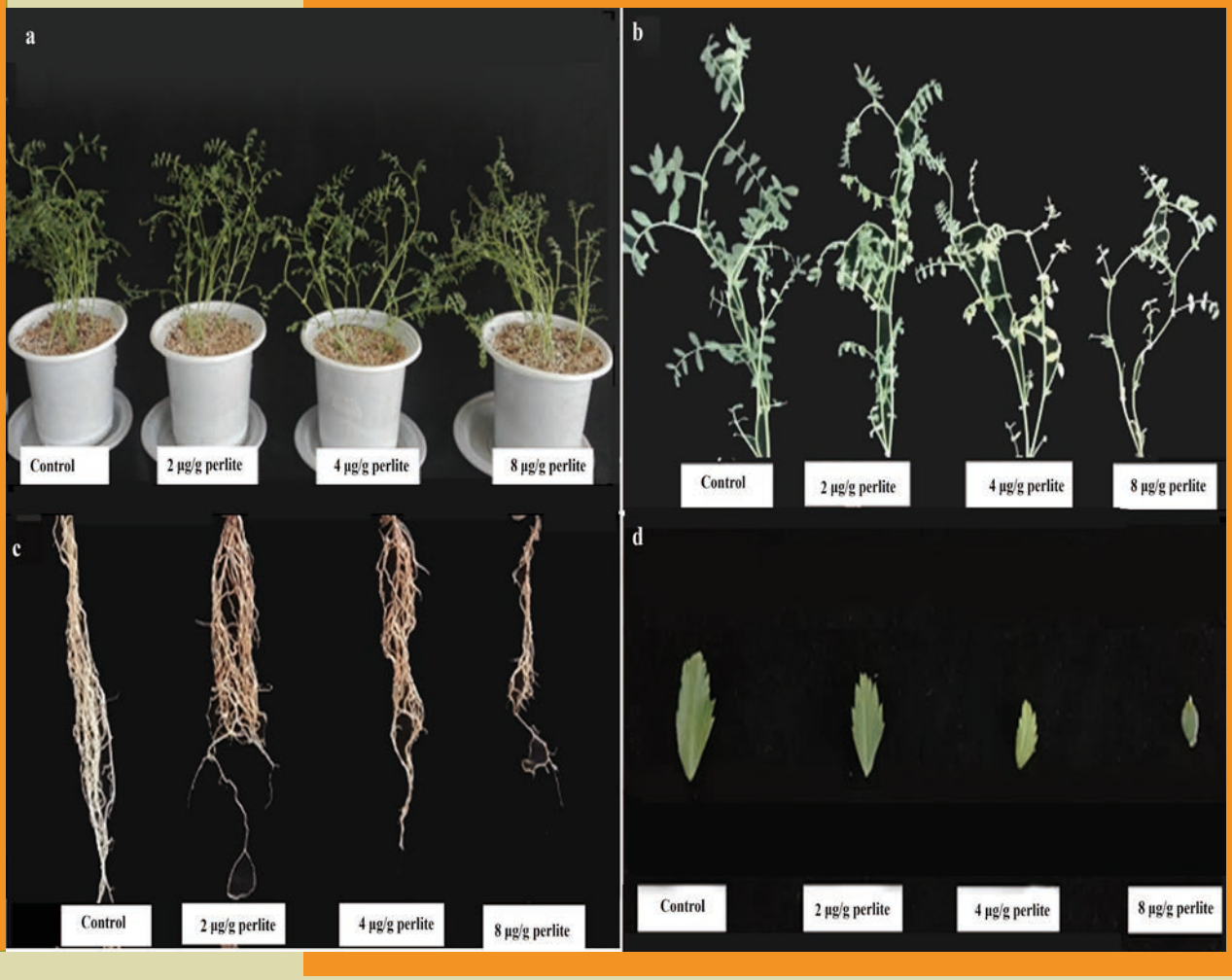

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Ovitek: Učinki kadmija na rast čičerke (*Cicer arietinum* L.) v normalnih razmerah (kontrola) in pri različnih koncentracijah kadmija: a) sejanke, b) nadzemni deli, c) korenine, d) listna površina (kontrola, 2, 4 in 8 µg Cd g^{-1} perlita) (Foto: Maryam Kolahi, 1–18)
*Cover: Effect of cadmium on chickpea (*Cicer arietinum *L.) growth under normal and various concentrations of cadmium. a) Seedlings, b) Aboveground parts, c) Roots, d) Leaf areas (control, 2, 4 and 8 µg Cd g^{-1} perlite) (Photo: Maryam Kolahi, 1–18)*

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Pinched sunflowers (*Helianthus annuus* 'Teddy Bear') produce high-quality flowers under high nitrogen fertilizer

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Pinched sunflowers (*Helianthus annuus* 'Teddy Bear') produce high-quality flowers under high nitrogen fertilizer

Abstract: This study was investigated the effect of removing the central bud (pinching) and different levels of nitrogen fertilizer urea on some morphological and physiological traits of ornamental sunflower. This study was conducted as a factorial experiment in a randomized complete block design with four replications on ornamental sunflower (*Helianthus annuus* 'Teddy Bear') at Horticulture Farm, Department of Horticulture, Ferdowsi University of Mashhad, Iran, in 2020-2021. The first factor was pinching in two levels (pinching and non-pinching) and the second factor was using urea at four levels (0, 200, 300, and 400 kg ha^{-1}) in the form of water-soluble fertilizer. Results showed that the highest flower dry mass (59.25 g) was observed in pinched plants fertilized by 400 kg ha^{-1} of urea. Besides, the application of a high level of urea fertilizer and pinching treatment increased the amount of total chlorophyll and chlorophyll b. By removing the central bud, the amount of N, P, K, Ca, Zn, and Fe elements in the leaf increased by 1.5, 1.6, 1.3, 1.9, 1.4, and 1.5 times, respectively. Therefore, pinching and the adding of urea fertilizer at 400 kg $ha^{-1}$is recommended for the production of high-quality sunflower plant 'Teddy Bear'.

Key words: flowering period, head diameter, nutrient elements, photosynthesis, plant height

Pincirane sončnice (*Helianthus annuus* 'Teddy Bear') dajejo visoko kakovostna socvetja pri gnojenju z velimi količinami dušikovih gnojil

Izvleček: V raziskavi je bil preučevan učinek odstranjevanja (pinciranja) osrednjega socvetja in različnih odmerkov gnojenja z ureo na nekatere morfološke in fiziološke lastnosti okrasnih sončnic. Raziskava je bila izvedena kot popolni faktorski bločni poskus s štirimi ponovitvami na okrasnih sončnicah (*Helianthus annuus* 'Teddy Bear') na Horticulture Farm, Department of Horticulture, Ferdowsi University of Mashhad, Iran, v rastni sezoni 2020-2021. Prvi dejavnik je obsegal dve ravni pinciranja (pincirano in ne pincirano), drugi dejavnik pa štiri različne odmerke uree (0, 200, 300, and 400 kg ha^{-1}) v obliki vodotopnega gnojila. Rezultati so pokazali, da je bila dosežena največja suha masa socvetij (59,25 g) pri pinciranih rastlinah in uporabi 400 kg ha^{-1} of uree. Večji odmerek uree je pri pinciranih socvetjih povečal vsebnost celokupnega klorofila in klorofila b. Pri odstranitvi osrednega socvetja se je vsebnost N, P, K, Ca, Zn in Fe v listih povečala za 1,5; 1,6; 1,3; 1,9; 1,4 in 1,5 krat. Zaradi naštetega priporočamo pinciranje in gnojenje s 400 kg ha^{-1} uree za vzgojo kakovostnih sončnic 'Teddy Bear'.

Ključne besede: cvetenje, premer koška, hranila, fotosinteza, višina rastlin

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1 INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an annual plant belonging to the Asteraceae family. This plant is native to North America and has medicinal, nutritional, and ornamental uses (Sehrawat et al., 2003) beside of its usage as a biodegradable source in biodiesel fuels (Saba et al., 2016). According to the specialized institute of cut flowers, in some sunflower cultivars, such as 'Pro-Cut Gold' and 'Sunrich Lemon', the stems are so long. In contrast, other sunflower cultivars produce lateral shoots and have short stems and uniform flowers (Dole, 2002). Removing the central bud (pinching) is considered one way to stimulate the plant to produce lateral branches and increase the number of stems per plant (Wien, 2015; Cheema, 2018). Depending on the stage of plant growth, pinching can be beneficial or harmful for plants (Smakel, 2006), as pinching of the different sunflower cultivars at the right time enhanced flower production three to four times (Wien, 2012a). However, pinching delays flowering and reduces flower size (Cheema, 2018) and the formation of flowers for 7-10 days (Wajid et al., 2007). Wien (2016b) reported that pinching the 'Sunrich Orange' cultivar, led to the production of smaller flowers but appropriate stem length. The smaller size of the flower, but with the marketable stem length, allows the florists to use them in arranging the flower bouquets properly. The study results of Badge and Panchbhai (2018) revealed that pinching the African marigold (*Tagetes erecta* L.) plants (15 days after transplanting) lead to the production of maximum flower yield in comparison to other treatments. The maximum nitrogen, phosphorus, and potassium content and uptake, as well as yield parameters, were obtained by pinching the plants 15 days after transplanting and foliar application of gibberellic acid at 300 mg l^{-1} (Badge et al., 2015). Prakash et al. (2016) reported that pinching the African marigold (*Tagetes erecta*) affects the plant height, number of lateral branches, number of flowers, and number of days to 50 % of flowering.

Adequate nutrition with essential elements, especially with nitrogen, is very important for the successful development of plants. Nitrogen is an essential nutrient that plays a role in the structure of various proteins, enzymes, coenzymes, nucleic acids, and cytochromes (Hassegawa et al., 2008), as well as, involving in the cell division and expansion, thereby increasing leaf length and width (Kumari, 2011, Lehri et al., 2011). Besides, this element plays a crucial role in the formation of chlorophyll and has a vital function in supplying carbohydrates and photosynthesis (Wajid et al., 2007). The effect of nitrogen on plant growth and development has often been linked to increased photosynthesis because the appropriate amount of nitrogen determines plant yield (Mekonnen et al., 2002). Studies indicated that the increase in growth and yield of the sunflower plant is dependent upon the adequate supply of nitrogen (Ali et al., 2004; Ali, 2015). The results of Oad et al. (2018) study indicated that sunflower plants treated with foliar application of urea (1 %) after 35 days of sowing in addition to recommended soil applied urea (130 kg ha^{-1}) led to the highest plant height, head diameter, grains per head, seed index, and grain yield. Ali et al. (2014) reported that the application of 80 kg ha^{-1} nitrogen fertilizer resulted in an increased plant height and head diameter of the sunflower plants. In another study, a significant increment in crop growth, biomass, dry matter production, and biological yield resulted in 100 kg ha^{-1} of N rate application (Saifullah, 1996), but Handayati and Sihombing (2019) recommended the application of 150 kg ha^{-1} nitrogen for the cultivation of this plant.

Considering the effect of pinching and nitrogen on the reproductive and vegetative traits of the sunflower plant, the present experiment was aimed to investigate the effect of removing the central bud (pinching) and different levels of nitrogen fertilizer (urea) on flowering, flower size, plant height, and other morphological and physiological traits of ornamental sunflower (*Helianthus annuus* 'Teddy Bear').

2 MATERIALS AND METHODS

The field experiment was conducted at Research Farm, Department of Horticulture, Ferdowsi University of Mashhad, Iran, in 2020-2021. Before planting, chemical analysis of the soil was done at an upper 0-30 cm zone, the results of which are shown in Table 1.

This experiment was conducted as a factorial experiment in a randomized complete block design with four replications on ornamental sunflower (*Helianthus annuus* 'Teddy Bear'). The first factor was removing or not removing the central bud (pinching); and the second factor was applied in four levels of adding urea fertilizer ($CO(NH_2)_2$): 0, 200, 300, and 400 kg ha^{-1} in the form of water-soluble fertilizer. The sunflower seeds were purchased from the Dutch Hemogenetic Company and sown in April 2020. Four weeks later, the seedlings with four true leaves were planted at spacing 50 × 20 cm. Ten days after transplanting, urea fertilizer was applied three times (weekly) with irrigation water according to the mentioned levels. Then, one month after transplanting, the pinching treatment was applied.

During the experiment, the number of days to flowering (vegetative period), and the duration of the flowering period (flowering period) were recorded. The number of flowers per plant was counted, and the head

Table 1: The physical and chemical properties of the soil

Depth	Soil Texture	Sand	Clay	Loam	pH	EC	N	P	K	Fe	Zn	Ca
(cm)			(%)			(dS m^{-1})			(mg kg^{-1})			
0-30	Sandy loam	40	33	27	7.5	1.3	610	606	6251	24716	52	29371

diameter and the stem diameter of each treatment were measured with a digital caliper. In 50 % of the flowering stage, the number of leaves per plant and the plant height were calculated. At this stage, the rates of photosynthesis and transpiration were also measured using a portable photosynthesis system (Li-6400) from 9:00 to 11:00 AM under natural conditions.

Fresh leaf tissue was used for the measurement of chlorophyll contents. 0.2 g fresh leaf was crushed in 10 ml of methanol 96 %. The resulted solution was filtered through Whatman filter paper and then centrifuged at 2500 rpm for 10 minutes. The supernatant optical absorption was then read at 653, and 666 nm using a spectrophotometer (model CE2502, BioQuest, UK) method (Sukran et al., 1998). Finally, the chlorophyll pigments were obtained using the following equations:

$$Chl_a\ (\mu g.ml^{-1}) = 15.65\ A_{666} - 7.340\ A_{653}$$
$$Chl_b\ (\mu g.ml^{-1}) = 27.05\ A_{653} - 11.21\ A_{666}$$
$$Chl_{Total} = Chl_a + Chl_b$$

After applying the treatments, at the beginning of the reproductive phase, N, P, K, Zn, Fe, and Ca elements in the sunflower leaf were measured. The amount of nitrogen in the plant was measured using the Kjeldahl method (Bremmer and Mulvaney, 1982). Concentrations of P, K, Ca, Zn, and Fe were analyzed by an inductively coupled plasma optical emission spectrometer (ICP-OES, Perkin-Elmer Optima 5300 DV) in plant samples (Van de Wiel, 2003).

The flowers were collected and dried during the flowering period to record the flower dry mass. After flowering, the leaf area was measured using the leaf area meter (Model Li-Cor-1300, USA). The specific leaf area (SLA = leaf area leaf dry $mass^{-1}$) and the leaf area ratio (LAR = leaf area total dry $mass^{-1}$) were also calculated. To measure the dry mass of plant components (stems, roots, leaves, and flowers) and the total dry mass, the plant samples were dried at 70 °C until the sample mass was held constant. Then the dry mass of different plant parts was recorded.

2.1 DATA ANALYSIS

Data were analyzed with One Way ANOVA using JMP® (v.8) software (SAS institute, 1989-2021), and means were compared based on the LSD test at the 5 % of probability level.

3 RESULTS

The results of ANOVA revealed that urea application and pinching have significant effects on different traits of the sunflower plant including mineral uptake, vegetative and generative traits, photosynthesis and transpiration rate, chlorophyll contents, and dry mass of different parts of sunflower (data not shown).

3.1 ELEMENT UPTAKE

The element content of sunflower shoots was affected by urea application and pinching, and not by their interaction. The use of urea led to an increase in N content in shoots as well as P, K, Ca, Zn, and Fe contents. As the amount of urea fertilizer increased, the accumulation of these elements in the shoots also increased. Using urea fertilizer at 400 kg ha^{-1} induced mineral accumulation 2.4, 2.6, 1.7, 2.3, 4.1, and 4.2 times more than the control for N, P, K, Ca, Zn, and Fe, respectively (Table 2). Contrariwise, these element contents decreased when pinching was applied. Pinched plants had 1.5, 1.6, 1.3, 1.9, 1.4, and 1.5 times less amount of N, P, K, Ca, Zn, and Fe than non-pinched sunflower plants, respectively (Table 2).

3.2 VEGETATIVE TRAITS

The interaction of pinching × urea fertilizer had a significant effect on vegetative traits including plant height, stem diameter, leaf number, leaf area, SLA, and LAR. Sunflower plants had the biggest height (153 cm) when grown using 400 kg ha^{-1} of urea fertilizer and not pinched, while pinched plants without urea fertilizing showed the lowest height (129 cm). The same results were obtained for stem diameter growth with 28.98 and 20.90 mm, respectively. The leaf number increased by urea application and pinching (27.5-30.0), whereas, the lowest number of leaf production (13.0) was recorded in non-pinched plants without urea. The biggest leaf area (16130.25 cm^2) showed in 400 kg ha^{-1} of urea applica-

Table 2: The simple effect of pinching and urea fertilizer treatments on element content in sunflower shoots

Factors	Treatments	N	P	K	Ca	Zn	Fe
				(mg kg^{-1})			
Urea fertilizer (kg ha^{-1})	0	940.00d*	820.00d	12346.0d	9167.0d	8.0000d	93.000d
	100	1088.00c	968.00c	16934.0c	11939.3c	11.3750c	108.500c
	200	1212.50b	1094.00b	19746.0b	20584.0b	17.8750b	333.875b
	400	2292.00a	2172.00a	20847.8a	20798.0a	32.8750a	393.125a
Pinching	-	1659.50a	1539.50a	19499.4a	20623.1a	20.3750a	277.000a
	+	1106.75b	987.50b	15437.4b	10621.1b	14.6875b	187.250b

*Means followed by similar letters in each trait and for each factor didn't have any significant difference based on LSD test ($p \leq 0.01$)

tion and pinching treatment, while non-pinched plants grown without urea fertilizer expanded their leaf to the minimum amount (8953.65 cm^2). SLA was the highest when 400 kg ha^{-1} of urea fertilizer with pinching (302.46 cm^2 g^{-1}) and 300 kg ha^{-1} of urea fertilizer without pinching (301.37 cm^2 g^{-1}) was applied and the lowest amount of SLA was shown in the 300 kg ha^{-1} of urea application with pinching (266.60 cm^2 g^{-1}) treatment (Table 3). The highest amount of the LAR was obtained in two treatments include 400 and 300 kg.ha^{-1} of urea fertilizer + pinching (73.64 and 72.97 cm^2.g^{-1}, respectively), and the lowest amount was recorded for plants with no urea fertilizing with (62.11 cm^2.g^{-1}) or without (62.36 cm^2 g^{-1}) pinching (Table 3).

3.3 GENERATIVE TRAITS

We obtained the highest number of flowers (77.75) in pinched plants fertilized by 400 kg ha^{-1} of urea fertilizer, and non-pinched plants produced the less flower number (21.25-26.00) in all levels of urea fertilizer (Table 4, A). The head diameter had the highest amount (146.68-15.1.59 mm) when pinching was not applied in plants of urea fertilizer in 0, 200, and 300 kg ha^{-1}, and the lowest amount (97.42 mm) was recorded in the pinched plants with no urea using. The number of days to first flower appearance and the duration of the flowering stage were affected by urea fertilizer, that is, the increase in urea levels led to prolongation of the vegetative and generative period and low levels of urea stimulate the entering to and shortening of the generative stage. Duration of the flowering stage also was increased by pinching up to 6 days compared to the non-pinched plants (Table 4, B).

3.4 CHLOROPHYLL CONTENTS, PHOTOSYNTHESIS, AND TRANSPIRATION RATE

The content of chlorophyll$_b$ and total chlorophyll was affected by the interaction of urea fertilizer × pinching, while the chlorophyll content was not influenced by interaction but was affected by simple effect of them. The plants which were grown under 400 kg ha^{-1} of urea fertilizer with (0.28 µg g^{-1} FM) or without pinching (0.24 µg

Table 3: The interaction effect of pinching × urea fertilizer on vegetative traits of the sunflower plant

Pinching	Urea fertilizer (kg ha^{-1})	Plant height (cm)	Stem diameter (mm)	Leaf number	Leaf area (cm^2)	SLA** (cm^2 g^{-1})	LAR** (cm^2 g^{-1})
-	0	139.33abc*	25.49abcd	13.00b	8953.65d	294.81bc	62.36c
-	200	143.66abc	26.25abc	22.00ab	9976.30cd	298.51ab	69.07ab
-	300	147.00ab	26.37abc	21.00ab	12407.76b	301.37a	72.97a
-	400	153.00a	28.98a	30.33a	9856.37cd	273.78cd	64.12bc
+	0	129.00c	20.90d	22.33ab	11416.25bc	276.35c	62.11c
+	200	133.00bc	21.45cd	27.50a	12969.25b	296.16ab	69.29ab
+	300	138.00abc	23.02bcd	26.66a	12813.25b	266.60e	65.21b
+	400	147.33ab	28.01ab	30.00a	16130.25a	302.46a	73.64a

*Means followed by similar letters in each trait do not have any significant difference based on the LSD test ($p \leq 0.01$)
**SLA: The specific leaf area, LAR: The leaf area ratio

Table 4: The interaction effect of pinching × urea fertilizer (A) and simple effect of them (B) on generative traits of the sunflower plant

(A)				(B)			
Pinching	Urea fertilizer (kg ha^{-1})	Flower number	Head diameter (mm)			Day to1st flowering (day)	Duration of flowering (day)
-	0	23.50d*	146.68a	Urea fertilizer (kg ha^{-1})	0	44.0000D	32.3750D
-	200	21.25d	151.59a		200	46.1250C	34.1250C
-	300	26.00d	148.68a		300	48.7500B	36.7500B
-	400	25.50d	119.27b		400	52.7500A	40.7500A
+	0	45.50c	97.42c	Pinching			
+	200	46.75c	106.84bc		-	44.9375A	32.9375B
+	300	63.50b	111.24bc		+	50.8750A	39.0625A
+	400	77.75a	110.04bc				

*Means followed by small (interaction effect) and capital (simple effect) letters in each trait does not have a significant difference based on the LSD test ($p \leq 0.01$)

g^{-1} FM) had the highest amount of chlorophyll b and the lowest was related to not using urea fertilizer for pinched and non-pinched plants (0.11-0.13 µg g^{-1} FM). In the same manner, total chlorophyll content was the highest in non-pinched plants treated by 400 kg ha^{-1} of urea fertilizer (0.45 µg g^{-1} FM), and the lowest amount was recorded in the pinched and non-pinched plants without urea fertilizing (0.25-0.26 µg g^{-1} FM)(Table 5, A). Unlike the chlorophyll$_b$ and total chlorophyll, the amount of chlorophyll only was affected by urea fertilizer and pinching. Urea fertilizer at 300 kg ha^{-1} (0.19 µg g^{-1} FM) and pinching (0.17 µg g^{-1} FM) provoked chlorophyll$_a$ accumulation. There was a trend for photosynthesis and transpiration rate, increasing urea levels from zero to 400 kg ha^{-1} enhanced the amounts of photosynthesis from 6.19 to 11.39 µmol mol^{-1} CO_2 and transpiration rate from 1.44 to 2.65 mmol.mol^{-1} H_2O, respectively. Pinching significantly led to a decrease in photosynthesis and transpiration rate (Table 5, B).

3.5 DRY MASS OF PLANT ORGANS

Leaf, head, root, and total dry mass of sunflower was affected by the interaction of urea fertilizer × pinching, as the highest amount of them was recorded on pinched plants were fertilized by 400 kg ha^{-1} of urea fertilizer, 53.33, 59.25, 39.99, and 219.04 g, respectively. Non-

Table 5: The interaction effect of pinching × urea fertilizer on chlorophyll b and total chlorophyll content (A) and simple effect of them on photosynthesis, transpiration rate and Chlorophyll a content (B) in sunflower plant

(A)				(B)				
Pinching	Urea fertilizer (kg ha^{-1})	Chlorophyll$_b$ content (µg g^{-1} FM)	Total chlorophyll content (µg g^{-1} FM)			Chlorophyll$_a$ content (µg g^{-1} FM)	Photosynthesis (µmol.mol^{-1} CO_2)	Transpiration rate (mmol mol^{-1} H_2O)
-	0	0.11c*	0.25d	Urea fertilizer (kg ha^{-1})	0	0.13C	6.19D	1.44D
-	200	0.15bc	0.30cd		200	0.15BC	7.67C	1.78C
-	300	0.11c	0.37b		300	0.19A	8.96B	2.08B
-	400	0.24a	0.45a		400	0.17AB	11.39A	2.65A
+	0	0.13c	0.26d	Pinching				
+	200	0.16bc	0.31c		-	0.15B	9.07A	2.09A
+	300	0.18b	0.31c		+	0.17A	8.09B	1.88B
+	400	0.28a	0.42ab					

*Means followed by small (interaction effect) and capital (simple effect) letters in each trait does not have a significant difference based on the LSD test ($p \leq 0.01$)

pinched plants had the lowest amount of leaf (30.37 g), head (33.75 g), root (22.78 g), and total (143.57-153.70 g) dry matter. Indeed, the total dry matter was not much significantly affected by urea fertilizer levels (Table 6, A).

Urea fertilizer and pinching had a significant effect on stem dry mass as a simple effect. The application of urea at 300 kg ha^{-1} induced the highest dry matter in the stem, while the lowest amount was detected when no urea fertilizer was used. Pinching increased stem dry mass to 1.2 times (62.03 g) in comparison with non-pinching (Table 6, B).

