrednostih relativne zračne vlage (55 in 75 %). Ugotovljeno je bilo, da so uporabljeni vzorci imeli le rahel insekticidni učinek na odrasle osebke riževega žužka in niso primerni za širšo uporabo za zatiranje skladiščnih škodljivcev, kot je na primer rižev žužek. Razlog slabe učinkovitosti, kljub veliki vsebnosti SiO_2, je lahko tudi v tem, da so kremenovi delci precej veliki in jih je težje zdrobiti ali ‚zmleti' na manjši/finejši prah, ki bi najverjetneje bolje učinkoval pri zatiranju škodljivih žuželk (Alexander in sod., 1944c).

4 RAZPRAVA

Zatiranje koloradskega hrošča je od samega začetka temeljilo na intenzivni rabi insekticidov, kar je posledično vodilo v razvoj odpornosti proti določenim aktivnim snovem. Danes, ko je razpoložljivih insekticidov vse manj, zlasti v EU, moramo razmišljati o novih možnostih in rešitvah, ki bi bile tudi potencialno učinkovite in manj škodljive za okolje in ljudi (Balaško in sod., 2020).

V članku smo našteli, kategorizirali in izpostavili širok nabor različnih vrst inertnih prahov, ki se uporabljajo predvsem za zatiranje skladiščnih škodljivcev (Golob, 1997; Eroglu in sod., 2017; Bohinc in sod., 2020ab, itd.). Omenjena prašiva bi lahko bila potencialno učinkovita tudi proti koloradskemu hrošču (Boiteu in sod., 2012). Ideje o uporabi inertnih prahov kot sredstev za zatiranje najrazličnejših škodljivcev so imeli že stari Egipčani. Gre za alternativno metodo, ki bi lahko pripomogla k učinkovitejšemu integriranemu varstvu rastlin, ki temelji na celostnemu zatiranju škodljivih organizmov. Za uspešno implementacijo v praksi je ključnega pomena učinkovitost uporabljene metode. Inertne prahove ločimo po skupinah (prahovi s kremenom, brez kremena, naravni SiO_2, itd.). Za učinkovito insekticidno delovanje inertnih prahov so ključnega pomena velikost delcev, oblika delcev, uniformnost delcev, relativna zračna vlaga, temperatura ter ostale pomembnejše spremenljivke, ki vplivajo na delovanje prahov. Inertni prahovi na žuželko delujejo tako, da jo izsušijo (pepel, zeolit, ipd.) ali pa jo mehansko poškodujejo (diatomejska zemlja, kremen, ipd.), kar posledično pomeni postopno izgubo vode pri škodljivemu organizmu. Nekatera sredstva delujejo tudi na dihalne poti (Alexander in sod., 1944a). Uporaba inertnih prahov na prostem predstavlja uporabnikov večji izziv, saj morajo upoštevati še ostale okoljske dejavnike, ki negativno vplivajo na nanesena sredstva (izpiranje, odnašanje, ipd.). Zato bo potrebno še veliko dela in poskusov, da bodo uporabniki inertne prahove učinkoviteje nanašali na rastline in da bodo ti učinkovito delovali na ciljne organizme. Uporaba inertnih prahov ima po našem mnenju precejšen potencial na področju nekemičnega zatiranja škodljivih organizmov. Skupaj z ostalimi metodami IVR bi lahko pripomogla k bolj zdravemu ekosistemu z večjo biotsko raznovrstnostjo in manjšim številom škodljivcev.

5 ZAKLJUČKI

Tudi sto let po vnosu v Evropo je koloradski hrošč najpomembnejši škodljivec krompirja na Stari celini. Z oženjem nabora sintetičnih FFS in pojavom odpornosti škodljivca na številne sintetične insekticide se nabor učinkovitih sredstev, ki bi tega škodljivca učinkovito zatrli, naglo zmanjšuje. Inertni prahovi predstavljajo eno izmed alternativ, ki bi lahko bila ob sočasni uporabi drugih metod IVR potencialno učinkovita tudi pri uporabi proti koloradskemu hrošču na krompirju. Glede na rezultate dosedanjih raziskav bi lahko bil eden od učinkovitejših inertnih prahov za zatiranje tega pomembnega škodljivca lesni pepel, ki spada med najstarejše znane insekticidne snovi. Za optimizacijo uporabe lesnega pepela ali drugih vrst inertnih prahov in njihovo čim večjo učinkovitost v naravnih razmerah pa bodo potrebe še nadaljnje raziskave.

6 ZAHVALA

Prispevek je nastal v okviru aplikativnega projekta L4-3178 Razvoj in optimizacija nekemičnih načinov zatiranja rastlinskih škodljivcev z namenom njihove implementacije v sisteme trajnostnega kmetijstva, ki ga financirata Javna agencija za raziskovalno dejavnost RS (ARRS) in Ministrstvo za kmetijstvo, gozdarstvo in prehrano RS (MKGP).

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