The percentage of dry matter allocation in different parts of the plant showed no distinctive difference between treatments. On average, the highest to the lowest percentage of dry matter allocation were for stem (32.7 %), head (26.0 %), leaf (23.4 %), and root (17.9 %), respectively (Table 7).

Table 7: The percentage of dry matter allocation in different parts of the sunflower plant under different levels of urea fertilizer and pinching

Pinching	Urea fertilizer (kg ha^{-1})	Dry mass (%)			
		Leaf	Head	Stem	Root
-	0	20.5	27.0	33.2	19.2
-	200	20.6	28.2	32.2	19.0
-	300	25.6	23.1	35.7	15.6
-	400	25.6	24.0	34.1	16.2
+	0	22.8	26.8	32.3	18.1
+	200	22.8	27.8	30.7	18.8
+	300	22.7	28.0	30.4	18.9
+	400	26.5	22.8	33.0	17.7
Mean		23.4	26.0	32.7	17.9

4 DISCUSSION

Besides the high cost of chemical fertilizer, the environmental impacts of their application are the important reason for the need to determine the exact amount of fertilizers. In our experiment, the interaction of different amounts of urea fertilizer (CO $(NH_2)_2$) and the removal of apical bud (pinching) had distinctive results on sunflower 'Teddy Bear' growth and development. The nitrogen fertilizer that used in this experiment is quite soluble and converts to ammonia in several days. So, as expected, a rise in the urea levels caused an increase in the nitrogen uptake. Similarly, the uptake of nitrogen is enhanced in broccoli plants when nitrogen fertilizer amounts increase (Vagen, 2003). In addition, the amount of P, K, Zn, Fe, and Ca was enhanced in the sunflower shoots by increasing the urea levels (Table 2). Confirmed results were reported by Karitonas (2003) and Yildirim et al. (2007) on broccoli plant, that an increase in the uptake of P, K, Fe, and Ca were shown by adding nitrogen fertilizer. Similarly, lettuce and tomato plants which were foliar sprayed by urea had higher amounts of N and K (Padem and Alan, 1995), and N, K, and Fe (Alan and Padem, 1994), respectively. All studied nutrient elements (i.e., N, P, K, Ca, Zn, and Fe) play several important functions and critical roles within plants; metabolism, and catabolism processes, so, increasing in their uptake by plants can explain the significant differences in the studied traits in this experiment. The availability of nitrogen in the soil increase

Table 6: The interaction effect of pinching × urea fertilizer (A) and simple effect of them (B) on the dry mass of different parts of the sunflower plant

(A)

Pinching	Urea fertilizer (kg ha^{-1})	Leaf dry mass (g)	Head dry mass (g)	Root dry mass (g)	Total dry masst (g)
-	0	30.37e*	33.75e	22.78e	143.57c
-	200	33.42de	37.14de	28.50cde	144.42c
-	300	41.17ab	45.75bcd	30.88bcd	170.03bc
-	400	36.00cde	40.00cde	25.07de	153.70c
+	0	41.31bcd	45.90bcd	32.84bc	183.78b
+	200	43.79bc	48.66bc	36.04ab	187.16b
+	300	48.06ab	53.40ab	35.70ab	196.48ab
+	400	53.33a	59.25a	39.99a	219.04a

(B)

		Stem dry mass (g)
Urea fertilizer (kg ha^{-1})	0	53.83B
	200	55.60AB
	300	60.87A
	400	57.19AB
Pinching	-	51.71B
	+	62.03A

*Means followed by small (interaction effect) and capital (simple effect) letters in each trait does not have any significant difference based on the LSD test ($p \leq 0.01$)

RuBisCO contents in leaves, even though some climate and soil factors including light, air humidity, and soil pH showed considerable influences on the fraction of nitrogen allocated to RuBisCO regionally (Luo et al., 2021). Many scientists believe that the higher uptake of essential nutrients by plants as a result of the urea application is related to the positive influence of nitrogen on the chemical properties of the soil (Malhi et al., 2006; Haydon et al., 2007; Choudhury et al., 2011; Ai et al., 2017; Adekiya et al.,2018; Pasley et al., 2019). Ewulo et al. (2009) stated the possible reason for this is related to more microbial soil activity induced by urea application that causes more production and mineralization of organic matter in the soil. The reduction in the soil pH is another probable reason for higher element uptake by urea application that is shown in the Adekiya et al. (2018) report. As the sunflower plants like the slightly acidic soils, this reduction in pH can improve elements uptake as the soil pH of the experiment site was close to neutral, 7.5 (Table 1).

'Teddy Bear' cultivar of sunflower is a dwarf cultivar and mature plants grow up maximum 140 cm. Urea application up to 400 kg ha^{-1} had a positive influence on plant height, and pinching reduced its effect. The suppressive effect of pinching on the plant height has been previously reported for different cultivars of sunflowers (Wien, 2016b; Cheema, 2018). Increasing the plant height and the stem diameter by using urea fertilizer is related to more leaf area production (Milford et al. 2000), while increasing the amount of chlorophyll in the sunflower leaves, followed by increasing photosynthesis and dry matter production, is closely related to higher uptake of various elements, including iron and zinc. It has been reported that iron is involved in the structure of chlorophylls, cytochrome, and nitrogenase enzymes, and zinc is involved in the activity of enzymes associated with chlorophyll formation and consequently increase photosynthesis, accelerating the formation of growth compositions such as tryptophan as the raw material of auxins (Haydon et al., 2007). Enhanced dry matter production in non-pinched plants under more urea fertilizer, is probably due to increased water and mineral absorption by extended roots and rapid growth (Solangi et al., 2015). Steer et al. (1986), also reported an increscent in N uptake and dry matter production by enhancing nitrogen fertilizer levels, as the application of low amounts of nitrogen fertilizer reduced leaf expansion and also the accumulation of dry matter in sunflower. We also obtained the higher dry matter of leaf, root, and head amounts in pinched plants in positive relation with urea levels, while there was an optimum level at 300 kg ha^{-1} in non-pinched plants (Table 6).

Leaf area, SLA, and LAR traits were the highest in 400 kg ha^{-1} of urea fertilizer application with pinching (Table 3). Leaf area is a critical index for plant growth as it is associated with important criteria including light interception, photosynthesis, transpiration, and evapotranspiration rates (Goudriaan and Van Laar, 1994; Zahoor et al., 2010). Leaf growth in earlier stages needs more nitrogen amounts (Evans, 1989; Johnson et al., 2010) and leaf area is limited when nitrogen is deficient by affecting cell division and enlargement (Roggatz et al., 1999). Pinching also had a positive effect on leaf area expansion in chrysanthemum 'Snowball' (Ona et al., 2015). An increase in leaf number after pinching is reported by others on different herbaceous plants (Sehrawat et al., 2003; Tomar et al., 2004; Sudarshan, 2004; Salyh, 2013; and Ona et al., 2015). It seems that it might be related to the fact that pinching alters the direction of growth from upward to lateral parts of the plant (Salyh, 2013).

The findings of this study indicated that pinching and urea application extended vegetative and flowering stages up to eight more days (Table 4. B). The number of days to flowering increased up to 70 days in pinched ornamental sunflowers, while non-pinched plants started to flower after 63 days (Wien et al., 2016). The same results were reported by Ona et al. (2015) for chrysanthemum 'Snowball' and other species (Ahmad et al., 2007; Ryagi et al., 2007; Salyh, 2013). The pinching effect on delayed flowering is due to delay in flower initiation and bud physiological maturity (Naresh and Singh, 2012) because the growth rate in axillary buds is slower than apical buds.

In pinched plants, flower number was affected by urea levels in a positive trend, while head diameter indicated a negative trend. Flower diameter was independently by cultivar decreased by pinching intensity (Burnett, 2017; Cheema, 2018). Other studies also confirmed these results (Ryagi et al., 2007; Habiba, 2012; Salyh, 2013; Ona et al., 2015). The removal of the shoot apex leads to the activation of dormant axillary buds below it to form branches Naresh and Singh (2012). Flower disk diameter of sunflower (Wien, 2016b) and the flower size of chrysanthemum (Ona et al. 2015) were reduced by pinching due the competition between branches and flowers. They have revealed that the number of the branches in a unit area has a negative linear relationship with head size in sunflower (Majid and Schneiter, 1987; Robinson et al., 1980; Wien, 2016b).

5 CONCLUSION

This study demonstrated that adding urea in the soil and pinching improved photosynthetic traits by increasing leaf area and number, SLA, LAR, and total chlorophyll content. The findings also revealed that the

interaction of pinching × urea application at 400 kg ha^{-1} is the best combination of investigated variation sources for the cultivation of sunflower 'Teddy Bear'. The suitable amount of dry matter production (219.04 g), number of flowers (77.7), head diameter (110.04 cm), and plant height (147.3 cm) are the important reasons for this recommendation.

6 AUTHOR CONTRIBUTION STATEMENT

All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

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8 CONFLICT OF INTEREST

The authors certify the following:

- This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue;

- The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

9 DATA AVAILABILITY

The data used to support the findings of this study are available from the corresponding author upon request.

10 REFERENCES

Adekiya, A.O., Aboyeji, C.M., Dunsin, O., Adebiyi, O.V., & Oyinlola, O.T. (2018). Effect of urea fertilizer and maize cob ash on soil chemical properties, growth, yield, and mineral composition of okra, *Abelmoschus esculentus* (L.) Oench. *Journal of Horticultural Research*, *26*(1), 67–76. https://doi.org/10.2478/johr-2018-0008

Ahmad, I., Ziaf, K., Qasim, M., & Tariq M. (2007). Comparative evaluation of different pinching approaches on vegetative and reproductive growth of carnation (*Dianthus caryophyllus*). *Pakistan Journal of Agricultural Sciences, 44*(4), 563-570.

Ai, Z., Wang, G., Liang, C., Liu, H., Zhang, J., Xue S., & Liu, G. (2017). The effects of nitrogen addition on the uptake and allocation of macro and micronutrients in *Bothriochloa ischaemum* on loess plateau in China. *Frontiers in Plant Science, 8*, 1476. https://doi.org/10.3389/fpls.2017.01476

Alan, R., & Padem, H. (1994). The influence of some foliar fertilizers on growth and chemical composition of tomatoes under greenhouse conditions. In: Proc. Solanacea in Mild Winter Climates. *Acta Horticulturae*, *366,* 397–404. https://doi.org/10.17660/ActaHortic.1994.366.49

Ali, A.B., Altayeb, O.A., Alhadi, M., & Shuang-En, Y. (2014). Effect of different levels nitrogen and phosphorus fertilization on yield and chemical composition hybrid sunflower grown under irrigated condition. *Journal of Environmental Sciences, 1*, 7-14.

Ali, H., Randhawa, S.A., &Yousaf, M. (2004). Quantitative and qualitative traits of sunflower (*Helianthus annus* L.) as influenced by planting dates and nitrogen application. *International Journal of Agriculture and Biology, 6,* 410-412.

Ali, M. (2015). *The response of sunflower cultivars to nitrogen fertilizer*. Annual Report (2000-01). Oil Seed Crops Research Program. Wad Medani, Sudan.

Badge, S., Panchbhai, D., & Gajbhiye, R. (2015). Nutrient content, uptake, and yield in African marigold (*Tagetes erecta* Linn.) as influenced by pinching and foliar application of gibberellic acid. *The Indian Journal of Agricultural Sciences, 49*(6), 534-538. https://doi.org/10.18805/ijare.v49i6.6681

Badge, S., & Panchbhai, D.M. (2018). Yield and benefit: cost ratio of African marigold influenced by pinching and growth regulator. *International Journal of Pure and Applied Bioscience, 6*(1), 1148-1153. https://doi.org/10.18782/2320-7051.5456

Bange, M.P., Hammer, G.L., & Rickert, K. G. (1997). Effect of radiation environment on radiation use efficiency and growth of sunflower. *Crop Science, 37*, 1208-1214. https://doi.org/10.2135/cropsci1997.0011183X003700040030x

Bremmer, J.M., & Mulvaney, C.S. (1982). "Total nitrogen", In: A.L. Page, R.H. Miller and D.R. Keeny, (Eds.), *Methods of Soil Analysis,* American Society of Agronomy and Soil Science Society of America, Madison, pp. 1119-1123.

Burnett, R.B. (2017). *Pinching and spacing effects on cut sunflowers (Helianthus annuus) production in East Texas*. Electronic Thesis and Dissertations 121:35-49.

Chantal, K., Ongor, B.T, Bandushubwenge, D., Soter, N., & Felix, SH. (2018). Effects of different nitrogen fertilizer levels on sunflower growth and yield attributes. *Pakistan Journal of Nutrition*, *17*, 557-562. https://doi.org/10.3923/pjn.2018.557.562

Cheema B.I. (2018). *Effect of pinching and spacing on the growth and development of sunflowers (Helianthus annuus) in east Texas* [master's thesis]. Texas: Stephen F. Austin State University. Department of Agriculture 70p.

Devecchi M. (2013). Post-harvest physiology of cut flowers of sunflowers 'sunrich orange' (*Helianthus annuus*): first experimental results. Paper presented at the *VIII International Symposium on Postharvest*

Physiology of Ornamental Plants. FAO, *669,* 381-388.
Dole J. (2002). ASFCG national cultivar flower seed trials. *Cut Flower Quarterly*, *15*(1), 7-22. https://doi.org/10.17660/ActaHortic.2005.669.50

Evans J.R. (1989). Photosynthesis and nitrogen relationships in leaves of C3 plants. *Oecologia*, *78*, 9–19. https://doi.org/10.1007/BF00377192

Ewulo, B.S., Babadele, O.O., & Ojeniyi ,S.O. (2009). Sawdust ash and urea effect on soil and plant nutrient contents and yield of tomato. *American-Eurasian Journal of Sustainable Agriculture, 3*(1), 88–92.

Goudriaan,, J., & Van Laar, H.H. (1994). *Modelling Potential Crop Growth Processes*. – Kluwer Academic Publ., Dordrecht. 239 pages. https://doi.org/10.1007/978-94-011-0750-1

Handayati, W., & Sihombing, D. (2019). Study of NPK fertilizer effect on sunflower growth and yield. *Paper presented at the AIP Conference Proceedings*. https://doi.org/10.1063/1.5115635

Hassegawa, R. H., Fonseca, H., Fancelli, A.L., Da silva, V.N., Schammass, E.A., Reis, T.A., & Correa, B. (2008). Influence of macro-and micronutrient fertilization on fungal contamination and fumonisin production in corn grains. *Food Control, 19*(1), 36-43. https://doi.org/10.1016/j.foodcont.2007.01.006

Haydon, M. J., & Cobbett, C.S. (2007). Transporters of ligands for essential metal ions in plants. *New Phytologist, 174*(3), 499-506. https://doi.org/10.1111/j.1469-8137.2007.02051.x

Johnson, I.R., Thornley, J.H., Frantz, J.M., & Bugbee, B. (2010). A model of canopy photosynthesis incorporating protein distribution through the canopy and its acclimation to light, temperature, and CO_2. *Annals of Botany, 106*, 735–749. https://doi.org/10.1093/aob/mcq183

Karitonas R. (2003). Development of a nitrogen management tool for broccoli. In: Proc. XXVI. IHC-Fertil. Strateg. Field Veg. Prod. *Acta Horticulture*, *627,* 125–129. https://doi.org/10.17660/ActaHortic.2003.627.15

Kumari, S. (2011). Effects of nitrogen levels on anatomy, growth, and chlorophyll content in sunflower (*Helianthus annuus* L.) leaves. *The Journal of Agricultural Science, 9,* 208-219. https://doi.org/10.5539/jas.v9n8p208

Lehri, S., Kurd, A., Rind, M., & Bangulzai, N. (2011). The response of *Gladiolus tristis* L. to N and P2O5 fertilizers. *SJA, 27*(2), 185-188.

Liu, T., Ren, T., White Ph. J., Cong R., & Lu J. (2018). Storage nitrogen co-ordinates leaf expansion and photosynthetic capacity in winter oilseed rape. *Journal of Experimental Botany*, *69*(12), 2995–3007. https://doi.org/10.1093/jxb/ery134

Luo, X., Keenan, T.F., Chen, J.M. et al. (2021). Global variation in the fraction of leaf nitrogen allocated to photosynthesis. *Nature Communications, 12***,** 1-8. https://doi.org/10.1038/s41467-021-25163-9

Majid, H.R., & Schneiter, A.A. (1987). Yield and quality of semidwarf and standard height sunflower hybrids grown at five plant populations. *Agronomy Journal*. 79:681–684. https://doi.org/10.2134/agronj1987.00021962007900040020x

Malhi, S.S., Lemke, R., Wang, Z., & Chhabra, B.S. (2006). Tillage, nitrogen and crop residue effects on crop yield, nutrient uptake, soil quality, and greenhouse gas emissions. *Soil Tillage Research, 90*(1-2), 171-183. https://doi.org/10.1016/j.still.2005.09.001

Mekonnen, A., Prasanna, R., & Kaushik, B. (2002). Cyanobacterial N2 fixation in presence of nitrogen fertilizers. *Indian Journal of Experimental Biology, 40*(7), 854-7.

Oad, R.K., Ansari, M.A., Kumar, J., & Menghwar, D.R. (2018). Effect of foliar applied urea on growth and yield of sunflower (*Helianthus annuus* L.). *Open Access Library, 5*(7), 1-13. https://doi.org/10.4236/oalib.1104668

Ona, A., Taufique, T., Roni, M., Jui N., & Uddin, A. (2015). Influence of pinching on growth and yield of snowball *Chrysanthemum*. *International Journal of Business, Social and Scientific Research, 3*(3), 174-178.

Ozer, H., Polat, T., & Oztruk, E. (2004). Response of irrigated sunflower hybrids to nitrogen fertilization: Growth, yield and yield components. *Journal of Plant Soil and Environment, 50*(5), 205-211. https://doi.org/10.17221/4023-PSE

Padem H., & Alan, R. (1995). The effect of foliar fertilizers on yield, chlorophyll and chemical content of lettuce (*Lactuca sativa* L.). *Atatürk University Journal of Agricultural Faculty*, *26*, 21–34.

Pasley, H.R., Cairns, J.E., Camberato, J.J., & Vyn, T.J. (2019). Nitrogen fertilizer rate increases plant uptake and soil availability of essential nutrients in continuous maize production in Kenya and Zimbabwe. *Nutrient Cycling in Agroecosystems*, *115*(3), 373-389. https://doi.org/10.1007/s10705-019-10016-1

Prakash, S., Anitha, P., Giridharan, M., Rajagopalan, A., & Rao, G.S. (2016). Impact of seasons and pinching on growth and flowering in African marigold (*Tagetes erecta* L.). *Journal of Tropical Agriculture, 54*(1), 50.

Robinson R.G., Ford J.H., Lueschen W.E., Rabas D.L., Smith L.J., Warnes D.D., Wiersma J.W. (1980). Response of sunflower to plant population. *Agronomy Journal, 72*, 869–871. https://doi.org/10.2134/agronj1980.00021962007200060003x

Roggatz, U., McDonald, A.J., Stadenberg, I., & Schurr, U. (1999). Effects of nitrogen deprivation on cell division and expansion in leaves of *Ricinus communis* L. *Plant, Cell and Environment, 22*, 81–89. https://doi.org/10.1046/j.1365-3040.1999.00383.x

Saba, T., Estephane, J., El Khoury, B., El Khoury, M., Khazma, M., El Zakhem, H., & Aouad, S. (2016). Biodiesel production from refined sunflower vegetable oil over KOH/ZSM5 catalysts. *Renew Energy, 90*, 301-306. https://doi.org/10.1016/j.renene.2016.01.009

Saifullah, N. (1996). *Exploration of yield potentials of three sunflower cultivars under varying nutritional status* [M.Sc Thesis]. Unpublished M. Sc Agronomy Thesis, Pakistan. University of Agriculture, Faisalabad.

Salyh, T. (2013). *Effect of nitrogen fertilization, planting media and pinching on the growth and volatile oil of geranium plants (Pelargonium graveolens L'Herit)*. [M.Sc thesis]. Iraq. University of Duhok. Department of Agriculture Engineering, 82p.

Sehrawat, S., Dahiya, D., Singh, S., & Rana, G. (2003). Effect of nitrogen and pinching on growth, flowering and yield of marigold (*Tagetes erecta* 'African Giant Double Orange'). *International Journal of Horticultural Science and Technology, 32*, 59-60.

Smakel, H.J. (2006). Pruning by pinching and disbudding. Documentary from Avocado Association Yearbook *Califonia USA*, *14*, 135 136.

Solangi, M., Suthar, V., Wagan, B., Siyal, A.G., Sarki, A., & Soothar, R.K. (2015). Evaluate the effect of nitrogen and phosphorus fertilizer doses on growth and yield of spinach (*Spinacia oleracea* L.). *Science International (Lahore), 28*(1), 379–383.

Sukran, D., Gunes, T., & Sivaci, R. (1998). Spectrophotometric determination of chlorophyll-A, B and total carotenoid contents of some algae species using different solvents. *Turkish Journal of Botany, 22*(1), 13-18.

Van de Wiel H.J. (2003). *Determination of elements by ICP-AES and ICP-MS*. National Institute of Public Health and the Environment (RIVM). Bilthoven, the Netherlands. 1-19.

Wajid, A., Ghaffar, A., Maqsood, M., Hussain, K., & Nasim, W. (2007). Yield response of maize hybrids to varying nitrogen rates. *Pakistan Journal of Agricultural Sciences, 44*(2), 217-220.

Wien, H. (2015). *Cut flower physiology*. Ithaca, Cornell University, Department of Horticulture.

Wien, H. (2016a). Pinching sunflower produces more stems-what about profits. *The Cut Flower Quarterly*, *28*(4), 14-15.

Wien, H.C. (2016b). Pinching ornamental sunflowers increases cut stem yield and reduces flower size. *Horttechnology, 26*(6), 762-766. https://doi.org/10.21273/HORTTECH03502-16

Yildirim E, Guvenc I., Turan M., & Karatas A. (2007). Effect of foliar urea application on quality, growth, mineral uptake and yield of broccoli (*Brassica oleracea* L., var. italica). *Plant, Soil and Environment, 53*(3), 120–128. https://doi.org/10.17221/2227-PSE

Zahoor, A., Riazi, M., Ahmad, S., Ali, H., Khan, M.B. et al. (2010). Ontogeny growth and radiation use efficiency of *Helianthus annuus* L. as affected by hybrids, nitrogenous regimes and planting geometry under irrigated arid conditions. *Pakistan Journal of Botany*, *42*(5), 3197-3207.

Zubillaga,, M.M., Aristi, J.P. & Lavado, R.S. (2002). Effect of phosphorus and nitrogen fertilization on sunflower (*Helianthus annus* L.) nitrogen uptake and yield. *Journal of Agronomy and Crop Science, 188*, 267-274. https://doi.org/10.1046/j.1439-037X.2002.00570.x

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Mycoviruses: trends in plant-fungus-mycovirus interactions and 'biocontrol' prospects in agriculture and the environment

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Mycoviruses: trends in plant-fungus-mycovirus interactions and 'biocontrol' prospects in agriculture and the environment

Abstract: Mycoviruses are cosmopolitan in plants, animals, fungi, bacteria, in soils, and water. There is a scarcity of information about them, which necessitated this review to provide some leads on where research should focus. Mycoviruses are able to persist in disparate types of hosts by utilizing diverse measures. They may engage either parasitic, pathogenic, or mutualistic tendencies. Mycoviruses employ many existential strategies that can be utilized by man. Hypovirulence may be induced in fungal hosts by mycoviruses via RNA silencing, alteration of genetic expression, and disruption of the transcriptome. Mycoviruses interact with killer phenotypes of yeasts and *Ustilago* spp. and proffer advantages to these fungi. Mycovirus interaction with some plants result in provision of thermal tolerance to plants. Based on their mode of microbe destruction mycoviruses may be used for waste disposal and termination of some life processes. For instance, grazer viruses completely oxidize the organic content of their host into carbon dioxide and inorganic nutrients, while lytic viruses release the organic material from their hosts without modification. Viruses may be utilized to facilitate the exchange of genetic material from one host to another. However, pathogenic mycoviruses exist especially in mushrooms.

Key words: control, disease complex, fungi synergy, integrated pest management, phage, relationship

Mikovirusi: trendi v interakcijah rastlina-gliva-mikovirus in izgledi 'biokontrole' v kmetijstvu in okolju

Izvleček: Mikovirusi so kozmopoliti v rastlinah, živalih, glivah, bakterijah, v tleh in vodi. O njih je le malo informacij, kar je bilo vodilo za ta pregled kot smernico za bodoče raziskave. Mikovirusi so sposobni bivati v različnih gostiteljih z različnimi načini preživetja. Uporabljajo lahko zajedalske, patološke ali mutualistične strategije, ki jih lahko koristimo tudi ljudje. Hipovirulenca je v glivnem gostitelju lahko vzpodbujena z mikovirusi preko RNA utišanja, spremembe izražanja genov in razgradnje transkriptoma. Mikovirusi sodelujejo z ubijalskimi fenotipi kvasovk in sneti (*Ustilago* spp.), kar daje prednosti tem glivam. Sodelovanje mikovirusov in nekaterih rastlin rezultira v njihovi toleranci na termperaturne spremembe. Na osnovi njihovega uničevanja mikrobov bi lahko mikoviruse uporabili za razgradnjo odpadkov in za zaključek nekaterih bioloških procesov. Na primer, virusi, ki se "pasejo" na mikrobih (grazer viruses) popolnoma oksidirajo organsko vsebino gostitelja do ogljikovega dioksida in anorganskih hranil med tem, ko litični virusi sproščajo organske snovi iz njihovih gostiteljev. Virusi se lahko uporabljajo za olajševanje izmenjave dednine iz enega gostitelja v drugega. Še posebej veliko patogenih mikovirusov živi v gobah.

Ključne besede: nadzor, bolezenski kompleks, glivno sodelovanje, integrirano uravnavanje škodljivcev, fag, odnosi

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1 INTRODUCTION

The discovery of bacteriophages and ultimately of mycoviruses/mycophages has been a great leap forward for researchers. Mycoviruses (mycophages) are a group of viruses that are naturally associated with fungi (including fungi associated with plants, mushrooms, microbes, soil, and water) (SDSU, 2021; Hu et al., 2022). Mycoviruses interact with four phyla of true fungi (eufungi): the Chytridiomycota (chytrids), Zygomycota (bread molds), Ascomycota (yeasts and sac fungi), and the Basidiomycota (club fungi). The relation of myoviruses with Pseudofungi like those in the Phyla Oomycota and Hyphochytridiomycota (in Kingdom Chromista i.e. some water moulds or Straminipila) and as well as slime moulds - other fungi-like organisms (Ghabrial and Suzuki, 2009; Pearson et al., 2009; Beakes et al., 2014; Xie and Jiang, 2014; Zhong et al., 2016; Calvalier-Smith, 2018; Myers et al., 2020; Zhou et al., 2021; Hough et al., 2023) was not covered in this review. Fungi are frequently infected with two or more unrelated viruses (Ghabriel and Suzuki, 2008; Howitt et al., 2006). Fungi may also act as vectors of viruses of higher life forms (Adams, 1991). The mycovirus-host fungus relationship take the form of mutualistism, commensalism, or parasitism.

Viruses associated with fungi or mycoviruses associated with higher life forms usually do not induce symptoms in their host fungi, except in the case of hypervirulence (increase in virulence of the symptoms of the infection of the fungus on its host: extremely or unusually virulent) and hypovirulence (decrease of the symptoms of the infection of the fungus on its host: extremely or unusually reduced virulence) (Ghabrial and Suzuki (2009). On the other hand, the diseases on some fungi and mushrooms/macrofungi are caused by the mycoviruses themselves. Ghabrial and Suzuki (2009) reported that mycoviruses are associated with latent infections of all major groups of plant pathogenic fungi. Some mycoviruses cause debilitating diseases and/or reduce the virulence of their phytopathogenic fungal hosts and these may lead to attenuation (hypovirulence) or enhancement of fungal virulence (hypervirulence).

Kong et al. (1997), Nuss (2005), Ong et al. (2016), García-Pedrajas et al. (2019), and Siddique (2020) reiterated that some mycoviruses reduce the virulence of the host fungus (hypovirulence), which can make the fungus less harmful to plants, whereas other mycoviruses have been shown to enhance the virulence of the host fungus (hypervirulence). However mycoviruses may be pathogenic on their hosts.

For instance, la France virus disease of cultivated mushrooms (*Agaricus bisporus* (J.E. Lange) Pilat was first reported in the late 1940s (Hollings, 1962; Ghabrial and Suzuki, 2009). Alvarez-Jubete et al. (2011) reported that Mushroom Virus X affects important traits associated with mushroom quality (including colour and appearance). Another instance is the effective virus-control of chestnut blight (caused by the fungus - *Cryphonectria parasitica* (Murrill) M.E. Barr) as a consequence of the infection of the fungus by the mycovirus - *Cryphonectria parasitica* hypovirus 1 (CHV1) in Europe (Hollings, 1962).

The natural distribution of mycoviruses seems to follow a normal distribution spectrum with avirulent, mutualistic, and virulent members being commonplace. Many mycoviruses have been shown to be mutualists.

Mycoviruses can alter host's tolerance to environmental stresses, e.t.c. Most of these mycoviruses have not been described to date or are unrelated to any known viruses. According to the PVEN (Plant Virus Ecology Network) (2011) viruses are widely distributed entities that can cause substantial mortality of plants and animals. Secondly, viruses can move genetic elements between hosts e.g. potentially between genetically engineered plants and non-target species.

Studies of host–mycovirus–vector interactions in nature offer both opportunities and challenges that will ultimately produce multi-faceted understanding of the role of mycoviruses in shaping ecological and evolutionary dynamics (Fargette et al., 2006; PVEN, 2011). Studies of pathogenic viruses have probably left out a vast majority of viruses. Mycovirus diversity is another area of mycovirology that has barely been explored. Virtually all plant (and perhaps all animal) species harbor pathogenic or mutualistic fungi in their tissues.

Kotta-Loizou (2019) pointed out that our current understanding of mycoviruses is not as detailed as in other fields of virology and currently not based on cutting-edge methodology. The general assumption is that much information is yet to be generated on mycoviruses especially considering that the majority of these mycoviruse are viruses of microorganisms (VOMs). With the advent of high-throughput sequencing and bioinformatics analysis pipelines in mycovirology, different types of mycoviruses are being discovered in all the four phyla of true fungi. Recent research has revealed an unexpected diversity of these mycoviruses, their interactions with plants, and modulation of some plant biotic and abiotic stresses.

Mycoviruses can be useful in molecular biology and biotechnology. We are just beginning to tap this potential. This appraisal was set up to document the literature on mycoviruses, diversity of currently known host-parasite interactions and biocontrol prospects possible in agriculture and the environment.

2 PLANT-FUNGI-MYCOVIRUS INTERACTIONS

Recently, researchers reported that viruses are the most abundant and dynamic entities in the hydrosphere (Weinbauer, 2004; Suttle, 2007) although Payet et al. (2014) contested that little is known about viruses in these water habitats. Viruses are major agents of microbial mortality and account for about 50% of bacterial mortality in the hydrosphere (Kirchman, 2018). Daily, between 20–50% of heterotrophic bacteria, cyanobacteria and phytoplankton are infected by viruses (Brussaard, 2004; Suttle, 2007).

Viral lysis releases organic cellular content and nutrients necessary for autotrophic and heterotrophic microbial life forms (Shelford et al., 2012). This essentially result in major changes in the biogeochemical nutrient (carbon, nitrogen and phosphorus) cycles and flow of energy in the oceans (Suttle, 2007; O'Malley, 2016). Kirchman (2018) stated that apparently viruses infecting fungi do not lyse their host and are rather transmitted from one fungus to another intracellularly, without being released into the external environment.

True mycoviruses demonstrate an ability to be transmitted and infect other still healthy fungi cells. The interaction between the mycovirus (*Cryphonectria parasitica* hypovirus 1 (CHV1)) with *Cryphonectria parasitica* (the causative agent of chestnut blight)), in Europe resulted in hypovirulence in the fungus. Thus the blight was controlled whenever a virulent strain of the virus attacked the plant.

However, this 'biocontrol' is restricted to a small number of plant vegetation compatibility groups (pVCGs). For instance, in North America plant vegetation incompatibility reactions prevent plant roots from fusing and exchanging their cytoplasmic content, thus hypovirulent strains of mycoviruses are hindered from spreading (See Anagnostakis et al., 1998). Hence in the USA, China and Japan this 'biocontrol' measure tends to fail due to a large number of different plant VCGs (Liu and Milgroom, 2007).

The natural host range of a mycovirus is supposed to be confined to taxa performing cytoplasmic fusion (Buck, 1986) but some mycoviruses can replicate in unrelated taxa not allowing anastomosis of the fungal hyphae. This is the case with two fungal species (*Sclerotinia homoeocarpa* Benn. and *Ophiostoma novo-ulmi* Braiser*)* associated with chestnut tree (Deng and Boland, 2003; Nuss et al., 2005). Chen et al. (1994) extended the natural host range of CHV1 to several phylogenetically unrelated fungal species associating with chestnut and supported their hypothesis using *in vitro* virus transfection techniques. In line with this, CHV1 can also propagate in the genera *Endothia* Murrill species (Cryphonectriaceae) and *Valsa* Fr. species (Diaporthales, Valsaceae) (Ghabriel and Suzuki, 2008).

Various studies revealed that the same mycovirus can be transmitted between different species of the same genus found in the same habitat. For instance the same mycovirus was transmitted between *Cryphonectria* spp. (i.e.; *Cryphonectria parasitica* and *Cryphonectria* sp.), *Sclerotinia* spp. (i.e.; *Sclerotinia sclerotiorum* (Lib.) de Bary and *Sclerotinia minor* Jagger), and *Ophiostoma* spp. (*Ophiostoma ulmi* (Buism.) Nannf. syn. *Ceratocystis ulmi* (Buism.) C. Moreau and *Ophiostoma novo-ulmi*) (Liu et al., 2003; Melzer et al., 2005).

Moreover, interspecies transmission has been reported between *Fusarium poae* (Peck) Wollenw and *Aspergillus* species (van Diepeningen et al., 2006). The mode of transmission in these instances is unknown and is still subject to guess work. Mycovirus infections are common even in humans as is the case with the mycoviruses in *Aspergillus fumigatus* Fresenius (i.e. AfuPmV-1) and *Talaromyces marneffei* Segretain, Capponi & Sureau) Samson, Yilmaz, Frisvad & Seffert (i.e. TmPV1) (Kotta-Loizou and Coutts, 2017; Lau et al., 2018).

Research on mycoviruses is hindered by many factors amongst which is the lack of appropriate infectivity assays (McCabe et al., 1999) and mixed infection or unknown numbers of infecting viruses. These situations make it difficult to ascribe a particular phenotypic change in the host to a particular virus under investigation. Moreover, neutral co-existence (likely due to co-evolutionary processes) may be in operation in a virus-fungus interaction (Araújo et al., 2003). These difficulties have hindered the studies on hypovirulent strains of mycoviruses. This is often due to lack of correlation between phenotypes and specific genomes or particular metabolic pathways (Xie et al., 2006).

Equilibrium offsetting conditions could also be responsible for changes in host-parasite relationships. Possibly, this is due to changes from mutual to neutral then to deleterious, and so on. Other relationships exist in the same habitat. Vidhyasekaran (2004) reported that satellite viruses are dependent on other viruses to supply the enzyme replicase and other enzymes necessary for replication. A satellite virus associated with Tobacco necrosis is not serologically related to Tobacco necrosis virus (TNV). TNV multiply indefinitely without causing the production of a satellite virus. However, the satellite virus is entirely dependent on TNV for its multiplication. The satellite virus has a viral coat and a small genome of its own. Both viruses are transmitted among roots by the fungus *Olpidium brassicae* (Woronin) P.A. Dang.

Sometimes satellite viruses also have satellite RNAs e.g., the satellite of Tobacco necrosis virus (TNV) has a

small satellite RNA that is dependent on Tobacco necrosis virus for replication and on the satellite virus for encapsulation (Vidhyasekaran, 2004). Moreover, various plant viruses (of the Tombusviridae) generate defective interfering RNA viruses during replication (Rubio et al., 1999). This new relationship may result in viral symptom amelioration (Roux et al., 1991; Kong et al., 1997) or intensification as observed in the case of the Turnip crinkle virus (Li et al., 1989; Kong et al. 1997). Hough et al. (2023) stated that mycoviruses have the ability to reduce the virulence of their hosts.

Rowley (2016), and Moonil et al. (2015) reported that asymptomatic associations with fungi and by mycoviruses are very common. Furthermore, fungi are often associated with unrelated viruses or 'defective dsRNA' and/or satellite dsRNA (Howitt et al., 2006; Ghabrial and Suzuki, 2009). Moreover, some viruses simply use fungi as vectors (which differentiate them from mycoviruses) since they do not replicate inside the fungus (Adams, 1991).

Tran et al. (2019) reported that very little is known about mycoviruses infecting *Monilinia* species although virus-like particles (VLPs) resembling those of partitiviruses, totiviruses, tobraviruses, and furoviruses have been reported from these hosts. McCabe et al. (1999) and Rowley (2016) argued that the virulence of a virus is ultimately limited by the need for the host to survive and thus permit the virus to replicate and continue to exist. This has not been proven.

Based on the obligate parasitic nature of viruses, the majority of mycoviruses should have some negative effect(s) on fungal growth or survival. This depends on the mode of infection and the population of the viruses. More than 250 mycoviruses infect true fungi in the aforementioned phyla (Bozarth, 1972; Rochon et al., 2004; Hacker et al., 2005; Ghabrial and Suzuki, 2009; Rowley, 2016; Tran et al., 2019; Xia et al., 2020). Many viruses can simultaneously infect a single fungus (Hollings, 1962).

Based on O'Malley (2016) viruses may operate in hosts with or without being pathogenic. De Filippis and Villarreal (2000) stated that a competition between different viral strains or individuals inside a host may result in selection of the fittest. Viruses have both general and specific requirements for replication and existence. The direction and extent of this change is determined by a combination of stochastic and environmental factors that are specific for a given time, space, and taxon.

Though viruses of plants have long been recognized as important components of plant ecosystems, only a few notable mycovirus have been studied in detail. Marzano et al. (2015) reported that a comprehensive picture of mycoviral diversity is lacking. Tran et al. (2019) lamented that the influence of mycoviruses on the ecosystem has not been well studied. For instance, the lack of studies on how some mycoviruses reduce the ability of their fungal host to cause plant diseases. Besides, it has been assumed that the natural host range of mycoviruses is confined to closely related vegetation-compatibility groups (VCGs) which allow fusion of cytoplasm (Buck, 1986). These assumptions may or may not be true, and are based on assumptions.

Zhang et al. (2020) attested that it is unclear how mycovirus that cause hypovirulence prevail in the field. Myers and James (2022) suggested the presence of mutualism between mycoviruses and their hosts. Pearson et al. (2009) agreed that our understanding of the interaction between mycoviruses and their hosts is largely limited to a few well-studied, possibly atypical systems. Coupled with the problem of mixed infections by multiple viruses (for example the mixed infection of *Botrytis cinerea* virus F (*BCVF*) and Botrytis virus X (*BVX*) in *Botrytis cinerea* Pers.) it may not be easy to ascribe a definite role to a mycovirus (Howitt et al., 2006). De Filippis and Villarreal (2000) emphasized that viral infection of a host may not necessarily involve tissue destruction, mortality or even full/partial mobilization of host antiviral mechanisms. Indeed, virus association with hosts may result in mutualistic relationships.

Most mycoviruses do not cause symptomatic infections in their hosts (Ghabrial et al., 2015; Khan et al., 2022). Symptom expression usually occur when there is hypersensitive reaction or incompatibility of the host and parasite. Rowley (2016) reported that fungal hosts defend themselves from mycoviruses using RNA interference (RNAi), which inhibit mycovirus replication. This may result in cell death thus blocking mycovirus transmission. De Filippis and Villarreal (2000) reported that disabling antiviral systems in fungi improves the chances of virus continuity. Bacteria hosts can employ abortive infection as a last resort to escape from the effects of bacteriophages (Weinbauer 2004). However, many mycoviruses interfer with fungal RNAi to prevent the inhibition of their replication. Interactions between vegetatively incompatible plants and fungal isolates culminate in programmed cell death (PCD) thus hindering any exchange of infected cellular contents (Nuss, 2011).

Biella et al. (2002) affirmed that mycovirus infection is influenced by the rate of PCD which could mean that mycoviruses may have developed mechanisms for delaying or hindering occurrence of PCD. RNA silencing (as a defence mechanism in fungi) invoked by fungi against viruses may be made inefficient by some viruses including mycoviruses (Segers et al., 2007). Furthermore, Moonil et al. (2015), and Rowley (2016) pointed out that some mycoviruses are associated with killer satellite virus particles which induce their fungus host to secrete

toxins that kill competing fungi. This host fungus beneficial mechanism is exhibited by the budding yeasts (*Sacharomyces cerevisiae* (Desm.) Meyen) in fermented foodstuffs.

These dsRNA satellite viruses are dependent on the Totiviridae mycoviruses for their stability. Alone, totiviruses have a minimal impact upon *S. cerevisiae*, but the additional presence of satellite RNAs provide additional capabilities to the virus which is an important example of a beneficial virus system. In fact, these killer systems are so beneficial to their hosts that in some cases, they have resulted in the loss of host RNAi systems (Drinnenberg et al., 2011; Moonil et al., 2015). Thus symptomless or latent mycoviruses may have unknown functions in their hosts. Somehow, some mycoviruses may act as extra-chromosomal genes that confer an advantage to the host as can be observed with the killer systems in yeast (Schmitt and Breinig, 2006).

Another example of beneficial relationship with a mycovirus, is a three-way symbiosis (among a mycovirus, an endophytic fungus, and tropical panic grass). The endophytic fungus (*Curvularia protuberata* Boedijn), panic grass (*Dichanthelium lanuginosum* (Elliott) Gould), and other plants can only survive high soil temperatures in the presence of the mycovirus (Márquez et al., 2007; Moonil et al., 2015). The mycovirus in turn obtains its basic necessities from its hosts. The mechanisms involves two distinct viral dsRNAs. A mutualistic relationship is also found in an interaction among *Trichoderma* Pers. species and their mycoviruses, and the host plant (Beilei et al., 2020).

The fungus is required for thermal tolerance of the plants. A parasite often tend to reduce its impact on its host, thus many parasites have co-evolved to an equilibrium state resulting in minimal impact. Therefore there is great variability in reactions between a single host and different viruses or dsRNAs.

Furthermore, Khan et al. (2022) reported that several types of virus-virus interactions (i.e.; synergistic, antagonistic, and mutualistic interactions) have been reported in fungal hosts. Co-infections of single fungal strains by over ten mycoviruses has been reported for several phytopathogenic fungi, which implies that much work has to be carried out to determine the type of relathionships that are created in such co-infections.

The effects of a mycovirus seems to be dependent on other factors like environment and presence of other invaders. For instance, Chu et al. (2002) reported a wide spectrum of reactions: reduced growth, increased pigmentation, reduced virulence, and a 60-fold decreased production of trichothecene mycotoxins associated with a dsRNA during a study of *Fusarium graminearum* Schwabe (syn *Gibberella zeae* (Schwein.) Petch) on wheat. Fine (1975) assumed that mycoviruses may be unable to persist if they lower the fitness of their hosts, because they are limited to vertical transmission only. In a detailed study of the effects of dsRNA on the fitness of asexual *Aspergillus* species, no beneficial effects were observed (Van Diepeningen et al., 2006) in vitro. In contrast Tran et al. (2007) observed higher growth rates of BVX-infected fungus compared to the same uninfected isolate.

It has been postulated that the virus environment is both multidimensional and continually changing thus constantly driving the increase in population fitness. It could also be argued that based on quantity of variables in the environment, viruses exhibit greater mobility through the space of their selective or adaptive environments than do more complex organisms (Moya, 1997).

De Filippis and Villarreal (2000) reported that the many levels of viral characters (point mutations, coding region products, multigene assemblages, behavioral traits, and even populational characters) can be considered as adaptations and may all endow their possessors with replication advantages. The adaptive viral characters favored within the relatively closed system of one individual host arise and persist due to intra-host selection pressure, the nature and strength of which is determined by the environmental conditions and other virus strains contained therein.

De Filippis and Villarreal (2000) reported that the host's cellular, tissue, and organismal environments are vitally important selective realms that contribute profoundly to the adaptation and diversity of viruses including mycoviruses. Also by disabling antiviral systems the virus reduces its own population decline. In the ecosystem the fittest mycovirus optimizes its utilization of host resources and does not maximize the utilization of host resources. This permits them to continue to persist despite the intrahost selection pressure. Thus the fittest individuals are not the ones that maximizes the use of host resources, rather the fittest individuals are those that optimizes the utilization of host resources.

To ensure continuity in most viral infections, less than 1 % of the susceptible host tissue is actually infected/harvested (Griffin, 1997). Such a host-parasite interaction could persist and be observed as any of the forms of guilds depending on the colorations and flavours added to it. In micro-ecosytems, the essential portion of the environment that is of most concern is the inorganic nutrients and energy derivable from the hosts. The mycovirus should therefore be properly adapted to avoid depleting these resources unnecessarily. In the case of bacteriophages, they impact the movement of nutrients and energy within the micro-ecosystems primarily by lysing bacteria and secondarily by encoding of exotoxins (a subset of which are capable of solubilizing the biolo-

gical tissues of living hosts/animals) (Weinbauer, 2004). Much has been reported already about viruses of plants, humans and animals so this will only be discussed briefly as antagonistic components of the micro-ecostystem. Kazinczi et al. (2004) pointed out that weeds, as alternative hosts of plant viruses can act as alternative nutrient sources for viruses and virus vectors. Weeds play important role in virus ecology and epidemiology. Alemu et al. (2002) reported that chronic infection with viruses is a major constraint that often force farmers to ban hot pepper production. This can result in decrease in the population of virus and mycovirus entities in an area. The presence of infected weeds throughout the year means, that they are reservoirs and sources of viruses for secondary spread. Yudin et al. (1986) reported that western flower thrips (*Frankliniella occidentalis* Pergande, 1895 - a known vector of tomato spotted wilt virus, was found to be associated with 48 plant species growing within the Kula vegetable-growing region on the island of Maui, Hawaii. This type of vector can be very vital for continual existence of mycoviruses even when the host plant and fungus are facing difficult times in the dry season. Weeds are widely infected by viruses. For instance, McGovern et al. (2008) reported that *Solanum viarum* Dunal (the invasive tropical soda apple) in Florida was infected by nine viruses which can in turn infect solanaceous crops.

3 IMPLICATION OF MYCOVIRUS INTERACTIONS WITH PLANTS IN CROP PROTECTION: TRENDS IN RESEARCH, APPLICATIONS, AND 'BIOLOGICAL' CONTROL POTENTIALS USING THESE AGENTS

We have just seen how the killer phenotypes can provide some advantages to yeasts and *Ustilago* (Pers.) Roussel species due to their interactions with viruses (Schmitt and Breing, 2002; Marquina et al., 2007). Killer isolates secrete proteinous toxins (mostly cell wall degrading enzymes) against sensitive cells of the same or closely related species, while the producing cells themselves are immune. These types of killer isolates could be beneficial in medicine, agriculture and industry (Schmitt and Breing, 2002).

We have also seen that three-part interaction provide thermal tolerance by the plant (Marquez et al., 2007). Another example is the A78 virus of *Aspergillus fumigatus* Fresen causing mild hypervirulence on *Galleria_mellonella* (L., 1758) (Greater wax moth) (Ozkan and Coutts, 2015). Likewise, TmPV1 associated with *T. marneffei* caused hypervirulence on *T. marneffei* in the mouse host (Lau et al., 2018).

Liu et al. (2022) reported that mycovirus *Stemphylium lycopersici* alternavirus 1 (SlAV1) from a necrotrophic plant pathogen (*Stemphylium lycopersici)* that causes altered colony pigmentation and hypovirulence by specifically interfering host biosynthesis of Altersolanol A, a polyketide phytotoxin.

Li et al. (2019) reported that most *Fusarium* mycoviruses establish latent infections, but some mycoviruses such as *Fusarium graminearum* virus 1 (FgV1), Fusarium graminearum virus-ch9 (FgV-ch9), *Fusarium graminearum* hypovirus 2 (FgHV2), and *Fusarium oxysporum* f. sp. dianthi mycovirus 1 (FodV1) cause hypovirulence. Khan et al. (2023) emphasized that among members of the genus *Sclerotinia*, a huge number of mycoviruses have been identified; some of them have a hypovirulent effect on the fitness of their fungal hosts.

Zhou et al. (2021) revealed that mycoviruses have been associated with plant adaptation to extreme environments, conferring heat tolerance to plants that contain fungal endophytes. They reported that endophytic fungi, can confer fitness to the host plants. It is unclear whether biological factors can modulate the parasitic and mutualistic traits of a fungus. Kotta-Loizou (2021) affirmed that in fungus-mycovirus-environmental interactions, the environment and both abiotic and biotic factors play crucial roles in whether and how mycovirus mediated phenotypes are manifest.

Connor (2021) reported that soybean leaf-associated gemycircularvirus-1 (SlaGemV-1) is capable of inducing hypovirulence in the highly pathogenic fungus *Sclerotinia sclerotiorum* as does the hypovirus 1 (CHV1) controlling *C. parasitica* in chestnut in Europe. It is an excellent model organism for studying hypovirulence in fungi (Anagnostakis et al., 1998; Liu and Milgroom, 2007).

Kirchman (2018) pointed out that viruses infecting fungi do not appear to lyse their host. The use of mycovirus can open many avenues for handling waste and decomposition, or terminating some life processes. For instance grazers completely oxidize the organic content of their host into carbon dioxide and inorganic nutrients. A third mode of employing viruses may theoretically be to facilitate the exchange of genetic material from one host to another. Most of these processes have been relatively poorly studied (Pearson et al., 2009).

Hypovirulence may be induced in hosts by mycoviruses via RNA silencing, alteration of genetic expression, and disruption of the transcriptome that can result in phenotypic changes like reduction in growth or changes in pigmentation (Nuss, 2005). Alterations of miRNAs expressions using viral suppressors of RNA silencing (VSRs) occurs by applying papain-like protease p29 (Segers et al., 2006) and potyvirus HC-Pro (Maia et

al., 1996). Also, *C. parasitica* when infected by the hypovirulence-inducing mycovirus undergoes RNA silencing thereby affecting the MAPK cascade and G-protein signaling. Moreover, direct disruption of the fungal transcriptome may occur (Nuss, 2005).

Proof of the ability of a mycovirus being able to control a pathogen in the field is either scarse or unavailable (Griffin 1986, MacDonald et al. 1991) but mycoviruses have been shown to be able to control fungi in modified environments (MacDonald et al., 1991; Milgroom et al., 2004).

Two major forms of defense signaling include: systemic acquire resistance (SAR) and induced systemic resistance (ISR). (Vidhyasekaran, 2015). Another theoritical approach usable to increase a plant resistance against pathogenic infection is resistance priming like that involved in SsHADV-1 allowing *S. sclerotiorum* to induce priming in plants. 'Priming is the process of inoculating plants, often the seeds, with beneficial microorganisms to improve nutrient use efficiency and to potentially improve resistance to pathogens' (Rakshit et al., 2015). Actually, Qu et al. (2020) demonstrated that SsHADV-1-infected, hypovirulent *S. sclerotiorum* is reprogrammed to act as a beneficial, bio-priming mycorrhiza in rapeseed due to *Sclerotinia* Fuckel stem rot reduction and improved yield. Mycoviruses have been shown to be involved in all forms of interactions (e.g. mutualism) with fungi hosts. In the future, mycoviruses may be required for manipulating micro-ecosystems within plants, humans, animals and so on. They are simple enough for direct insertion and removal of genes here and there if the right equipment is available. However, pathogenic mycoviruses have been reported and they can severely ravage host populations especially domesticated mushrooms e.g. la France disease on *Agaricus bisporus*. Thus, mycoviruses have to be controlled in fungus-fungus, fungus-plant, fungus-animal systems, etc.

Ruiz-Padilla et al. (2021) propounded that products based on microorganisms (including mycoviruses senso lato) can be used in biocontrol strategies alternative to chemical control. Keçeli (2017) reported that the use of mycoviruses in the treatment of invasive fungal infections in humans has not been suggested yet. Xie and Jiang (2014) suggested that fungal vegetative incompatibility is likely to be the limiting factor in the widescale utilization of mycoviruses to control crop diseases.

4 CONCLUSION

Past, present and future trends in mycovirus research are of interest to humans. They can reveal the prospects of mycoviruses in agriculture and the environment in terms of pathogen control and amelioration of the environment. Use of mycoviruse to induce hypovirulence in fungi host isolates has shown great potentials e.g. using the A78 virus of *Aspergillus fumigatus,* TmPV1 on *T. marneffei*, soybean leaf-associated gemycircularvirus-1 (SlaGemV-1) in *Sclerotinia sclerotiorum,* the hypovirus 1 (CHV1) in *Cryphonectria parasitica*. Hypovirulence may be induced in fungi hosts by mycoviruses via RNA silencing, alteration of genetic expression, and disruption of the transcriptome which can result in phenotypic changes like reduction in growth or changes in pigmentation. Moreover, direct disruption of the fungal transcriptome may occur. Another approach to increase a plant's resistance against pathogenic infection is resistance priming that may be required for manipulating micro-ecosystems within the plants. However, pathogenic mycoviruses have been reported and they can severely ravage host populations especially domesticated mushrooms

5 REFERENCES

Adams, M. J. (1991). Transmission of plant viruses by fungi. *Annals of Applied Biology, 118*(2), 479–492. https://doi.org/10.1111/j.1744-7348.1991.tb05649.x

Alemu T., Hamacher, J., & Dehne, H. W. (2002). The role of some weeds as hosts of Capsicum viruses in the rift valley parts of Ethiopia. *Meded Rijksuniv Gent Fak Landbouwkd Toegep Biol Wet., 67*(2), 283-9. Institute for Plant Diseases, University of Bonn, Nussallee 9, 53115 Bonn, Germany

Alvarez-Jubete, L., Bonnier, F., Byrne, H., Grogan, H., & Frias, J. M. (2011). Detection of mushroom Virus X (MVX) infection in asymptomatic mushrooms using FTIR microscopic imaging. *Poster Presentation at the 11th International Conference of Engineering and Food (ICEF11), May 2011, Athens, Greece*

Anagnostakis, S. L., Chen, B., Geletka, L. M., & Nuss, D. L. (1998). Hypovirus Transmission to Ascospore Progeny by Field-Released Transgenic Hypovirulent Strains of *Cryphonectria parasitica*. *Phytopathology. 88*(7), 598–604. https://doi.org/10.1094/PHYTO.1998.88.7.598

Araújo, A., Jansen, A. M., Bouchet, F., Reinhard, K., & Ferreira, L. F. (2003). Parasitism, the diversity of life, and paleoparasitology. *Memórias do Instituto Oswaldo Cruz, 98* (SUPPL. 1), 5-11. https://doi.org/10.1590/S0074-02762003000900003

Beakes, G.W., Honda, D. & Thines, M. (2014). Systematics of the Straminipila: Labyrinthulomycota, Hyphochytriomycota, and Oomycota. In: *The mycota systematics and evolution, VII part A*: (2nd ed.), (eds D.J. McLaughlin & J.W. Spatafora), pp 39-97. Berlin, Heidelberg: Springer-Verlag. https://doi.org/10.1007/978-3-642-55318-9_3. VIEW on Amazon

Beilei, W., Mei, Li, Chenchen, Liu & Xiliang, Jiang. (2020). The

insight of mycovirus from *Trichoderma* spp. *Agriculture Research and Technology: Open Access Journal 24*(2). DOI: 10.19080/ARTOAJ.2020.24.556258

Biella, S., Smith, M. L., Aist, J. R., Cortesi, P., & Milgroom, M. G. (2002). Programmed cell death correlates with virus transmission in a filamentous fungus. *Proceedings of Biological Science. 269*(1506), 2269–2276. https://doi.org/10.1098/rspb.2002.2148

Bozarth, R. F. (1972). Mycoviruses: a new dimension in microbiology. *Environmental Health Perspectives. 2*(1), 23–39. https://doi.org/10.1289/ehp.720223

Brussaard, C. P. D. (2004). Viral control of phytoplankton populations – a review. *Journal of Eukaryotic Microbiology, 51*, 125–138. https://doi.org/10.1111/j.1550-7408.2004.tb00537.x

Buck, K. W. (1986). *Fungal virology-An overview*. Boca Raton Florida: CRCPress, Boca Raton 1-84.

Cavalier-Smith, T. (2018). Kingdom Chromista and its eight phyla: a new synthesis emphasising periplastid protein targeting, cytoskeletal and periplastid evolution, and ancient divergences. *Protoplasma, 255,* 297-357. https://doi.org/10.1007/s00709-017-1147-3

Chen, B., Choi, G. H., & Nuss, D. L. (1994). Attenuation of fungal virulence by synthetic infectious hypovirus transcripts. *Science, 264*(5166), 1762–4. https://doi.org/10.1126/science.8209256

Cho, W. K., & Kook-Hyung, K., (2013). Mycoviruses. *Advances in Virus Research*, 2013.

Chu, Y. M., Jeon, J. J., Yea, S. J., Kim, Y. H., & Yun, S. H. (2002). Double-stranded RNA mycovirus from *Fusarium graminearum*. *Applied Environmental Microbiology, 68,* 2529–2534. https://doi.org/10.1128/AEM.68.5.2529-2534.2002

Connor, P. (2021). *A biocontrol pesticide derived from mycovirus-infected Sclerotinia sclerotiorum can induce plant resistance*. Electronic Theses and Dissertations. 5244. South Dakota State University, USA. https://openprairie.sdstate.edu/etd/5244

De FILIPPIS, V., & Villarreal, L. P. (2000). *Viral ecology. An introduction to the evolutionary ecology of viruses*. PMC7149709.: 125–208. https://doi.org/10.1016/B978-012362675-2/50005-7

Deng, F., Xu, R., & Boland, G. J. (2003). Hypovirulence-associated double-stranded RNA from *Sclerotinia homoeocarpa* is conspecific with *Ophiostoma novo-ulmi* Mitovirus 3a-Ld. *Phytopathology, 93*(11), 1407–14. https://doi.org/10.1094/PHYTO.2003.93.11.1407

Drinnenberg, I .A., Fink, G. R., & Bartel, D. P. (2011). Compatibility with killer explains the rise of RNAi-deficient fungi. *Science, 333*, 1592. https://doi.org/10.1126/science.1209575

Ezawa, T., Yoji, I., Hanako, S., Chikara, M., Tatsuhiro, E., Yoji, I., Hanako, S., & Chikara, M. (2015). Detection and characterization of mycoviruses in arbuscular mycorrhiza. *Methods in molecular biology: Plant Virology Protocols*. https://doi.org/10.1007/978-1-4939-1743-3_13

Fargette, D., Konaté, G., Fauquet, C., Muller, E., Peterschmitt, M., & Thresh, J. M. (2006). Molecular ecology and emergence of tropical plant viruses. *Annual Review of Phytopathology, 44*; 235-260. https://doi.org/10.1146/annurev.phyto.44.120705.104644

Fine, P.E.F. (1975). Vectors and vertical transmission: An epidemiological perspective. *Annals of New York Academy of Science, 266*, 173–194. https://doi.org/10.1111/j.1749-6632.1975.tb35099.x

García-Pedrajas, M.D., Cañizares, M.C., Sarmiento-Villamil, J.L., Jacquat, A.G., & Dambolena, J.S. (2019). Mycoviruses in biological control: from basic research to field implementation. *Epub, 109*(11), 1828-1839. https://doi.org/10.1094/PHYTO-05-19-0166-RVW

Ghabrial, S. A., & Suzuki, N. (2008). Fungal Viruses. In B. W. J. Mahy and M. H. V. Van Regenmortel (ed.), *Encyclopedia of virology, 3rd ed., vol. 2*. Elsevier, Oxford, United Kingdom. p. 284-291. https://doi.org/10.1016/B978-012374410-4.00563-X

Ghabrial, S. A., & Suzuki, N. (2009). Viruses of plant pathogenic fungi. *Annual Review of Phytopathology 47*, 353–84. https://doi.org/10.1146/annurev-phyto-080508-081932

Ghabrial, S. A., Castón, J. R., Jiang, D., Nibert., M. L., & Suzuki, N. (2015). 50-plus years of fungal viruses. *Virology, 479*, 356–368. https://doi.org/10.1016/j.virol.2015.02.034

Griffin, D.E. (1997). Virus-induced immune suppression. In: Nathanson N., editor. *Viral Pathogenesis*. Lippincott-Raven; New York: pp. 207–233.

Hacker, C.V., Brasier, C.M., & Buck, K.W. (2005). A double-stranded RNA from a *Phytophthora* species is related to the plant endornaviruses and contains a putative UDP glycosyltransferase gene. *Journal of Genetics and Virology, 86*, 1561–1570. https://doi.org/10.1099/vir.0.80808-0

Hammond, R. W., & Zhao, Y. (2000). Characterization of tomato protein kinase gene induced by infection by potato spindle tuber viroid. *Molecular Plant-Microbe Interactions, 13*, 903-910. https://doi.org/10.1094/MPMI.2000.13.9.903

Hollings, M. (1962). Viruses Associated with A die-back disease of cultivated mushroom. *Nature, 196*(4858), 962–965. Bibcode:1962Natur.196..962H. https://doi.org/10.1038/196962a0

Hough, B., Steenkamp, E., & Wingfield, B. (2023). Fungal viruses unveiled: a comprehensive review of mycoviruses. Kotta-Loizou, D.R.I. (Academic Editor). *Viruses, 15*(5), 1202. https://doi.org/10.3390/v15051202

Howitt, R. L., Beever, R. E., Pearson, M. N., & Forster, R. L. (2006). Genome characterization of a flexuous rod-shaped mycovirus, *Botrytis* virus X, reveals high amino acid identity to genes from plant ‚potex-like' viruses. *Archives of Virology, 151*(3), 563–579. https://doi.org/10.1007/s00705-005-0621-y

Hu, H. J., Wang, J. R., Cheng, X. H., Liu, Y., & Zhang, X.Y. (2022). Preliminary studies on the effects of oyster mushroom spherical virus China strain on the mycelial growth and fruiting body yield of the edible mushroom *Pleurotus ostreatus*. *Biology, 11*, 574. https://doi.org/10.3390/biology11040574

Kazinczi, G., Horváth, J., Takács, A. P., Gáborjányi, R., & Béres, I. (2004). Experimental and natural weed host-virus relations. *Community Agriculture and Applied Biological Science, 69*(3), 53-60.

Khan, H.A., Mukhtar, M., & Bhatti, M. F. (2023). Mycovirus-induced hypovirulence in notorious fungi Sclerotinia: a com-

prehensive review. *Brazilian Journal of Microbiology* (2023). https://doi.org/10.1007/s42770-023-01073-4

Khan, H. A., Telengech, P., Hideki, K., Muhammad, F. B., & Nobuhiro, S. (2022). Mycoviruses of pathogenic fungi: The current research landscape. mycovirus hunting revealed the presence of diverse viruses in a single isolate of the phytopathogenic fungus *Diplodia seriata* From Pakistan. *Frontiers in Cellular Infection Microbiology.* Section. Fungal Pathogenesis. *29* June 2022. https://doi.org/10.3389/fcimb.2022.913619

King, A. M. Q., Lefkowitz E. M., Adams, J., & Carstens, E. B. (2011). *Virus taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses (ICTV)*. San Diego, USA: Elsevier Academic.

Kirchman, D. L. (2018). *The ecology of viruses. Processes in microbial ecology, 2nd edn*. Oxford Academic. online ed. https://doi.org/10.1093/oso/9780198789406.003.0010

Kong, Q., Oh, J., Carpenter, C.D., & Simon, A.E. (1997). The coat protein of turnip crinkle virus is involved in subviral rna-mediated symptom modulation and accumulation. *Virology, 238*, 478–485. https://doi.org/10.1006/viro.1997.8853

Kotta-Loizou, I., & Coutts, R. H. (2017). Aspergilli: A comprehensive review. *Frontiers in Microbiology, 8*, 1699. PMC 5592211. PMID 28932216. https://doi.org/10.3389/fmicb.2017.01699

Kotta-Loizou, I. (2019). Mycoviruses: Past, Present, and Future. *Viruses, 11,* 361; www.mdpi.com/journal/viruses. https://doi.org/10.3390/v11040361

Kotta-Loizou, I. (2021). Mycoviruses and their role in fungal pathogenesis. *Current Opin Microbiology, 63,* 10-18. https://doi.org/10.1016/j.mib.2021.05.007

Lau, S. K., Lo, G. C., Chow, F. W., Fan, R. Y., Cai, J. J., Yuen, K. Y., & Woo, P. C. (2018). Novel partitivirus enhances virulence of and causes aberrant gene expression in *Talaromyces marneffei*. *Biology, 9*(3), e00947–18. https://doi.org/10.1128/mBio.00947-18. PMC 6016240.

Lenski, R. E., & Levin, B. R. (1985). Constraints on the coevolution of bacteria and virulent phage: a model, some experiments, and predictions for natural communities. *The American Naturalist, 125*(4), 585–602. https://doi.org/10.1086/284364. JSTOR 2461275.

Li, X.H., Heaton, L.A., Morris, T. J., Simon, A.E. (1989). Turnip crinkle virus defective interfering RNAs intensify viral symptoms and are generated de novo. *Proceeding Natnaiol Academy of Science of USA. 86*(23):9173-7. https://doi.org/10.1073/pnas.86.23.9173

Li, P., Bhattacharjee, P., Wang, S., Zhang, L., Ahmed, I., & Guo, L. (2019). Mycoviruses in *Fusarium* Species: An Update. *Frontier Cell Infection and Microbiology, 9,* 257. https://doi.org/10.3389/fcimb.2019.00257

Li, P., Wang, S., Zhang, L., Qiu, D., Zhou, X., & Guo, L. (2020). A tripartite ssDNA mycovirus from a plant pathogenic fungus is infectious as cloned DNA and purified virions. *Science Advances, 6*(14), eaay9634. Bibcode:2020SciA....6.9634L. https://doi.org/10.1126/sciadv.aay9634

Liu, Y. C., Linder-Basso, D., Hillman, B. I., Kaneko, S., & Milgroom, M. G. (2003). Evidence for interspecies transmission of viruses in natural populations of filamentous fungi in the genus *Cryphonectria*. *Molecular Ecology, 12*(6), 1619–1628. https://doi.org/10.1046/j.1365-294X.2003.01847.x

Liu, Y. C., & Milgroom, M. G. (2007). High diversity of vegetative compatibility types in *Cryphonectria parasitica* in Japan and China. *Mycologia, 99*(2), 279–284. https://doi.org/10.3852/mycologia.99.2.279

Liu, H., Wang, H., Liao, X.L., ... & Zhouqian, Q.Z. (2022). Pathogenic fungus into a biocontrol agent. *Proceeding Natnaiol Academy of Science of USA, 119*(50), e2214096119. https://doi.org/10.1073/pnas.2214096119.

Márquez, L. M., Redman, R. S., Rodriguez, R. J., & Roossinck, M. J. (2007). A virus in a fungus in a plant: three-way symbiosis required for thermal tolerance. *Science, 315*(5811), 513–515. Bibcode:2007Sci...315..513M. https://doi.org/10.1126/science.1136237

Marquina, D., Santos, A., & Peinado, J. M. (2002). Biology of killer yeasts. *International Microbiology, 5*(2), 65–71. https://doi.org/10.1007/s10123-002-0066-z

Marzano, S-Y. L., Hobbs, H. A., Nelson, B. D., Hartman, G.L., ... & Eastburn, D. M. (2015). Transfection of *Sclerotinia sclerotiorum* with in vitro transcripts of a naturally occurring interspecific recombinant of *Sclerotinia sclerotiorum* hypovirus 2 significantly reduces virulence of the fungus. *Journal of Virology, 89,* 5060–5071. https://doi.org/10.1128/JVI.03199-14

McCabe PM, Pfeiffer P, & Van Alfen NK 1999. The influence of dsRNA viruses on the biology of plant pathogenic fungi. *Trends in Microbiology, 7*(9), 377–81. https://doi.org/10.1016/S0966-842X(99)01568-1

McGovern, R. J., Polstonb, J. E. & Mullahey, J. J. (2008). pages 270-273.

Melzer, M. S., Deng, F., and Boland, G. J. (2005). Asymptomatic infection, and distribution of *Ophiostoma* mitovirus 3a (OMV3a), in populations of *Sclerotinia homoeocarpa*. *Canadian Journal of Plant Pathology, 27*(4), 610–615. https://doi.org/10.1080/07060660509507262

Mycovirus. (no date). From Wikipedia, the free encyclopedia. https://en.wikipedia.org/w/index.php?title=Mycovirus&oldid=1102583069.

Myers, J., Bonds, A., Clemons, R., Thapa, N., Simmons, D., Carter-Hous, D., Ortanez, J., Liu P., Miralles-Durán, A., & Desirò, A. (2020). Survey of early-diverging lineages of fungi reveals abundant and diverse mycoviruses. *Microbiology, 11,* e02027-20. https://doi.org/10.1128/mBio.02027-20

Myers, J. M., & James, T. Y. (2022). Mycoviruses. *Current Biology, 32*(4), R150-R155. PMID: 35231405. https://doi.org/10.1016/j.cub.2022.01.049

Nuss, D. L. (2005). Hypovirulence: Mycoviruses at the fungal–plant interface. *Nature Reviews Microbiology, 3,* 632–642. https://doi.org/10.1038/nrmicro1206

Nuss, D. L. (2011). Mycoviruses, RNA silencing, and viral RNA recombination. In *Advances in Virus Research.* https://doi.org/10.1016/B978-0-12-385987-7.00002-6

O'Malley, M. A. (2016). Studies in history and philosophy of science. Part C: Studies in history and philosophy of biological and biomedical sciences. *The Ecological Virus, 59,* 71-79. https://doi.org/10.1016/j.shpsc.2016.02.012

Ong, J.W.L., Li, H., Sivasithamparam, K., et al. (2016). Novel

endornalike viruses, including three with two open reading frames, challenge the membership criteria and taxonomy of the Endornaviridae. *Virology,499,* 203–211. https://doi.org/10.1016/j.virol.2016.08.019

Payet, J. P., McMinds, R., Burkepile, D. E. & Vega-Thurber, R. L. (2014). Unprecedented evidence for high viral abundance and lytic activity in coral reef waters of the South Pacific Ocean. *Frontiers in Microbiology, 5,* 493. https://doi.org/10.3389/fmicb.2014.00493

Pearson, M .N., Beever, R. E., Boine, B., & Arthur, K. (2009). Mycoviruses of filamentous fungi and their relevance to plant pathology. *Molecular Plant Pathology, 10*(1), 115–128. https://doi.org/10.1111/j.1364-3703.2008.00503.x

PVEN (Plant Virus Ecology Network). (2011). Pest management guidelines. 2011. Floriculture and ornamental nurseries. *Viruses and Viroid Diseases,* http://dx.doi.org/10.1016/j.virusres.2011.05.010

Ro, H. S., Lee, N. J., Lee, C. W., & Lee, H. S. (2006). Isolation of a novel mycovirus OMIV in *Pleurotus ostreatus* and its detection using a triple antibody sandwich-ELISA. *Journal of Virological Methods, 138*(1–2), 24–29. https://doi.org/10.1016/j.jviromet.2006.07.016

Rochon, D., Kakani, K., Robbins, M., & Reade, R. (2004). Molecular aspects of plant virus transmission by olpidium and plas¬modiophorid vectors. *Annual Reviews of Phytopathology, 42,* 211–241. https://doi.org/10.1146/annurev.phyto.42.040803.140317

Roux, L., Simon, A. E., and Holland, J. J. (1991). Effects of defective interfering viruses on virus replication and pathogenesis in vitro and in vivo. *Advances in Virus Research, 40,* 181-211. https://doi.org/10.1016/S0065-3527(08)60279-1

Rowley, P.A. (2016). *The hidden viruses of the fungal kingdom. Issue: Fungal diseases*. Microbiology society publication USA.

Rubio, T., Borja, M., Scholthof, H. B., Feldstein, P. A., Morris, T. J., & Jackson, A. O. (1999). Broad-spectrum protection against tombusviruses elicited by defective interfering RNAs in transgenic plants. *Journal of General Virology, 73,* 5070-5078. https://doi.org/10.1128/JVI.73.6.5070-5078.1999

Ruiz-Padilla, A., Rodríguez-Romero, J., Gómez-Cid, I., Pacifico, D., & Ayllón, M.A. (2021). Novel mycoviruses discovered in the mycovirome of a necrotrophic fungus. *Microbiology, 12*(3), e03705-20. https://doi.org/10.1128/mBio.03705-20

Keçeli, S.A. (2017). [*Mycoviruses and importance in mycology*] [Article in Turkish]. 51(4):404-412. https://doi.org/10.5578/mb.54128

Schmitt, M. J., & Breinig, F. (2002). The viral killer system in yeast: from molecular biology to application. *FEMS Microbiology Reviews, 26*(3), 257–76. https://doi.org/10.1016/S0168-6445(02)00099-2. PMID 12165427.

Schmitt, M. J., & Breinig, F. (2006). Yeast viral killer toxins: lethality and selfprotection. *National Review of Microbiology, 4*(3), 212-221. https://doi.org/10.1038/nrmicro1347

Segers, G. C., Zhang, X., Deng, F., Sun, Q., & Nuss, D. L. (2007). Evidence that RNA silencing functions as an antiviral defense mechanism in fungi. *Proceedings of National Academy of Science USA, 104,* 12902–12906. https://doi.org/10.1073/pnas.0702500104

Shelford, E. J., Middelboe, M., Møller, E. F., & Suttle, C. A. (2012). Virus-driven nitrogen cycling enhances phytoplankton growth. *Aquatic Microbiology and Ecology, 66,* 41–46. https://doi.org/10.3354/ame01553

Siddique, A.B. (2020). Viruses of endophytic and pathogenic forest fungi. *Virus Genes, 56,* 407–416. https://doi.org/10.1007/s11262-020-01763-3

Suttle, C. A. (2007). Marine viruses – major players in the global ecosystem. *National Review Microbiology, 5,* 801–812. https://doi.org/10.1038/nrmicro1750

Suzuki, N., Cornejo, C., Aulia, A., Shahi, S., Hillman, B. I, Rigling, D. .... & Suzuki, N. (2021). In-tree behavior of diverse viruses harbored in the chestnut blight fungus, *Cryphonectria parasitica. Journal of Virology, 95(*6), e01962-20. https://doi.org/10.1128/JVI.01962-20

Tran, T. T., Li, H., Duy, Q., Nguyen, M., Jones, G. K., & Wylie, S. J. (2019). Communication co-infection with three mycoviruses stimulates growth of a *Monilinia fructicola* isolate on nutrient medium, but does not induce hypervirulence in a natural host. *Viruses, 11,* 89. www.mdpi.com/journal/viruses. https://doi.org/10.3390/v11010089

van Diepeningen, A. D., Debets, A. J., & Hoekstra, R. F. (2006). *Fungal Genetics and Biology, 43*(6), 446-452. https://doi.org/10.1016/j.fgb.2006.01.014

Varga, J., Tóth, B., & Vágvölgyi, C. (2003). Recent advances in mycovirus research. *Acta Microbiologica et Immunologica Hungarica, 50*(1), 77–94. https://doi.org/10.1556/AMicr.50.2003.1.8

Vidhyasekaran, P. (2004). *Concise encyclopedia of plant pathology*. ISBN 1-56022-942-X (hard cover: alk. paper)—ISBN 1-56022-943-8 (pbk. : alk. paper). Food Products Press® The Haworth Reference Press Imprints of The Haworth Press, Inc. New York London Oxford. 619 pp. https://doi.org/10.1201/9781482277951

Weinbauer, M. G. (2004). Ecology of prokaryotic viruses. *FEMS Microbiology Reviews, 28,* 127–181. https://doi.org/10.1016/j.femsre.2003.08.001

Woodhall, J.V., Smith, J. E., Mills, P.R., & Sansford, C.E. (2009). *A UK commodity pest risk analysis for the cultivated mushroom, Agaricus bisporus, p: 59*. Commodity PRA for mushrooms CSL/Warwick HRI, December 18th 2007; revised 24 February 2009. CSL R File No. PPP 12011A

Xia, L. C., Li, M., Gao, Z.Y., Gong D., Hong X. Y., Jiang Y., ... & Hu MeiJiao. (2020). Mycoviruses of *Colletotrichum* spp.: a review. *Journal of Southern Agriculture, 51*(1), 123-132. Doi:10.3969/j.issn.2095-1191.2020.01.016.

Xie, J., Wei, D., Jiang, D., Fu, Y., Li, G., Ghabrial, S., & Peng, Y. (2006). Characterization of debilitation-associated mycovirus infecting the plant-pathogenic fungus *Sclerotinia sclerotiorum*. *The Journal of General Virology, 87*(Pt 1), 241–249. https://doi.org/10.1099/vir.0.81522-0

Xie, J., & Jiang, D. (2014). New insights into mycoviruses and exploration for the biological control of crop fungal diseases. *Annual Review of Phytopathology, 52,* 45-68. https://doi.org/10.1146/annurev-phyto-102313-050222

Yu, H. J., Lim, D., & Lee, H. S. (2003). Characterization of a novel single-stranded RNA mycovirus in *Pleurotus ostreatus*. *Virology, 314*(1), 9–15. https://doi.org/10.1016/S0042-6822(03)00382-9

Yudin, L. S., Cho, J. J., & Mitchell, W. C. (1986). Host range of

western flower thrips, *Frankliniella occidentalis* (*Thysanoptera*: Thrip, Cicacidae), with special reference to *Leucaena glauca*. *Environmental Entomology, 15*(6), 1292-1295(4). https://doi.org/10.1093/ee/15.6.1292

Zhang, H, Jiatao, X., Yanping, F., Jiasen, C., Zheng, Q., Zhenzhen, Z., ... & Daohong, J. (2020). A 2-kb mycovirus converts a pathogenic fungus into a beneficial endophyte for *Brassica*. *Protection and Yield Enhancement*. https://doi.org/10.1016/j.molp.2020.08.016

Zhong, J., Chen, D., Zhu, H.J., Gao, B.D., & Zhou, Q. (2016) Hypovirulence of *Sclerotium rolfsii* caused by associated RNA mycovirus. *Frontier of Microbiology, 7,* 1798. https://doi.org/10.3389/fmicb.2016.01798

Zhou, L., Li, X., Kotta-Loizou, I., Dong, K., Li, S., Ni, D., Hong, N., Wang, G., & Xucorresponding, W. (2021). A mycovirus modulates the endophytic and pathogenic traits of a plant associated fungus. *ISME Journal, 15*(7), 1893–1906. https://doi.org/10.1038/s41396-021-00892-3

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Study on the evolution of the fruit morphological and physico-chemical parameters of 'Majhoul' date palm during fruit growth

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Study on the evolution of the fruit morphological and physico-chemical parameters of 'Majhoul' date palm during fruit growth

Abstract: Date palm is an economically important species in the Middle East and North Africa. In Morocco, date palm is the main crop in the southeastern region, mainly in Draa-Tafilalet area. The 'Majhoul' is ranked among the worldwide best quality dates due to its large size and good texture. This work aimed to study the effect of three phases of flowering (early flowering, seasonal and late) on fruit quality of 'Majhoul' during its development. Experiments were carried out on an adult plantation in a modern palm grove in Tafilalet. Obtained results showed that, except for the chemical parameters of the fruit, there is a significant difference ($p \leq 0.01$) between the three flowering phases for the morphological parameters studied (fruit mass, size, and dimensions) during all the fruit development stages. The early flowering phase yielded fruits with higher parameters than the other flowering phases. The mean fruit size (volume) for all the fruit development stages was 22 cm^3 for the early flowering phase, whereas it was only 12.86 and 10 cm^3, respectively, for the seasonal and late flowering phases. The final fruit size was 19.70, 13.55, and 9.97 cm^3, respectively, for the early, seasonal, and late flowering phases.

Key words: Tafilalet area, date palm 'Majhoul', flowering phase, fruit development, fruit morphological and chemical parameters

Raziskava razvoja morfoloških in biokemičnih parametrov plodov dateljeve palme 'Majhoul' v rastni sezoni

Izvleček: Dateljeva palma je ekonomsko pomembna vrsta v Bližnjem vzhodu in severni Afriki. V Maroku je dateljeva palma glavna kulturna rastlina na jugovzhodnih območjih, v glavnem na območju Draa-Tafilalet. Sorta Majhoul je uvrščena med najboljše na svetu zaradi svoje kakovosti, velikih plodov in njihove dobre teksture. V raziskavi je bil preučevan učinek treh obdobij cvetenja (zgodnje cvetenje, cvetenje v glavni sezoni in pozno cvetenje) na razvoj in kakovost plodov. Poskus je potekal v odraslem nasadu z moderno vzgojno obliko v Tafilaletu. Rezultati so pokazali, da so bile z izjemo kemijskih parametrov plodov, značilne razlike ($p \leq 0{,}01$) med tremi obdobji cvetenja v vseh preučevanih morfoloških parametrih plodov (masa, velikost in dimenzije plodov) v vseh fazah razvoja. Zgodnja faza cvetenja je dala plodove, ki so imeli vrednosti vseh merjenih parametrov večje kot plodovi, nastali iz poznejših cvetenj. Povprečna vrednost velikosti plodov (volumen) nastalih po zgodnejm cvetenju je bila 22 cm^3 med tem, ko sta bili velikosti sezonskih in poznih plodov samo12,86 in 10 cm^3. Končne velikosti plodov so bile 19,70; 13,55 in 9,97 cm^3, za plodove nastale iz zgodnjega, sezonskega in poznega cvetenja.

Ključne besede: območje Tafilalet , dateljeva palma 'Majhoul', faze cvetenja, razvoj plodov, morfološki in kemični parametri plodov

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1 INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a perennial monocotyledon plant, which is part of the family of Palmaceae and the genus *Phoenix,* which includes 14 species that are native to tropical and subtropical regions of South Asia or East and North Africa (Dransfield et al., 2008; Shengji et al., 2010). It has been currently grown in the Middle East, North Africa, parts of Central and South America, India, and Pakistan (Al-Shahib & Marshall, 2003) and recently introduced in some African countries such as Namibia. Date palm has been an important fruit species in the Middle Eastern and North African countries for a long time (Marondedze et al., 2014). In Morocco, date palm occupies an area of around 52.000 ha and represents the backbone of agriculture of the Oasian regions, mainly Draa-Tafilalet area, which is the main production area in the country. The genetic diversity of date palm in Morocco consists of more than 223 varieties which are well known and represent 52 % of the total population. The rest (48 %) consists of 'khalts', hybrid seedlings. Traditional commercial varieties of good quality represent only 36 % of the national heritage. They consists of the varieties 'Majhoul', which represents 9 % of the national heritage, 'Bouffegous', which represents 15 %, 'Jihel' 12 % and 'Bouskri' which represents only 0.1 % (ORMVAT, 2015).

Dates fruit are oblong drupes or stone fruits with more or less fleshy and fibrous flesh, which represents 85-90 % of the total fruit mass and contains a single seed (Mansour, 2005; Lobo et al., 2014). They are a fundamental nutrient for the oasis populations. They are an important food source rich in sugars, proteins, dietary fiber, antioxidants, and minerals (magnesium, iron, potassium, etc.) (Amira et al., 2011; Rastegar et al., 2012). With an average annual production of 92976 tons in Morocco, the dates provide an average yearly value of 743.8 million dirhams and contribute 40 to 60 % of the income of the Oasian farms. Dates are the engine of the economy of the producing regions and an important cash source for the farmers of these regions and for the financing of their agricultural activities (ORMVAT, 2015). Dates have reached the international market with famous commercial varieties like 'Bouffegous' and 'Majhoul' (Chafi et al., 2015). Several studies have been conducted on the physico-chemical, biochemical, and biological constituents of date varieties (Hasnaoui et al., 2010; Elguerrouj et al., 2011; Chafi et al., 2015), and their results have classified the dates of the 'Majhoul' among the good quality dates with a large size and high sugar content (more than 70 %) (Acourene et al., 2001).

After the fruit set, there are five development stages in date palm, which are based on changes in fruit size, color, texture, and chemical composition. These development stages are known internationally as ''Hababouk" (immature fruits in the form of peas), ''Kimri" (large and green fruits), ''Khalal" (color stage of the fruit which becomes crisp when eaten), ''Rutab" (fruit ripening stage, soft fruit, and succulent texture) and ''Tamar" (full ripening stage and less humid flesh) (Al-Shahib & Marshall, 2003; Fadel et al., 2006). Marondedze et al. (2014) also reported that fruit development of date palm consists of morphological and physiological changes in the fruit, which occur as biological processes associated with cellular metabolic activities. Fruit growth and development in date palm also leads to morphological, physiological, and biochemical changes after fruit set (Lobo et al., 2013).

Date palm is a species where flowering does not occur simultaneously because the spathe emission is done gradually. Consequently, the flowering and pollination of date palm will also occur progressively over time. The growers in the producing regions distribute the flowering in three phases: an early flowering phase, a seasonal, and a late one. Therefore, fruit quality of these flowering phases have not been studied, and very little research has been carried out. However, in modern date palm groves in date palm growing regions of Morocco, producers of the 'Majhoul' have always used the practice of limiting clusters on clusters that are produced from early and late flowering phases and have always opted to maintain the seasonal flowering regimes in their production system. This research work aimed to study the effect of the three flowering phases on fruit development and quality of 'Majhoul' date palm during fruit growth, by harvesting fruit samples over time.

2 MATERIALS AND METHODS

2.1 THE SITE OF TRIALS

The experiment was set up in a modern date palm grove located in the Goulmima region, Tafilalet area (31°41' N, 4°57' W, and 1028 m elevation), and the trials were carried out on a 13-year-old plantation of 'Majhoul' date palm with an IGP (geographical protection index). The planting density is 7 x 6 m (238 palms per hectare). The irrigation system used on the farm is drip irrigation with two drip ramps per planting row and two drips per palm (one drip per ramp). Plants are irrigated once a week during January and February, twice a week during September, October, November, December, March, and April, three times a week during May, and once a day during June, July, and August.The irrigation dose is 500 l per date palm tree.The fertilization program used on the farm is presented in Table 1.

Table 1: Fertilization program used in the farm of trials on a 13-year old plantation of 'Majhoul' date palm in the Goulmima region, Tafilalet area, Morocco

Intake period	Fertilizer used	Dose provided (kg per ha per month)
December January February	Sulfuric acid	10
	Compost	5000
	Acide Humique	5
March April	Hydrocomplex	50
	Phosphoric Acid	5
May June	Hydrocomplex	50
	Phosphoric Acid	15
	Humic Acid	5
July August	Ammonium Sulphate	20
	Sulfiric Acid	10
	Potassium Sulfate	45

P = date palm tree

The pollinating variety is a 'khalt' which is also 13 years old, and the pollination is carried out manually by placing 5 to 7 spikelets of mature male inflorescence in the middle of the female inflorescence, which is slightly attached with a lace of leaflets to maintain the pollen inside the female inflorescence. The pollination period of each flowering phase of date palm in the farm of trials is presented in Table 2.

2.2 PARAMETERS STUDIED AND MEASURES AND OBSERVATIONS REALIZED

Morphological parameters studied included fruit size (volume), dimensions (length and diameter), and fruit mass. Fruit size is determined with a graduated cylinder of 100, 250 and 1000 ml, fruit dimensions are measured with a caliper and fruit, pulp mass and seed mass are measured with an electronic balance having an accuracy of 0.01 g. Fruit shape and color are determined by visual observation. The percentage of pulp relative to fruit and seed mass is determined according to Acourene et al. (2001):

% pulp = pulp mass/fruit mass x 100
Seed mass = fruit mass - pulp mass

The determination of the fruit dry mass is carried out on fruits; which are devoid of their seeds and dried in the oven at a temperature of 70 °C for 48 hours (Achour et al., 2003).

2.3 CHEMICAL ANALYSIS OF THE FRUITS

Chemical analysis of the fruits was carried out on the pH of the fruit juice and the content of total sugars in the fruits. The juice was extracted from the fruits according to the method of Chafi et al. (2015). The fruits were washed with ordinary water, and their seeds were removed. They were then ground very finely with a mortar, and the resulting crusher was added twice its mass in distilled water. The mixture was centrifuged for 20 minutes in a centrifuge; the supernatant was recovered and then filtered using a vacuum quenching. The filtrate was then adjusted with distilled water to 200 ml, and the resulting solution constituted the raw juice to be analyzed. The pH of the juice was determined using a pH meter, and the content of total sugars in the fruits was determined with a digital refractometer.

2.4 THE EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS OF DATA

Adopted experimental design was a completely random design with a single factor; the flowering phase with three repetitions on five date palm trees, which were randomly selected on the farm and pollinated homo-

Table 2: Pollination period of each one of the three flowering phases (early, seasonal and late flowerings) of 'Majhoul' date palm in the Goulmima region, Tafilalet area, number of clusters used per palm and dates of harvesting fruits for morphological measures and chemical analyzes

		Number of clusters used per palm tree of the study					
Flowering phase	Pollinating period	P1	P2	P3	P4	P5	Dates of harvesting fruits
Early flowering	From 23 to 28 February 2016	2	5	2	2	3	06/02/2016 ; 06/22/2016 ; 07/02/2016 ; 07/13/2016 ; 07/31/2016 ; 08/10/2016 ; 09/03/2016
Seasonal flowering	From 9 to 13 March 2016	3	4	5	3	3	
Late flowering	From 25 to 29 March 2016	3	4	0	4	0	

geneously for each flowering phase. Twenty fruits were randomly chosen per flowering phase and fruit harvesting stage, which coincides with a fruit development stage to make measures and analyses. The fruits were selected at a rate of 3 to 5 fruits per cluster at different heights and orientations of the cluster, and the harvested fruits were deprived of their scars. The aim was to carry out the measures of the morphological parameters and the chemical analysis of the fruits in the laboratory to follow the evolution of these morphological and chemical parameters from fruit set to fruit ripening. Table 2 shows the number of clusters selected per date palm of the study and per flowering phase, the number of fruit samples taken, and the dates of harvesting fruits. Fruit samples collected per fruit development stage and flowering phase were placed in white plastic bags, labeled and placed in an isothermal container, and brought back to the laboratory for analysis.

Statistical analysis of data was performed with the Minitab 16 software, the determination of the mean was made by ANOVA with a single factor, and the comparison of the means was performed with the Tukey test with an error of 5 %.

3 RESULTS AND DISCUSSION

3.1 EVOLUTION OF THE MORPHOLOGICAL PARAMETERS OF THE FRUITS DURING THEIR DEVELOPMENT

3.1.1 Evolution of the fruit size and dimensions

The evolution of fruit size and dimensions (length and diameter) in the three flowering phases (early flowering, seasonal and late) of 'Majhoul' date palm during fruit development in Tafilalet area is presented in Figure 1. It shows that fruit size and dimensions are higher in the early flowering phase than in the other phases. This is because the fruits of the early flowering phase have an 11 to 15 days growth advance compared to fruits of the seasonal flowering phase and 28 to 31 days compared to fruits of the late flowering phase. The mean and final values of the fruit size and dimensions in the three flowering phases and for all the fruit harvesting dates are presented in Table 3, and statistical analysis of data has shown that for these parameters, there is a significant difference ($p \leq 0.001$) between the three flowering phases. Several authors have also reported that the stages of fruit development in date palm lead to physical and physiological changes in the fruit, and modifications in color and texture of the fruit from fruit set to fruit ripening (Al-Shahib & Marshall, 2003; Fadel et al., 2006; Lobo et al., 2014). These morphological and physiological changes in the fruits of date palm provide a promising approach for characterizing their development and quality parameters (Marondedze et al., 2014).

For the sixth (July 31 2016) and seventh (August 10 2016) fruit harvesting dates fruit size is not different between the seasonal and late flowering phases, while it is different between these phases for all the other fruit harvesting dates. This convergence in fruit size between these two flowering phases results in low fruit growth in the seasonal flowering phase and high fruit growth in the late flowering phase (Figure 1a). Whereas the difference in fruit size between the seasonal and late flowering phases during the first six fruit harvesting dates (from

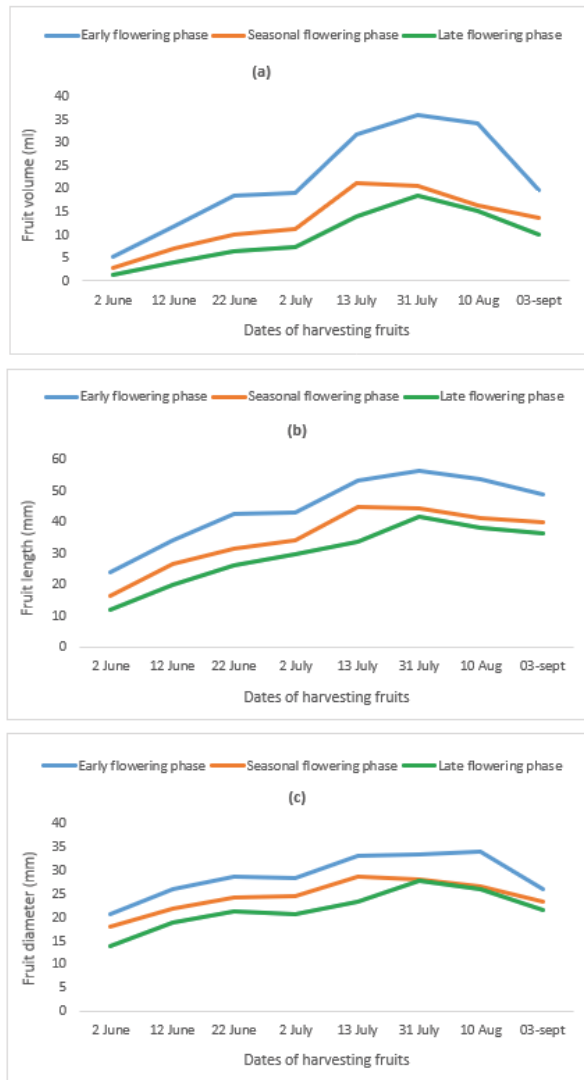

Figure 1: Evolution of the fruit size (volume) (a) and dimensions (b and c) in the early flowering phase, seasonal and late one during fruit growth in 'Majhoul' date palm in the Goulmima region, Tafilalet area, Morocco

Table 3: Mean and final values of fruit size and dimensions in the three flowering phases (early flowering, seasonal and late phases) of 'Majhoul' date palm in the Goulmima region, Tafilalet area.

	Early flowering phase			Seasonal flowering phase			Late flowering phase			
	Fruit length (cm)	Fruit diameter (cm)	Fruit size (cm^3)	Fruit length (cm)	Fruit diameter (cm)	Fruit size (cm^3)	Fruit length (cm)	Fruit diameter (cm)	Fruit size (cm^3)	
Mean value of the fruit parameter for all the fruit harvesting stages	4.43 ± 3	2.87 ± 2	22.02 ± 4	3.47 ± 3	2.43 ± 2	12.86 ± 3	2.95 ± 2	2.15 ± 2	9.55 ± 2.5	**
Final value of the fruit parameter on September 3 2016	48.76 ± 6	26.07 ± 5	19.70 ± 5	39.78 ± 5	23.29 ± 4	13.55 ± 3	36.10 ± 5	21.44 ± 5	9.97 ± 2.5	**

** Significative difference at $p \leq 0.001$

June 2 to July 31 2016) is due to difference in fruit growth between the two flowering phases. Moreover, the difference in the final fruit size between the two flowering phases on September 3 2016 (Figure 1a) is due to the loss of water in the fruits as they are in the ripening phase. Regarding fruit dimensions, fruit length is also the same for the seasonal and late flowering phases at the time of the sixth fruit harvesting stage (Figure 1b), and fruit diameter during the sixth and seventh fruit harvesting stages is also the same for these flowering phases (Figure 1c). This overlap at the time of the sixth fruit harvesting date can be only explained by the difference in fruit growth between these flowering phases, which is due to a delay of about 16 days between the two flowering phases.

3.1.2 Evolution of the fruit, pulp mass and seed mass

Figure 2 presents the evolution of the fruit mass, and pulp mass and seed mass in the three flowering phases during fruit development. It shows that for all the fruit harvesting stages, the mass of fruit, pulp and seed in the early flowering phase is higher than the mass of these elements in seasonal and late flowering phases. This is due to fact that the fruits of the early flowering phase have an 11 to 15 days growth advance compared to the seasonal flowering phase and 28 to 31 days growth advance compared to the late flowering phase. The mean and final values of fresh mass of the fruit, pulp and seed and the mean and final dry mass of the fruit of the three flowering phases for all the fruit harvesting stages are presented in Table 4. Moreover, statistical analysis of data showed that for these parameters of the fruit, there is a significant difference ($p \leq 0.01$) between the fruits of the three flowering phases.

During the sixth (July 31 2016) and seventh (August 10 2016) fruit harvesting dates, the seasonal and late flowering phases yielded fruits with similar fruit and pulp fresh mass, whereas they were different during the other fruit development stages (Figure 2a and b). In the case of seeds, it is only during the last fruit harvesting stage (September 3 2016) that their mass is similar in the three flowering phases. However, it is different between the flowering phases in the other fruit harvesting dates, except for the seventh fruit harvesting date where seed mass of the seasonal flowering phase is close to that of the late flowering phase (Figure 2c). This is due to favorable climatic conditions for fruit development during the early flowering phase, which are favorable to fruit development during the early stages of fruit growth. Some authors have also reported that favorable climatic conditions, which coincide with the early flowering phase, promote the development of growth hormones, mainly

gibberellic acid, which induces the accumulation of reserves in the fruit pulp (El-Otmani et al., 2015). Fruit dry

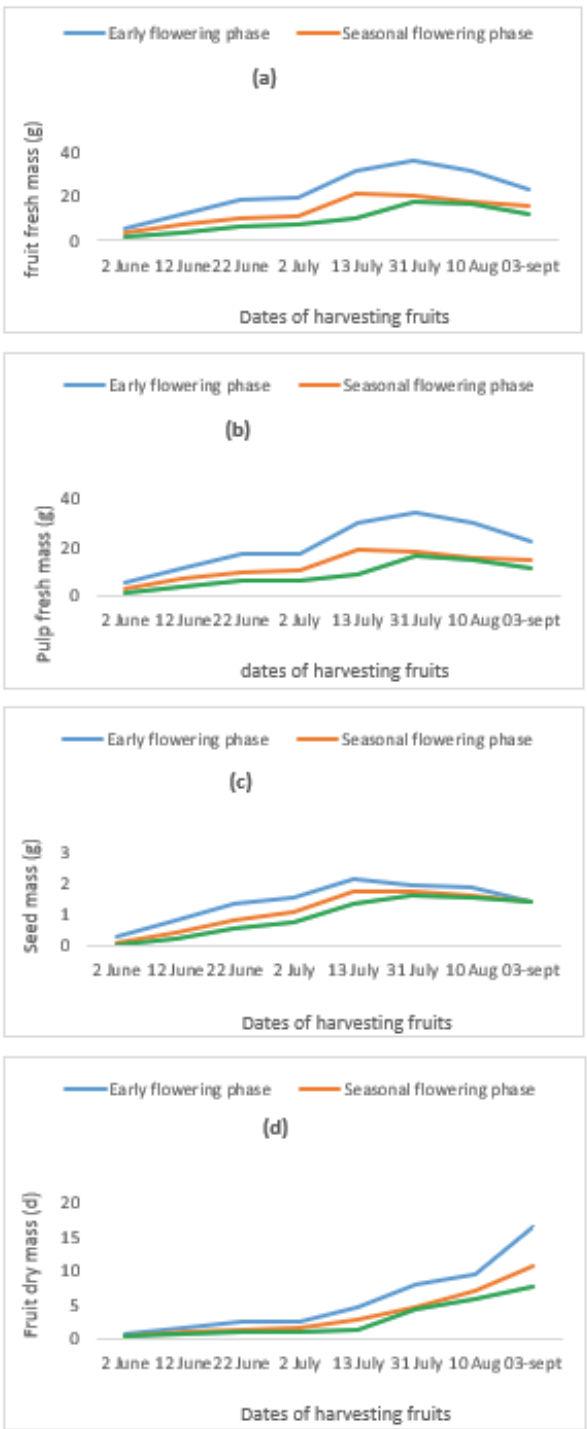

Figure 2: Evolution of the fruit fresh mass (a), pulp fresh mass (b), seed mass (c) and fruit dry mass (d) of the fruits of the early, seasonal and late flowering phases during fruit development of 'Majhoul' date palm in the Goulmima region, Tafilalet area

Table 4: Mean and final values of the fruit and pulp fresh mass, seed mass and fruit dry mass of the fruits of the early, seasonal and late flowering phases of 'Majhoul' date palm in the Goulmima region, Tafilalet area

	Early flowering phase				Seasonal flowering phase				Late flowering phase			
	Fruit fresh mass (g)	Pulp fresh mass (g)	Seed mass (g)	Fruit dry mass (g)	Fruit fresh mass (g)	Pulp fresh mass (g)	Seed mass (g)	Fruit dry mass (g)	Fruit fresh mass (g)	Pulp fresh mass (g)	Seed mass (g)	Fruit dry mass (g)
Mean value of the fruit parameter for all the fruit harvesting stages	22,43 ± 3	20,95 ± 2.5	1,42 ± 1.2	5,73 ± 2.5	13,32 ± 3	12,21 ± 2	1,11 ± 1.2	3,65 ± 2	9,44 ± 2	8,56 ± 1.5	0,91 ± 0.5	2,70 ± 1.4 *
Final value of the fruit parameter on September 3 2016	23.33 ± 3	21.88 ± 2.5	1.42 ± 1.2	16.44 ± 3	16.01 ± 3.5	14.85 ± 2.5	1.40 ± 1.2	10.72 ± 3	12.15 ± 2.5	11.08 ± 2	1.38 ± 1	7.72 ± 2 *

* Significative difference at $p \leq 0.01$

mass is almost similar during the first four harvesting stages in the seasonal and late flowering phases (Figure 2d). This is due to fruit development of these flowering phases, which took the same pace during the early stages of fruit development because the two flowering phases are separated only for a short period.

3.2 EVOLUTION OF THE CHEMICAL COMPOSITION OF THE FRUITS DURING THEIR DEVELOPMENT

The evolution of the chemical composition of the fruits during their development is presented in Figure 3. It shows that the pH of the fruit juice has a similar evolution for the three flowering phases from the second fruit harvesting stage to the last, while it's different between the flowering phases for the first fruit harvesting stage (Figure 3a). The content of total sugars in the fruits also has a similar evolution for the three flowering phases during all the fruit harvesting stages (Figure 3b). The mean and final values of the pH of the fruit juice and the content of total sugars in the fruits of the three flowering phases and for all the fruit harvesting stages are presented in Table 5. Moreover, statistical analysis of data showed that there is no significant difference ($p > 0.05$) between the three flowering phases for the two parameters. This is probably because the flowering phase does not affect the pH of the fruit juice and the content of total sugars in the fruits; however, the fruit harvesting stage affects these parameters in the three flowering phases. Several authors have also reported that the chemical composition of the fruits varies according to the stages of fruit development (Salman Haidar et al., 2013), and fruit development in date palm consists of biological processes which are associated with chemical changes in the cell from fruit set to ripening stage (Lobo et al., 2013; Marondedze et al., 2014).

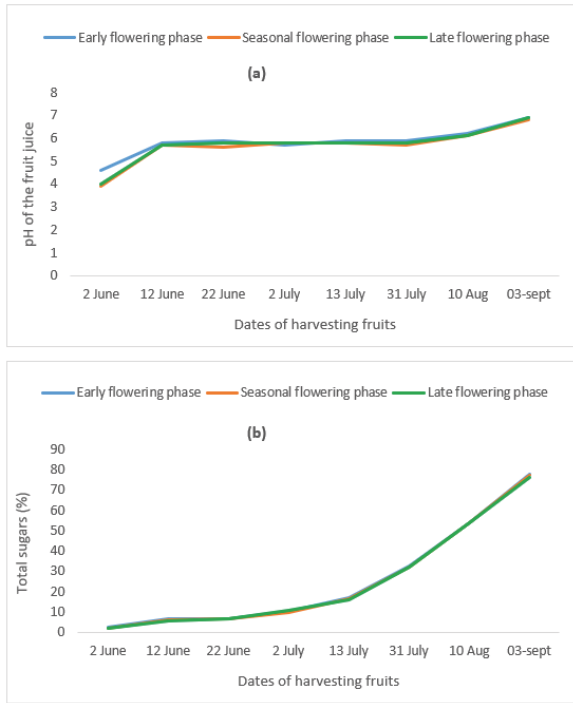

Figure 3: Evolution of the pH of the fruit juice (a) and the content of total sugars in the fruits (b) of the three flowering phases (early flowering, seasonal and late) of 'Majhoul' date palm in the Goulmima region, Tafilalet area

4 CONCLUSIONS

For all studied morphological parameters of the fruit (fruit size and dimensions and fruit mass), there is a difference in their evolution between the three flowering phases during fruit development, and along this evolution, the parameters of the early flowering phase are higher than those of the other flowering phases. This is partly because the fruits of the early flowering phase have

Table 5: Mean and final values of the pH of the fruit juice and the content of total sugars in the fruits of the three flowering phases (early flowering, seasonal and late) of 'Majhoul' date palm in the Goulmima region, Tafilalet area

	Early flowering phase		Seasonal flowering phase		Late flowering phase		
	pH of the fruit juice	Content of total sugars in the fruits (%)	pH of the fruit juice	Content of total sugars in the fruits (%)	pH of the fruit juice	Content of total sugars in the fruits (%)	
Mean value of the fruit chemical parameter for all the fruit harvesting stages	5.86 ± 3	25.89 ± 3	5.68 ± 2.5	25.49 ± 4	5.74 ± 2.5	25.36 ± 4	ns
Final value of the fruit chemical parameter on September 3 2016	6.90 ± 4	77.80 ± 11	6.80 ± 3.5	76.90 ± 11	6.90 ± 4	76.00 ± 11	ns

ns: No significant difference at $p > 0.05$

a remarkable 11 to 31 days of growth advance compared to other flowering phases. On the other hand, favorable climatic conditions for fruit growth (mild temperatures and long days) during the spring season which coincides with the early stages of fruit development of date palm in the region of study. However, for the content of total sugars in the fruits and the pH of the fruit juice, their evolution during the fruit harvesting stages is similar for the three flowering phases, while their values vary from one fruit harvesting stage to another and for the three flowering phases. This is because the flowering phase does not affect these parameters, while the fruit development stage affects these parameters.

Based on these results, we can suggest that growers keep only the early flowering phase clusters for their cluster-limiting operation when the number of clusters of this flowering phase is sufficient. Moreover, when the number of clusters of the early flowering phase is not sufficient, the choice of clusters to be retained in the limitation operation can be made on the clusters of the early and seasonal flowering phases to obtain a good fruit yield and quality and an early entry into production.

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6 REFERENCES

Achour, M., Ben Amara, S., Ben Salem, N., Jebali, A., Hamdi, M. (2003). Effet des conditionnements sous vide et sous atmosphère modifiée sur la conservation des dattes DegletNor en Tunisie. *Fruits, 58*(4), 205-212. https://doi.org/10.1051/fruits:2003008

Acourene, S., Buelquedji, M., Tama, M., Taleb, B. (2001). Caractérisation, évaluation de la qualité de la datte et identification des cultivars rares de palmier dattier de la région des Ziban. *Recherche Agronomique, 5(8)*, 19-39.

Al-Shahib, W. & Marshall, R. J. (2003). The fruit of the date palm: Its possible use as the best food for the future. *International Journal of Food Science and Nutrition, 54*, 247-259. https://doi.org/10.1080/09637480120091982

Amira, E., Flamini, G., Saafi, E. B., Issaoui, M., Zeyene, N., Ferchichi, A., Hammami, M., Hedal, A. N., Achour, L. (2011). Chemical and aroma volatile compositions of date palm fruits at three maturation stages. *Food Chemistry, 127*, 1744-1754. https://doi.org/10.1016/j.foodchem.2011.02.051

Chafi, A., Benabbes, R., Bouakka, M., Hakkou, A., Kouddane, N., Berrichi, A. (2015). Pomological study of dates of some date palm varieties cultivated in Figuig oasis. *Journal of Materials and Environmental Science, 6*(5), 1266-1275.

Dransfield, J., Uhl, N. W., Asmussen, C. B., Baker, W. J., Harley, M. M., Lewis, C. E. (2008). *Genera Palmarum, the evolution and classification of palms*. Kew, UK, Kew: Royal Botanic Gardens.

Elguerrouj, M., Paquot, M., Robert, C., Benjouad, A., Bouakka, M., Hakkou, A. (2011). Physicochemical composition of two varieties of Moroccan palm date fruit. *Asian Journal of Chemistry, 23*, 1932–1936.

El-Otmani, M., Bagayogo, S., El-Fadl, A., Benismail, M. C. (2015). Effect of regulated deficit irrigation on vegetative growth, fruiting, stomatal conductance and water use efficiency in 'nules' clementine under arid conditions of the souss valley of morocco. *Acta Horticulturae, 1065*, 1757-1766. https://doi.org/10.17660/ActaHortic.2015.1065.225

Fadel, M. A., Kurmestegy, L, Rashed, M., Rashed, Z. (2006). Fruit color properties of different cultivars of dates (*Phoenix dactylifera* L.). *Agricultural Engineering International CIGR Journal, 3*, 1-9.

Hasnaoui, A., Elhoumaizi, M. A., Hakkou, A., Wathelet, B., Sindic, M. (2010). Physico-chemical characterization, classification and quality evaluation of date palm fruits of some Moroccan cultivars. *Biology Journal of Scientific Research, 3*(1), 139-149. https://doi.org/10.3329/jsr.v3i1.6062

Lobo, M. G., Elhadi, M. Y., Kader, A. A. (2013). Biology and postharvest physiology of date Fruit. In M. Siddiq, S. M. Aleid & A. A. Kader (Eds.), *Dates: Postharvest science, processing technology and health benefits* (pp. 57-80). UK, John Wiley & Sons Ltd Publisher. https://doi.org/10.1002/9781118292419.ch3

Mansour, H. M. (2005). *Morphological and genetic characterization of some common Phoenix dactlifera L. cultivars in Ismailia region*. M.Sc. Thesis. Suez Canal University, Faculty of Science, Department of Botany, Egypt.

Marondedze, C., Gehring, C., Thomas, L. (2014). Dynamic changes in the date palm fruit proteome during development and ripening. *Horticulture Research, 1*, 1-14. https://doi.org/10.1038/hortres.2014.39

ORMVAT (2015). *Bilan Phoenicicole au titre de la compagne agricole 2014-2015.* Office Régional de Mise en Valeur Agricole de Tafilalet (ORMVAT).

Rastegar, S., Rahemi, M., Baghizadeh, A., Gholami, M. (2012). Enzyme activity and biochemical changes of three date palm cultivars with different softening pattern during ripening. *Food Chemistry, 134*, 1279-1286. https://doi.org/10.1016/j.foodchem.2012.02.208

Salman Haidar, M., Iqrar, A. K., Summar, A. N., Jaskani, M. J., Rashad, W. K., Nafees, M., … Pasha, I. (2013). Fruit developmental stages effects on biochemical attributes in date palm. *Pakistan Journal of Agricultural Scences, 50*(4), 577-583.

Shengji, P., Sanyang, C., Lixiu, G., Henderson, A. (2010). *Phoenix* Linnaeus. *Flora China, 23*,143-144.

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Investigating the growth characteristics, oxidative stress, and metal absorption of chickpea (*Cicer arietinum* L.) under cadmium stress and *in silico* features of HMAs proteins

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Investigating the growth characteristics, oxidative stress, and metal absorption of chickpea (*Cicer arietinum* L.) under cadmium stress and *in silico* features of HMAs proteins

Abstract: Heavy metal contamination can have a strong effect on the morphological and physiological characteristics of plants. In the present study, *Cicer arietinum* L. (chickpea) was exposed to different concentrations of cadmium (control, 2, 4, 8 µg Cd g^{-1} perlite) and the effect on plant growth and antioxidant enzymes were evaluated. The observed morphological changes in chickpea plant included stunted growth, reduced root system development and plant color change. A significant increase in enzyme activity of peroxidase, superoxide dismutase, catalase, and ascorbate peroxidase was observed at 4 µg Cd g^{-1} perlite, with a subsequent decrease when concentration was increased to 8 µg Cd g^{-1} perlite in the leaves of the plants. The highest cadmium levels were determined at a concentration of 8 µg Cd g^{-1} perlite. With the addition of 2 µg Cd g^{-1} perlite, manganese uptake in the aboveground part of the plant increased significantly, but then decrease at higher cadmium concentrations. In addition, zinc and copper levels decrease in the presence of cadmium. These results indicate that chickpea has a relatively high adsorption capacity for cadmium in aboveground tissues and special precautions should be taken when growing chickpea. *In silico* analysis led to the identification of 13 heavy metal ATPases (HMAs) in chickpea. These proteins contain 130 to 1032 amino acids with 3 to 18 exons. They are involved in the transfer of cadmium and zinc and help in heavy metal detoxification of plants. Bioinformatics studies have been conducted to better understand the mechanism by which the plant is able to combat heavy metal stress.

Key words: cadmium, chickpea, HMAs, oxidative stress

Preučevanje rastnih značilnosti, oksidativnega stresa in prevzema kovin pri čičerki (*Cicer arietinum* L.) v razmerah kadmijevega stresa in *in silico* lastnosti HMAs proteinov

Izvleček: Onesnaženje s težkimi kovinami ima lahko močan učinek na morfološke in fiziološke lastnosti rastlin. V raziskavi je bila čičerka (*Cicer arietinum* L.) izpostavljena različnim koncentracijam kadmija (kontrola, 2, 4, 8 µg Cd g^{-1} perlita). Ovrednoteni so bili učinki na rast rastlin in na antioksidacijske encime. Opažene morfološke spremembe čičerke so bile zavrta rast, zmanjšan razvoj koreninskega sistema in spremembe v barvi rastlin. Značilna porast aktivnosti encimov peroksidaze, superoksid dismutaze, katalaze in askorbat peroksidaze je bila opažena pri 4 µg Cd g^{-1} perlita s posledičnim upadom, ko se je koncentracija povečala na 8 µg Cd g^{-1}perlita v listih tretiranih rastlin. Največja vsebnost kadmija je bila določena pri obravnavanju z 8 µg Cd g^{-1} perlita. Pri dodatku 2 µg Cd g^{-1} perlita se je privzem mangana v nadzemnih delih rastlin značilno povečal a se je pri večjih koncentracijah kadmija zmanjšal. Dodatno so se v prisotnosti kadmija vsebnosti cinka in bakra zmanjševale. Ti izsledki kažejo, da ima čičerka relativno veliko sposobnost privzema kadmija v nadzemna tkiva in moramo na to biti pozorni, če jo gojimo v s kadmijem onesnaženem okolju. *In silico* analize so vodile k prepoznavanju 13 ATPaz (HMAs), povezanih s težkimi kovinami. Ti proteini vsebujejo 130 do 1032 amino kislin s 3 do 18 eksoni. Vključeni so v prenos kadmija in cinka in pomogajo v rastlinah pri detoksikaciji težkih kovin. Za bolje razumevanje mehanizmov s katerimi rastline premagujejo stres težkih kovin so bile izvedene tudi bioinformacijske raziskave.

Ključne besede: kadmij, čičerka, HMAs, oksidativni stres

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1 INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a major legume crop that is consumed globally especially on the Africa and Asia continents (Kaur et al., 2022). Chickpea has a very high nutritional content and is one of the cheapest sources of protein and an important source of minerals (manganese, molybdenum, phosphorus and potassium) and vitamins (Mohanty et al., 2022), so measures need to be taken to avoid its contamination with heavy metals such as cadmium.

Cadmium (Cd) is one of the most important contaminants due to its high toxicity and high water solubility and is readily absorbed by the root system of many plants (Zulfiqar et al., 2022). High levels of Cd can have detrimental effects on plant physiological and biochemical processes, leading to reduced growth, impaired nutrient uptake, and disruption of cellular functions. Moreover, Cd toxicity inhibits plant growth by affecting cell division, cell elongation, and differentiation processes (Tuver et al., 2022). It disrupts hormone balance, leading to stunted root and shoot growth, reduced biomass production, and impaired reproductive development. Cd toxicity can interfere with the uptake and transport of essential nutrients such as iron, calcium, magnesium, and zinc (Zhou et al., 2022). It can bind to transporters, enzymes, and carrier proteins, thereby disrupting nutrient homeostasis and causing nutrient deficiencies. Furthermore, Cd toxicity negatively impacts photosynthesis, reducing the efficiency of light absorption, electron transport, and carbon assimilation (Zulfiqar et al., 2022).

Plants have evolved several mechanisms to mitigate the toxic effects of cadmium (Cd) and minimize its accumulation in their tissues. One crucial strategy is the sequestration of cadmium into vacuoles, which serves as a storage site for toxic metals (Jogawat et al., 2021). On the other hand, Cd toxicity leads to the generation of reactive oxygen species (ROS) in plant cells, causing oxidative stress (Zhang et al., 2019). Antioxidant enzymes, such as superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT), scavenge and neutralize ROS, protecting cellular components from oxidative damage (Faria et al., 2022). Moreover, Plants possess transporters that can efflux Cd ions from the cytoplasm to the extracellular space or restrict their entry into specific tissues. ATP-binding cassette (ABC) transporters and heavy metal ATPases (HMAs) are involved in Cd transport across cell membranes. These transporters play a crucial role in minimizing the accumulation of Cd in sensitive tissues and facilitating its sequestration (Tian et al., 2023).

HMAs belong to the P-type ATPase superfamily and are localized in the plasma membrane or tonoplast (vacuolar membrane) of plant cells. HMAs play a crucial role in the detoxification of cadmium by actively transporting it out of sensitive cellular compartments or sequestering it into vacuoles. This process contributes to reducing the concentration of free cadmium in the cytoplasm and minimizing its toxic effects on plant growth and development. HMAs function as efflux pumps, actively transporting Cd ions out of the cytoplasm and extruding them from the cell or into specific compartments, such as the vacuole (Fang et al., 2016). By pumping Cd out of sensitive cellular regions, HMAs reduce the concentration of free cadmium in the cytoplasm and minimize its toxic effects on cellular processes (Satoh-Nagasawa et al., 2012). HMAs participate in the regulation of metal ion homeostasis in plants. They are involved in maintaining the balance between essential metals (such as zinc and copper) and non-essential heavy metals (such as Cd) (Fang et al., 2016). This regulation ensures that essential metals are properly acquired and utilized while minimizing the uptake and accumulation of toxic metals like Cd. HMAs interact with metal chelators, such as phytochelatins (PCs), which are small peptides that bind to heavy metal ions, including Cd. This process contributes to the detoxification and sequestration of cadmium in less sensitive cellular compartments (Tian et al., 2023).

The purpose of the study is to get insights how chickpea plants respond to cadmium, a harmful heavy metal that can contaminate soil and negatively affect plant health .Understanding the mechanisms of Cd toxicity in plants is crucial for developing strategies to mitigate its adverse effects. The first objective is therefore to examined the impact of Cd on the growth characteristics, activity of oxidative enzymes, Cd, zinc (Zn), copper (Cu) and manganese (Mn) content in chickpea.

The next objective of this study was to gain a better understanding of the role that HMAs play in chickpea, particularly under conditions of cadmium stress and to provide insights into how chickpea plants respond to cadmium by Bioinformatics analyses such as number of genes, proteins, gene loci, cellular location, phylogenetic relationship, three-dimensional protein structure, conserved domains, similar template and catalytic site.

2 MATERIALS AND METHOD

2.1 PROPAGATION AND CADMIUM EXPOSURE

Chickpea (*Cicer arietinum* L.) seeds were germinated in sterilized Cucupite and Perlite in a greenhouse on the photoperiod of 8 h light and 16 h darkness. Seedlings with leaves were planted in pots (diameter, 12 cm and height 15 cm) under controlled conditions and watered

with distilled water every 3 days. Cadmium chloride were added in four concentrations (control, 2, 4, and 8 µg Cd g^{-1} perlite) calculated per g of perlite. Plants were watered with Hoagland nutrient solution (Hoagland and Snyder 1933) without cadmium chloride (field capacity was considered). After 10 days of Cd treatment plants were harvested for further investigations.

2.2 GROWTH PARAMETERS

The fresh and dry mass of the roots and above ground parts were determined (mg). Plantlet height, leaf area, root length, shoot length and internode length were measured. Stomatal densities on the lower and upper epidermis were evaluated.

2.3 ENZYME ASSAYS

Enzyme extracts were prepared from fresh chickpea leaves (1 g) with phosphate potassium buffer (5 ml). Homogenous samples were prepared by pulverizing followed by centrifugation (4 °C, 25 min, 15000 rpm) and storage at -80 °C. Catalase enzyme activity was determined by mixing phosphate buffer (2.5 ml, pH 7.5) and hydrogen peroxide (1%, 0.1 ml) in an ice bath and the addition of enzyme extract (0.1 ml) and the rate of disappearance of H_2O_2 is followed by observing the rate of decrease in the absorbance at 240nm via spectrophotometer.

Peroxidase enzyme activity was determined based on the method by Koroi (1989). The reaction mixture consisted of acetate buffer (0.2 M, 2 ml, pH 5), benzidine (0.02 M, 100 ml), hydrogen peroxide (3 %, 200 µl) and enzyme extract (25 µl). The absorption was determined at 530 nm. Ascorbate peroxidase (EC11.1.11.1) activity was determined spectrophotometrically (Nakano and Asada, 1987). To the enzyme extract (100 µl) was added K_2HPO_4 (0.5 M, 2.5 ml), ascorbate (0.5 mM, 0.1 ml), EDTA (0.1 mM, 0.1 ml) and H_2O_2 (1 %, 0.2 ml) and the absorbance read at 290 nm. Specific enzyme activity was reported as units/g fresh mass (Nakano and Asada 1987). Total soluble protein was determined utilizing the Bradford assay with bovine serum albumin (BSA) as standard. The absorbance was read at 595 nm (Bradford, 1976).

2.4 CADMIUM AND OTHER ELEMENTS MEASUREMENT

Plant samples were oven dried (72 h, 60 °C) and the dry mass determined. Dried samples were ashed (550 °C, 8 h). The digested extract (1N HCl, 1 mL; nitric acid, 97 %, 1 ml, 1 h) was made to a final volume of 20 ml and the cadmium, zinc, copper and manganese content of the samples measured (Chellaiah, 2018) utilizing a Flame Atomic Absorption Spectrometer (GBC, SAVANTAA scientific equipment, Australia) which has a detection limit of 0.007 µg ml^{-1}. Cd (II), Zn (II), Cu (II) and Mn (II) standard solution were prepared using their nitrate salts in nitric acid. Bioconcentration factor (BCF) computed as heavy metal accumulated in each plant tissue to that dissolved in the soil medium (Bose and Bhattacharyya 2008).

Root bioconcentration factor: BCF = root/soil
Shoot bioconcentration factor: BCF = shoot/soil
TF = BCFshoot/BCFroot

2.5 BIOINFORMATICS ANALYSIS

The gene database of NCBI was searched utilizing the keyword „HMA". Gene characteristics included location, exon count and conserved domain. Protein sequences were used for localization prediction from the Localizer and protein tertiary structure predicted by Phyre2. Potential tunnels within each protein and catalytic pocket were predicted utilizing CAVER Web. The Jones-Taylor Thornton model was selected to obtain the phylogenies tree of HMAs from chickpea and *Arabidopsis* using the neighbor-joining (NJ) method, with a bootstrap test performed using 1000 iterations in MEGA5 (Tamura et al., 2007). Multiple sequence alignments were performed utilizing the muscle algorithm of mega 7 software to detect conserved residues (Kumar et al., 2016). HMAs from *Arabidopsis* were highlighted in green. Some information has been mentioned below:

XP_004509102.1: Probable cadmium/zinc-transporting ATPase HMA1, chloroplastic [*Cicer arietinum*], P_004487939: Cadmium/zinc-transporting ATPase HMA3-like isoform X1 [*Cicer arietinum*], XP_027189340: Cadmium/zinc-transporting ATPase HMA2-like isoform X2 [*Cicer arietinum*], XP_012573401: Putative inactive cadmium/zinc-transporting ATPase HMA3 [*Cicer arietinum*], XP_004488108: Cadmium/zinc-transporting ATPase HMA3-like [*Cicer arietinum*], XP_012573132: Copper-transporting ATPase HMA4-like [*Cicer arietinum*], XP_012574029: Copper-transporting ATPase HMA4-like isoform X1 [Cicer arietinum], XP_027192934: Copper-transporting ATPase HMA4-like isoform X2 [*Cicer arietinum*], XP_004500941: Cation-transporting ATPase HMA5-like [*Cicer arietinum*], XP_004511583: Probable copper-transporting ATPase HMA5 [*Cicer arietinum*], XP_004504792: Copper-transporting ATPase PAA1, chloroplastic [*Cicer arietinum*], XP_004504659:

Copper-transporting ATPase RAN1 [*Cicer arietinum*], XP_004501429: Copper-transporting ATPase PAA2, chloroplastic [*Cicer arietinum*].

Q9SH30 (Protein: Probable copper-transporting ATPase HMA5, Gene: HMA5, Organism: *Arabidopsis thaliana* (L.)Heynh., P0CW78 (Protein: Cadmium/zinc-transporting ATPase HMA3, Gene: HMA3, Organism: *Arabidopsis thaliana*, Q9SZW4 (Protein: Cadmium/zinc-transporting ATPase HMA2, Gene: HMA2, Organism: *Arabidopsis thaliana*, Q4L970 (Protein: Copper-exporting P-type ATPase, Gene: copA, Organism: *Staphylococcus haemolyticus* Schleifer & Kloos, 1975 *(*strain *JCSC1435*), O32220 (Protein: Copper-exporting P-type ATPase, Gene: copA, Organism: *Bacillus subtilis (Ehrenberg 1835)* Cohn 1872 *(*strain *168)*, Q9S7J8 (Protein: Copper-transporting ATPase RAN1, Gene: RAN1, Organism: *Arabidopsis thaliana*), Q9M3H5 (Protein: Probable cadmium/zinc-transporting ATPase HMA1, chloroplastic, Gene: HMA1, Organism: *Arabidopsis thaliana*).

2.6 STATISTICAL ANALYSES

Data analyses were performed using the SPSS 20 software package (SPSS Inc., Chicago, USA). All experimental data were presented as the mean ± SD. One-way ANOVA was used to test differences between various means followed by the post hoc Tukey test (homogeneity of variances and data normally distributed). The level of significance was set at $p < 0.05$ for all tests.

3 RESULTS

3.1 GROWTH CHARACTERISTICS IN THE ABOVEGROUND PARTS OF CHICKPEA SEEDLINGS AFFECTED BY CADMIUM

Observed morphological changes in chickpea seedlings exposed to cadmium included changes in plant length, coloration and leaf size. Results indicated that stem color changed to a bright green-yellow. Moreover, changes were observed in leaf color (yellow) due to cadmium exposure. There was a significant reduction in shoot and root length. Shorter and less dense roots were observed in the treated samples (Table 1). The fresh and dry mass of the shoots and roots in chickpea plants were also significantly affected by cadmium with the lowest seedling mass being observed at high cadmium concentrations. Plants treated with 2 µg Cd g^{-1} perlite had a decline in leaf area which was less than half that of the control. At cadmium levels of 2 µg Cd g^{-1} perlite, the length of the first internodes increased, whereas at higher concentrations, there was a decrease, while the length of the

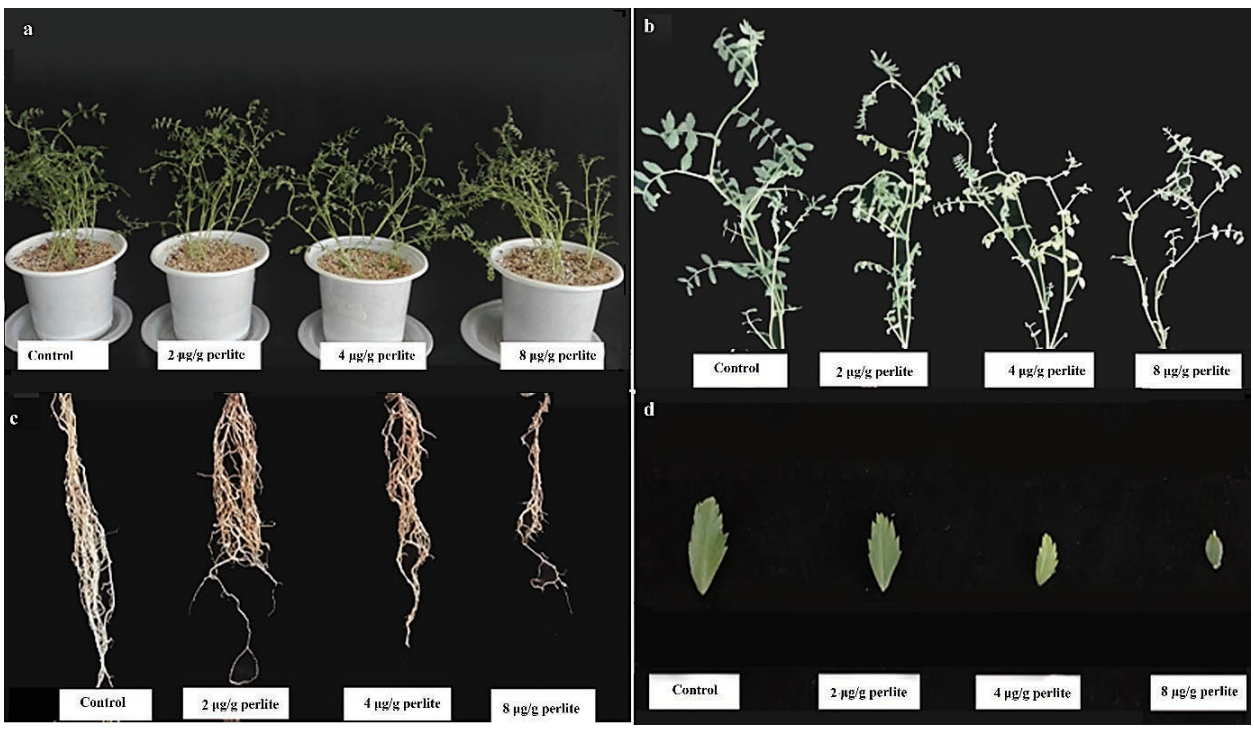

Fig. 1: Effect of cadmium on chickpea (*Cicer arietinum* L.) growth under normal and various concentrations of cadmium. a Seedlings, b Aboveground parts, c Roots, d Leaf areas (control, 2, 4 and 8 µg Cd g^{-1} perlite)

second internodes showed only a significant reduction at high concentrations of cadmium (Fig. 1, Table 1). Furthermore, with the addition of cadmium (4 µg Cd g^{-1} perlite), stomatal densities on the lower epidermis increased significantly but subsequently declined while higher concentrations of cadmium (Table1).

3.2 EFFECT OF CADMIUM ON SOD, POD AND CAT ACTIVITIES IN THE AERIAL PARTS OF CHICKPEA SEEDLINGS

Cadmium stress resulted in a significant increase in POD enzyme activity. The highest ascorbate activity was observed in cadmium treatments at 4 and 8 µg Cd g^{-1} perlite. Further increase in cadmium exposure resulted in a decline in POD activity which was however still significantly higher than that of the control and plantlets treated with 4 µg Cd g^{-1} perlite. The lowest enzyme activity was observed in the controls (Fig. 2a). SOD enzyme activity significantly increased in chickpea with the highest enzyme activity being observed in plantlets treated with 4 µg Cd g^{-1} perlite with the lowest enzyme activity being observed in the control (Fig. 2b). There was a significant increase in catalase enzyme activity. The highest catalase activity was also observed in plants treated with 4 µg Cd g^{-1} perlite with a subsequent decline when cadmium chloride concentration was increased to 8 µg Cd g^{-1} perlite. The lowest level of enzyme activity was observed in the control (Fig. 2c). Investigation of ascorbate peroxidase enzyme activity showed that this enzyme was also affected by cadmium exposure. The highest ascorbate activity was observed in cadmium treatments with 4 and 8 µg Cd g^{-1} perlite (Fig. 2d). Oxidative enzyme activity (SOD, APX or CAT) was shown to increase in the leaves of plants exposed to cadmium. Increased SOD activity is associated with an increase in the formation of superoxide, which activates gene expression by signal induction.

3.3 MEASUREMENT OF CADMIUM CONTENT AND ELEMENTAL CHANGES IN THE AERIAL PARTS OF CHICKPEA SEEDLINGS AFFECTED BY CADMIUM

The cadmium content in aerial parts of chickpea grown in different concentrations of cadmium chloride increased significantly. The highest concentrations were observed at cadmium chloride concentration of 8 µg Cd g^{-1} perlite. A doubling of cadmium accumulation was observed in the aerial parts of the plant when the cadmium content of the medium was increased from 2 to 4 µg Cd g^{-1} perlite (Fig. 3a). Moreover, elemental composition was significantly affected by cadmium levels (Fig. 3). Chickpea cultivated in cadmium-containing media showed a significant difference in the amount of manganese present in the aerial part of the plant.

With the addition of cadmium, manganese uptake increased significantly by approximately three times,

Table 1: Effect of Cd (Control, 2, 4 and 8 µg Cd g^{-1} perlite) on morphometric features in chickpea (*Cicer arietinum* L.) Values with different letters are significantly different at $p < 0.05$

Parameters	Control	2 µg Cd g^{-1} perlite	4 µg Cd g^{-1} perlite	8 µg Cd g^{-1} perlite
Plant length (cm)	62.76 ± 1.36^{a}	58 ± 0.0709^{a}	42.56 ± 1.78^{b}	37.93 ± 1.78^{b}
Shoot length (cm)	29.33 ± 0.66^{a}	25.65 ± 0.779^{b}	22.55 ± 1.35^{bc}	21.16 ± 1.092^{c}
Root length (cm)	35 ± 0.57^{a}	30.86 ± 0.69^{b}	18.56 ± 0.92^{c}	16.6 ± 0.83^{c}
Plant fresh mass (g)	4.0367 ± 0.043^{a}	3.442 ± 0.238^{b}	3.084 ± 0.169^{b}	1.715 ± 0.042^{c}
Shoot fresh mass (g)	2.291 ± 0.11^{a}	1.6317 ± 0.14^{b}	1.297 ± 0.061^{c}	0.682 ± 0.014^{d}
Root fresh mass (g)	2.24 ± 0.078^{a}	1.9 ± 0.1^{b}	1.3167 ± 0.109^{c}	0.99 ± 0.003^{d}
Shoot dry mass (g)	1.987 ± 0.01^{a}	1.4783 ± 0.11^{b}	1.0447 ± 0.029^{c}	0.606 ± 0.002^{d}
Root dry mass (g)	2.01 ± 0.04^{a}	1.696 ± 0.063^{b}	1.123 ± 0.069^{c}	0.823 ± 0.062^{d}
Leaf area (mm^2)	103.33 ± 1.76^{a}	48.33 ± 2.18^{b}	20.66 ± 0.666^{c}	18.33 ± 1.201^{c}
First internode length (cm)	1.1 ± 0.264^{b}	1.766 ± 0.0577^{a}	1.3 ± 0.3^{b}	0.833 ± 0.838^{c}
Second internode length (cm)	2.433 ± 0.513^{a}	2.266 ± 0.503^{a}	1.766 ± 0.808^{b}	1.633 ± 0.850^{b}
Stomatal densities on the upper epidermis	35 ± 0.545^{ab}	31 ± 0.564^{b}	24.33 ± 0.413^{c}	39.33 ± 0.633^{a}
Stomatal densities on the lower epidermis	29.6667 ± 0.448^{c}	39 ± 0.653^{b}	46 ± 0.765^{a}	37.33 ± 0.985^{b}

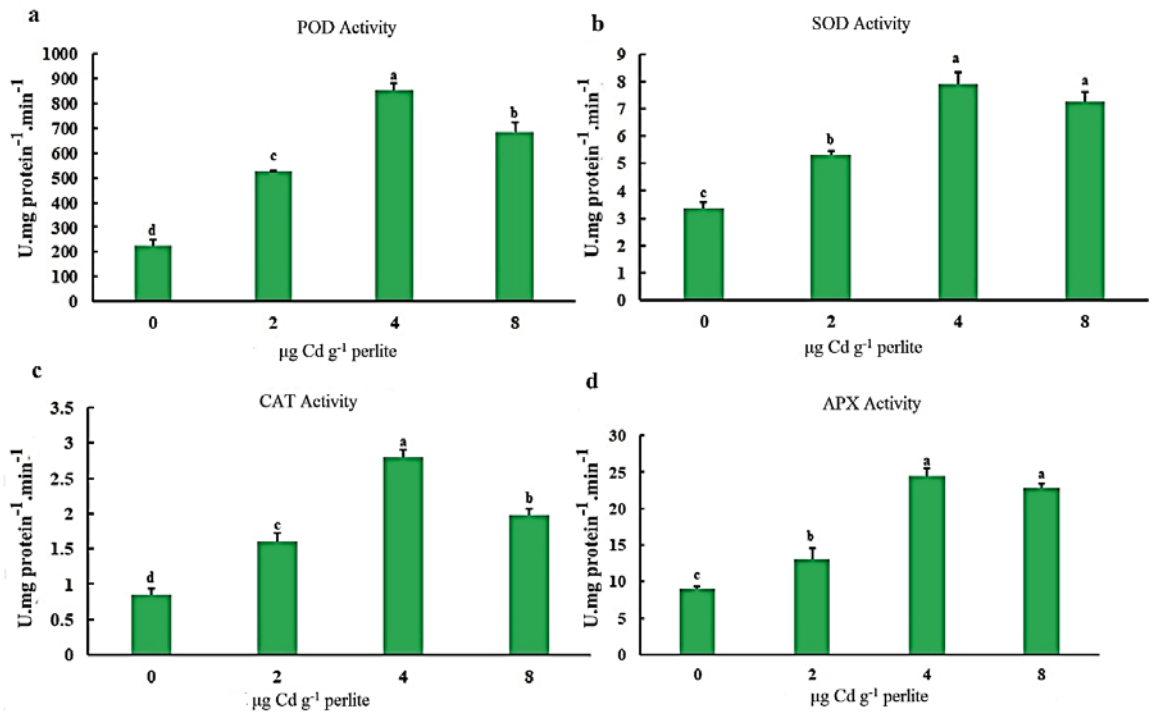

Fig. 2: The activities of a Peroxidase (POD), b Superoxide dismutase (SOD), c Catalase (CAT) and d Ascorbate peroxidase enzymes in aboveground parts of chickpea (*Cicer arietinum* L.). Values with different letters are statistically significantly different at $p < 0.05$ (One-way ANOVA, post hoc Tukey test)

while higher concentrations of cadmium reduced the amount of manganese in chickpea plants (Fig. 3b). Increase in the levels of cadmium in the culture also caused changes in the amount of zinc present in the aerial parts of pea plants. Increasing the levels of cadmium in the medium resulted in a decline in zinc (Fig. 3c). Increasing cadmium concentration, also decreased the levels of copper present in the aerial parts of chickpea seedlings. The lowest amount of copper was observed in high-cadmium seedlings (Fig. 3d). The BCF and TF values is greater than one at 8 µg Cd g^{-1} perlite (Fig. 3 e,f).

3.4 BIOINFORMATICS

In the current bioinformatics study of chickpea under cadmium stress, HMA proteins were chosen. *In silico* analysis of chickpea HMAs showed that of the 13 HMA identified, there were three proteins for each HMA3 and HMA4, two proteins for HMA5 and one protein for HMA 2, 6, 7, 8 (Table 2). The ATPase PAA2, chloroplastic, copper-transporting ATPase RAN1, and copper-transporting ATPase PAA1, chloroplastic identified in chickpea were identified as HMA6, HMA7, HMA8, in Arabidopsis, respectively. HMA7 and HMA8 all contribute to copper transport. The HMA 1, HMA 3, g HMA 2, HMA 4, HMA 5, PAA1, RAN1 and PAA2 genes are located on chromosomes 7, 1 and 7, 1, 6 and 7, 5 and 8, 6, 6, 5 respectively (Table 2). These proteins contain 130 to 1032 amino acids with 3 to 18 exons. The confidence level of predicting the three-dimensional structure of chickpea HMAs proteins is shown in Table 3. Their cellular locations are often in the nucleus and chloroplast. Using phyre2, their three-dimensional structure was determined. The protein templates and organisms used to predict the three-dimensional structure of these proteins are listed in Appendix 1. Among these templates, c3rfuC was used to predict all 13 proteins in a study related to copper-transporting PIB-type ATPase from the gram-negative bacterium *Legionella pneumophila* subsp. *pneumophila Brenner DJ,* Steigerwalt AG, McDade JE 1979. The patterns of c3j08A and c3j09A are also related to the p-type ATPase copper transporter CopA. Five (5) templates including copper-transporting proteins ATPase ATP7A, apoWLN5-6, domains 3 and 4 of human ATP7B, apo HMA domain of copper chaperone for superoxide dismutase and C2H2 type zinc finger (region 641-673) of human zinc finger protein 473 belong to humans. In total, the HMA studied in chickpeas were found to contain nine domains which are common in the 13 HMAs.

The COG4087 domain is listed as Soluble P-type ATPase and pfam00122 as E1-E2_ATPase are present

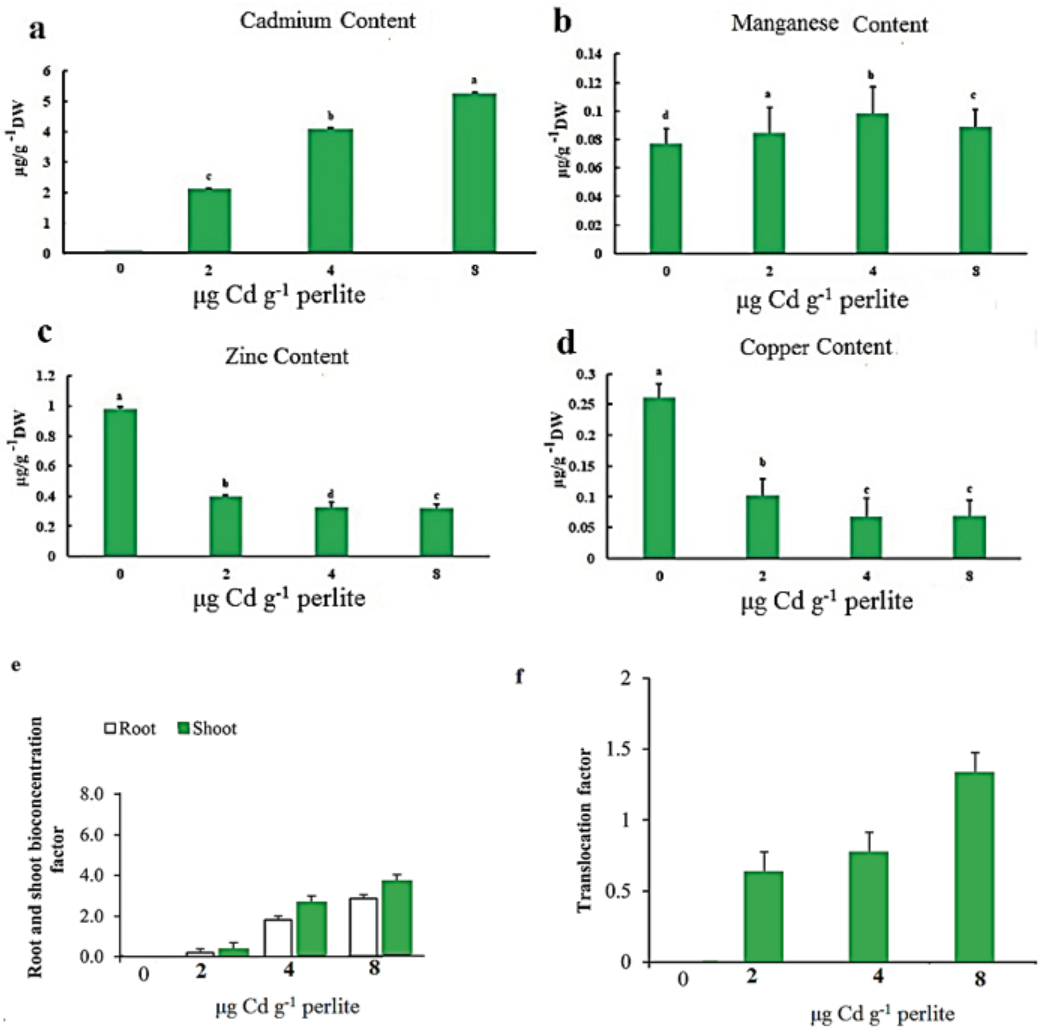

Fig. 3: Effects of different cadmium treatments on a Accumulation of cadmium, b Manganese, c Zinc, d Copper content in the aerial parts of chickpea seedlings after 10 days of cadmium treatment. Values with different letters are statistically significantly different at $p < 0.05$. (One-way ANOVA, post hoc Tukey test). Bioconcentration factor (e) and translocation factor (f). BCF values > 1 indicate that the concentration in the organism is greater than that of the medium. Translocation factor (TF) values more than one can be considered potential as Cd accumulators for phytoremediation. Mean plant tissues BCF are averages of five BCF values (n = 5) ± SEM

in ten HMAs. Following the prediction of the three-dimensional structure for chickpea HMAs, the longest tunnels for each protein and catalytic pocket predicted by CAVER Web for ion passing was determined. The longest and shortest tunnels predicted belonged to cadmium/zinc-transporting ATPase HMA3-like and cation-transporting ATPase HMA5-like, respectively. The putative inactive cadmium/zinc-transporting ATPase HMA3 was the largest HMA with 1032 amino acids and a short tunnel having a length of 41.7. No tunnel was predicted for copper-transporting ATPase PAA2, chloroplastic and copper-transporting ATPase PAA1, chloroplastic with 934 and 884 amino acids.

The three-dimensional structure with the longest predicted tunnel allowing for the passage of ions represented in color is illustrated in Fig 4. Based on the software used to analyze 8 of the 13 HMA chickpeas, the catalytic site was determined. From the proposed envelope for the HMAs the catalytic position for interaction with ions was determined. For XP_027192934, three catalytic sites with Asp residues at positions 522, 729, 733 with 40 % similarity over a specific reference of active site type and metal ion-binding site were identified. These catalytic sites can be evaluated and compared based on their pocket score. The neighboring residues of the catalytic position are also presented in the Table 3. In

Table 2: An overview of the features of chickpea HMAs proteins structure, genes loci, conserved Protein Domain Family, cellular location, Phyre2 confidence (residues modelled at > 90 % confidence), templates used for 3D prediction and longest tunnel predicted by the Caver Web for transport ions

Protein	Length	Gene	Exon count	Conserved domain	Location	Template pattern	Longest tunnel
XP_004509102.1	839	101490857 Chromosome: Ca7	13	COG4087 TIGR01512 pfam00122	Chloroplast	c3rfuC,c1mhsA,c3j08A,c5mrwF,c4umwA,c3j09A	70.1
XP_004487939	834	101492022 Chromosome: Ca1	9	COG2608 COG4087 pfam00122	Nucleus	c3rfuC, c3j08A, c4umwA, c3j09A	85.5
XP_027189340	569	101492022 chromosome: Ca1	9	cl21460 COG2608	-	c4umwA, c3rfuC, c3j08A, c3j09A	29
XP_012573401	1032	101505376 Chromosome: Ca7	11	COG2608 TIGR01512 pfam00122	Nucleus	c3rfuC,c2emcA, c3j08A,c4umwA,c3j09A	41.7
XP_004488108	832	101497233 Chromosome: Ca1	9	COG2608 COG4087 pfam00122	Nucleus	c3rfuC, c3j08A, c4umwA, c3j09A	107.3
XP_012573132	853	101504726 Chromosome: Ca6	7	COG2217 cd00371 pfam00122	-	c3rfuC, c4u9rA, c3j08A, c3j09A	43.8
XP_012574029	958	101515614 Chromosome: Ca7	10	COG2217 COG2608 COG4087 pfam00122	Nucleus	c2ew9A, c3rfuC, c2rmlA, c2ropA, c3j08A, c3j09A	72
XP_027192934	849	101515614 Chromosome: Ca7	10	cd02094 cd00371 cl00207	Nucleus	c3rfuC, c4u9rA, c3j08A, c3j09A	95.6
XP_004500941	130	101507723 Chromosome: Ca5	3	pfam00122	-	c3rfuC, c3j08A, c3j09A, c2kijA, c2hc8A	11.3
XP_004511583	998	101498342 Chromosome: Ca8	7	COG2217 COG4087 cd00371 pfam00122	Nucleus	c2ew9A, c3rfuC, c2rmlA, c2ropA, c3j08A, c3j09A	94.3
XP_004504792	934	101496348 Chromosome: Ca6	17	COG2217 COG4087 cd00371 pfam00122	Chloroplast	c3rfuC, c4u9rA, c3j08A, c3j09A	-
XP_004504659	995	101509532 Chromosome: Ca6	10	COG2217 COG4087 pfam00122	Nucleus	c2ew9A, c3rfuC, c2rmlA, c2ropA, c3j08A, c2crlA, c3j09A	95.9
XP_004501429	884	101500347 Chromosome: Ca5	18	COG2217 COG4087 cd00371	Nucleus, Chloroplast	c3rfuC, c3j08A, c3j09A	-

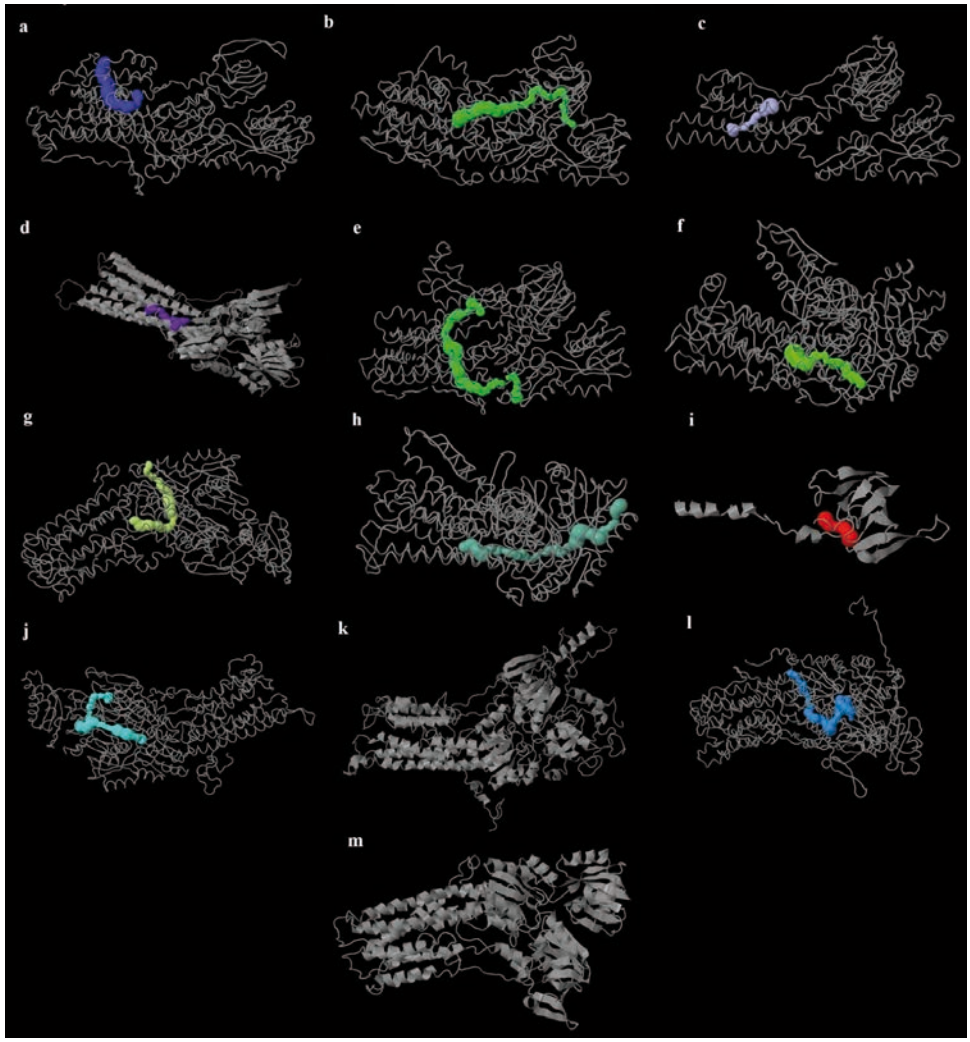

Fig. 4: An overview of the 3D model of chickpea HMAs generated by Phyre2 software. The structures were predicted using coordinate templates represented in Table 2. Colored regions in 3D structure represent the longest tunnel. a XP_004509102.1, b XP_004487939, c XP_027189340, d XP_012573401, e XP_004488108, f XP_012573132, g XP_012574029, h XP_027192934, i XP_004500941, j XP_004511583, k XP_004504792, l XP_004504659, and m XP_004501429

most cases, the amino acid Asp residue is introduced. For XP_012574029 and XP_004504659 the predicted pocket score was 100 % with XP_004504659 having an active site and three metal ion-binding sites (Table 3).

In the phylogenic tree of the HMAs (Fig. 5), comparison of the protein sequences of chickpea HMA with *Arabidopsis* revealed great similarity between these proteins in chickpea and *Arabidopsis*. HMA 2 and 4 are very similar to *Arabidopsis* and are next to HMA 3 chickpeas. HMA 3 chickpea is adjacent to HMA 3 *Arabidopsis*. HMA 1 2 3 chickpea are all involved in cadmium and zinc transfer and are in close proximity to each other in the tree. The P-type ATPases of *Arabidopsis* are very similar to the copper-transporting ATPase PAA2 chickpeas. Copper-transporting ATPase PAA1 pea is very similar to *Arabidopsis* P-type ATPases. In chickpea, copper-transporting ATPase RAN1 resembles copper-transporting ATPase HMA5, which is adjacent to copper-transporting ATPase RAN1 *Arabidopsis*. Cation-transporting ATPase HMA5-like and copper-transporting ATPase RAN1 are also in the vicinity of copper-transporting ATPase RAN1 Arabidopsis.

4 DISCUSSION

Heavy metal pollution is a significant environmental problem. Increasing our knowledge of the mechanisms by which plants are able to mitigate heavy metal stress could assist in creating new tools applicable to

Table 3: Index, residue, accession code of the reference entry, sequence identity to the reference entry, type, description, neighborhood and pocket score features of chickpea HMAs proteins structure

Protein accession number	Index	Residue	Accession code of the reference entry	Sequence identity	Type	Description	Neighborhood	Pocket score
XP_004511583	656	Asp	Q9SH30	73.8 %	active site	4-aspartylphosphate intermediate	VFDKT VFDKT	100 %
	860	Asp	Q9SH30	73.8 %	metal ion-binding	Magnesium	VGDGI VGDGI	
	864	Asp	Q9SH30	73.8 %	metal ion-binding	Magnesium	INDSP INDSP	
XP_004488108	591	Asp	P0CW78	50.2 %	metal ion-binding	Magnesium	VGDGI VGDG	33 %
XP_012573401	590	Asp	Q9SZW4	54.6 %	metal ion-binding	Magnesium	LGDGL VGDGL	28 %
XP_012574029	838	Asp	Q9SH30	56.4 %	metal ion-binding	Magnesium	VGDGI VGDGI	100 %
XP_012573132	522	Asp	Q4L970	41.3 %	active site	4-aspartylphosphate intermediate	VFDKT VFDKT	6 %
	730	Asp	Q4L970	41.3 %	metal ion-binding	Magnesium	VGDGI VGDGI	
XP_027192934	522	Asp	O32220	41.6 %	active site	4-aspartylphosphate intermediate	VFDKT VLDKT	68 %
	729	Asp	O32220	41.6 %	metal ion-binding	Magnesium	VGDGI VGDGI	
	733	Asp	O32220	41.6 %	metal ion-binding	Magnesium	INDSP INDAP	
XP_004487939	392	Asp	P0CW78	49.8 %	active site	4-aspartylphosphate intermediate	AFDKT AFDKT	13 %
	591	Asp	P0CW78	49.8 %	metal ion-binding	Magnesium	IGDGI VGDGL	
XP_004504659	649	Asp	Q9S7J8	73.4 %	active site	4-aspartylphosphate intermediate	IFDKT IFDKT	100 %
	138	Cys	Q9S7J8	73.4 %	metal ion-binding	Copper	AACVN AACVN	
	869	Asp	Q9S7J8	73.4 %	metal ion-binding	Magnesium	VGDGI VGDGI	
	873	Asp	Q9S7J8	73.4 %	metal ion-binding	Magnesium	INDSP INDSP	
XP_004509102	467	Asp	Q9M3H5	68.2 %	active site	4-aspartylphosphate intermediate	AFDKT AFDKT	25 %
	701	Asp	Q9M3H5	68.2 %	metal ion-binding	Magnesium	INDAP INDAP	

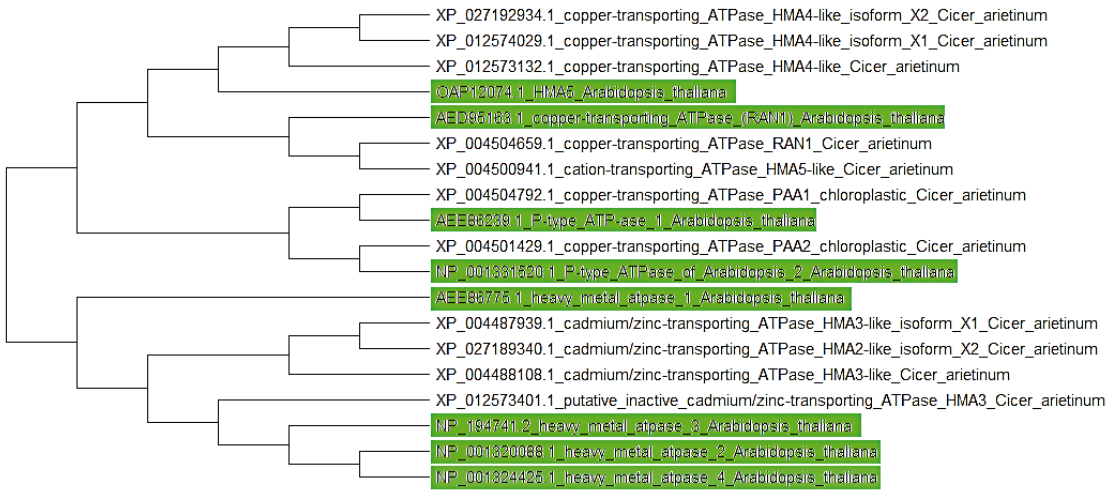

Fig. 5: Phylogenic tree of HMAs from chickpea and *Arabidopsis*. A phylogenetic tree was constructed using the neighbor-joining (NJ) method, with a bootstrap test performed using 1000 iterations in MEGA5 with the amino acid sequences of HMAs. HMAs from *Arabidopsis* are highlighted in green

phytoremediation. It is important to further research processes involved in heavy metal detoxification and signaling pathways in plants so as to identify useful targets for biotechnological applications thereby increasing plant fitness in heavy metal polluted sites (Dala-Paula et al., 2018).

Cadmium exposure reduced leaf area, shoot and root length. The effect of cadmium ion suppression on root expansion extends through its effect on cell growth (Hassan et al., 2008). Cadmium attaches to the cell wall and the middle lamella, increasing the bonding between the wall components, ultimately leading to growth inhibition and a decline in cell and organ development. Cadmium also alters water proportions in plants causing physiological dryness, which leads to metabolic dysfunction and production of ROS. These factors reduce growth and impact on plant length and mass (Zulfiqar et al. 2022). Many studies on the mechanism of cadmium blockage on cell growth have shown degradation of cell membranes by cadmium and changes in the degree of cell exchange and cellular depletion (Bücker-Neto et al., 2017). The observed changes in plants exposed to cadmium may be as a result of multiple nutritional deficiencies being experienced by the plant.

Nutrients serve an essential role in the formation, expansion, and operation of chloroplasts. Cd-phytotoxicity affects the synthesis and extensibility of cell walls (Gomes et al., 2011). Cell wall thickening in root endodermal tissue affords a greater surface area over which cadmium accumulation can occur thereby limiting its transportation to the shoot (Zulfiqar et al., 2022). Chlorosis observed in the leaves of bean plants exposed to cadmium may be due to loss of magnesium which is an integral structural feature of the porphyrin ring present in chlorophyll. Physiological changes observed in leaves are due to the associated toxic effects of cadmium including mesophyll curvature, decreased leaf thickness and a reduction in the composition of intercellular spaces of spongy parenchyma (Tuver et al., 2022). At higher doses of cadmium, the thickness of palisade and spongy tissues is reduced. A decline in the dimensions and composition of the main mid-vein bundle suggests that cadmium alters leaf expansion (Cregeen et al., 2015).

A study of the effect of heavy metals on the cell death of *Halophila stipulacea* (Forssk.) Asch leaves showed that high concentrations of metal causes necrosis of the epidermal cells and mesophyll, inhibiting surface growth of the leaves. High levels of heavy metal accumulation in plant cells inhibits the process of respiration and energy reactions, which are associated with cell growth (Ayangbenro, 2017). A decline in cell division and growth could also be a contributing factor to the observed morphological changes. Additionally, a decrease in photosynthetic rates has been observed in plants exposed to elevated levels of heavy metals. Higher concentrations of cadmium commonly result in root injury, damage to photosynthetic machinery, inhibition of plant growth, reduced nutrient and water uptake (Tuver et al., 2022). Cadmium may exert its inhibitory effect in different ways, namely binding specific groups of proteins and lipids thereby inhibiting normal function and possibly inducing free radical formation due to oxidative stress. The former may occur at transport and channel proteins of cell membranes disturbing the uptake of many other macro- and microele-

ments whereas the latter is due to the inactivation of antioxidant enzymes by cadmium (Long et al., 2017).

The results showed that oxidative enzymes activity (SOD, APX, POD and CAT) increased in the leaves of chickpea exposed to cadmium. Similar observations have been observed in CAT and POD enzymes present in cereals and squash (Ashraf, 2003). Increased activity of these enzymes is a consequence of lipid peroxidation. The effect of cadmium on growth and antioxidant enzymes in two varieties of *Brassica napus* showed that cadmium decreased the growth indices, nitrate reductase activity and leaf water potential while antioxidant enzyme activities increased. The highest level of enzyme activity was in relation to SOD enzymes, which showed more than 80 % increase in activity. The least increase in enzyme activity was observed in the catalase enzyme (Irfan et al., 2014). Increasing the absorption and accumulation of heavy metals in plants causes changes in cell metabolism, oxidative stress and cell destruction which is induced by ROS. Cadmium can induce mineral stress that reduces plant dry mass (Zhou et al., 2022). Tabarzad et al. (2017) showed that wheat seedlings grown in the presence of cadmium had changes in the level of SOD and POD activity. The observed decline in enzyme activity suggests a weakening of the oxygen and superoxide water scavenging system. Reduced activity of the other antioxidant enzymes in some tissues, is due to poor performance in oxygenate decomposition in cadmium treated tissues. ROS activity increased significantly under cadmium stress due to an increase in wall oxidation. Reduced SOD activity is justifiable as cadmium is known to be an enzyme inhibitor (Tabarzad et al., 2017).

Schutzendubel (2001) showed the inhibition of SOD, POD and total inactivation of APX in pine roots after 48 days of cadmium treatment. An increase in the activity of these enzymes under cadmium stress has been observed in other studies (Schutzendubel et al., 2001). Li et al. (2013) examined the effect of cadmium stress on growth and antioxidant enzymes and lipid oxidation in two Kenaf (*Hibiscus cannabinus* L.) species. In the study, glutathione reductase activity (GR) was greater than that of the control. The general trend was that of an increase in SOD, CAT and POD activities in the roots of cadmium-stressed plants followed by a decline. POD activity however remained relatively unchanged at all stress levels (Zhou et al., 2022). Ulusu et al. (2017) investigated the antioxidant capacity and cadmium accumulation of stressed parsley. In the study, enzyme activity increased for catalase and ascorbate peroxidase, (75 to 150 μM cadmium), while decreasing at 300 μM. The results showed that antioxidant enzymes activity was suppressed due to the accumulation of cadmium in parsley leaves and increased non-enzymatic antioxidant activity (Ulusu et al., 2017). Pereira et al. (2002) studied the activity of antioxidant enzymes in *Crotalaria juncea* L. which showed that under the influence of cadmium, catalase activity did not show any significant changes in the root. At concentrations of 2 mM cadmium, catalase activity in the leaves increased 6 fold compared to the control. Increased activity of some antioxidant enzymes exposed to metals reveal the crucial role that these enzymes play in detoxification (Pereira et al. 2002). Various antioxidant cycles under normal physiological metabolism, results in the production and scavenging of reactive oxygen species which is in a state of dynamic equilibrium (Zhou et al., 2022). Kisa (2018) studied the response of antioxidant systems to stress induced by heavy metals in the leaves and roots of tomato which showed that cadmium treatment significantly increased the activity of the APX and SOD enzymes. Antioxidant scavenging systems are connected with ROS detoxification which is a defense mechanism employed by plant tissue to combat oxidative stress (Kisa, 2018). Tomato plants exposed to cadmium showed significantly higher SOD. Catalase activity was however reduced.

The cadmium content in aerial parts of chickpea grown in different concentrations of cadmium increased significantly. Research conducted by Tang et al. (2022) revealed that cadmium concentrations in the seeds of beans from different regions and varieties is based on complex genetic factors and the environment. For different legume varieties, environmental factors such as climate, soil, agricultural and geological techniques, in comparison to genetic factors, are more important in the accumulation of heavy metals such as Cd. Compared to the genus and plant species, the accumulation of heavy metals seems to be more influenced by the genetic potential of the plant (Tang et al. 2022). The ability to absorb and distribute cadmium to the aerial regions of the plant is related to its attachment to the extracellular matrix, root flow, intracellular detoxification and transfer efficiency (Akhtar and Macfie, 2012). Cadmium is absorbed in the root of the plants subsequently accumulating in the aerial parts, which often limits the absorption and distribution of other elements (Gomes et al., 2013). Cadmium binds to the functional epidermis through direct binding to ion carriers via production of oxygen species that are associated with membrane affects (Altaf et al., 2022). Ling Liu et al. (2012) showed that legumes can increase the accumulation of cadmium in adjacent plants. Cadmium increase in plants was a direct result of planting crops in proximity to legumes. The study suggests that the system of cultivation of beans should be redesigned to prevent food contamination with cadmium (Liu et al., 2012). Vijendra et al. (2016) showed that in Moth bean (*Vigna aconitifolia* L.) cadmium concentrations in-

creased significantly in the leaves and roots. Cadmium reaches the aerial sections via the xylem of the plant (Vijendra et al., 2016). At concentrations of 0.04 to 0.32 mM, cadmium is non-polluting in soil. Knowledge about the distribution of cadmium in plant tissues is important to better understand the tolerance mechanism and accumulation of heavy metals in plants. Cadmium in plants is transferable through apoplast pathways of the stems and leaves (Benavides et al., 2005). Cadmium affects membrane potential, protein pump activity and can limit corn growth (Karcz & Kurtyka, 2007).

The result indicated that increasing cadmium concentration, also decreased the levels of copper and zinc present in the aerial parts of chickpea seedlings. Further studies also showed that zinc and copper along with cadmium have an antagonistic effect and that these minerals act in a competitive manner in relation to the transfer processes. Heavy metals, such as copper (Cu), zinc (Zn), manganese (Mn), and iron (Fe), serve as essential micronutrients for an array of metabolic processes. These micronutrients serve as cofactors, participate in cellular redox reaction and affects protein structure (Schutzendubel & Polle, 2002). At toxic levels Cu will however interfere with physiological processes. Zn also serves as a micronutrient but can be toxic if present at high concentrations (Schutzendubel & Polle, 2002). To minimize the potential effects of excess metal contaminants, the plant utilizes various homeostasis mechanisms which include the use of specialized transport proteins which serve as carriers mediating the transfer of heavy metals across cell membranes (Lee et al., 2007). Cadmium has a negative effect on the absorption of essential nutrients. It reduces ATPase activity and decreases the exchange of ion H^+/K^+ in the plasmalema surface (Brzoska & Moniuszko-Jakoniuk, 2001). Page and Feller (2005) showed that the transfer of zinc, manganese, cobalt and cadmium in the leaves and roots of wheat were selective. When other minerals are in close proximity to cadmium, the amount of zinc in the root decreases (Page and Feller, 2005). Santos et al. (2014) showed that in the family of legumes, lead and cadmium adsorption was competitive. In this study, the concentration of zinc was eight times higher than that of cadmium, which indicates that zinc adsorption is preferable to cadmium. In plants treated with zinc and lead, lower concentrations of cadmium were observed in plant tissues in comparison to plants treated with cadmium alone. Zinc and lead along with cadmium compete for the sites of absorption and transfer (dos Santos et al., 2014). Chen et al. (2007) showed that manganese reduces the toxic effects of cadmium in corn. This suggests that manganese can be utilized to manage cadmium contamination (Chen et al., 2007). Zinc acts as a micro-element that is essential for plant growth and is part of the structure of regulatory enzymes and proteins. Zinc is very important in reducing cadmium toxicity and decreases the oxidative stress induced by cadmium. Some studies describe zinc and phosphorus interactions in plants (Marques et al., 2013). The phosphorus content in the aerial parts of plants treated with cadmium is related to the low zinc content in these sections. The negative correlation between zinc and phosphorus content in the shoots of cadmium treated plants explains the high content of phosphorus in these plants (Sarwar et al., 2010). Analysis of cadmium and manganese content in this study supports the competitive theory of absorption of these two elements. The precise mechanism for promoting growth and reducing the toxic effects of cadmium is not well known. The uptake of various cations (K^+, Ca^{2+}, $Mg2^+$, Mn^{2+}, Zn^{2+}, and Fe^{2+}) is severely affected by the presence of cadmium (Linger et al., 2005).

Different types of proteins and adsorption carriers for cadmium are known such as NRAMP family (Thomine et al. 2000), P-type ATPase (Morel et al., 2009), ABC transporter (Kim, Gustin et al., 2004), CAX family, ZIP family (Pence et al., 2000), LCT transporter and CE family (Guerinot, 2000). Researchers report that cadmium has an antagonistic and synergistic effect on the microelements and macro elements in wheat. Many studies on the effect of cadmium inhibition on cell growth suggests the destruction of cell membranes and changes in mineral levels (Rietra et al., 2017). Jibril et al. (2017), showed that the content of micronutrients and macro elements in different varieties of lettuce is significantly affected by cadmium levels. The study showed that cadmium (12 mg l^{-1}) reduced essential elements by 72, 69, 56, 61 and 52 % (nitrogen, phosphorus, potassium and calcium, respectively). Copper content was higher in the root than the shoot of cadmium treated plants. This therefore reduces the effect of cadmium toxicity. Indeed, cadmium increases the absorption of copper, but prevents it from transferring to the shoots (Jibril et al., 2017). Gomez et al. (2013) examined the effect of cadmium on nutrient distribution in *Pfaffia glomerata* (Spreng.) Pedersen. Plants were cultured with different minerals and cadmium concentration was simultaneously increased over a 20 day period. The study showed that cadmium strongly affects the distribution of microelements and macroelements in the roots and shoots. Despite the high toxicity of cadmium, the micro and macro nutrients present in plants are able to survive in contaminated environments (Gomes et al., 2013).

Present study detected that at low concentrations of cadmium, the amount of manganese increased. With an increase in cadmium concentration, the level of manganese decreased in chickpea. Manganese plays a role in many biochemical functions, such as activating

enzymes involved in respiration, redox reactions, intracellular electron transfer systems, and the Hill reaction in chloroplasts, amino acid synthesis, and regulation of hormones (He et al., 2022). Manganese concentration was higher in the shoots than the root of plants treated with cadmium. The transfer of manganese to the shoot may in fact be a tolerance mechanism that reduces the effects of cadmium toxicity on photosynthesis. Research suggests that cadmium and manganese compete for the same membrane carriers (Socha & Guerinot, 2014). Dias et al. (2013) showed that at cadmium concentrations of 5 and 10 µm there was a significant decline in the mineral content of lettuce leaves. At high concentrations of cadmium, a significant decline in manganese in the roots was observed. Cadmium appears to interfere with the transmission of macro and micro elements in the leaf (Dias et al., 2013). According to Guerinot, members of the ZIP and NRAMP or Ca channels and transporters which are responsible for the uptake of essential elements are involved in the transport of cadmium via the same route (Guerinot, 2000). Imbalance in nutrient level and growth inhibition is ultimately due to competition between nutrients and toxic metals for binding sites in the cell. Sun and Shen (2007) explained that the decrease in concentrations of Mn, Fe, Mg, S, and P in the leaves of Cd-sensitive cultivars under cadmium stress is a contributing factor to the decline in photosynthesis and the decrease of cabbage growth (Sun & Shen, 2007).

Heavy metal ATPases (HMAs), belong to the large P-type ATPase family located in the plasma membrane or tonoplast. They play an important role in the transport of metals in plants and provide resistance to the uptake and transportation of metals. The identified HMAs may contribute to the mechanisms by which chickpea plants manage, detoxify, or tolerate cadmium exposure. Understanding the structure, function, and localization of these HMAs could offer new strategies for enhancing cadmium tolerance in chickpea, a crucial crop in many parts of the world. HMAs are classified based on substrate binding with one group bound to copper and silver and the other to cadmium, lead and cobalt (Chkadua et al., 2022). HMAs 9 and 8 have been studied in rice and *Arabidopsis*, respectively. AtHMA1–4 in *A. thaliana* and OsHMA1–3 in *Oryza sativa* L. are in the first group and AtHMA5–8 and OsHMA4–9 in the second group. The expression of each of these genes is sensitive to heavy metals as indicated by mutagenesis. Typical P1B-ATPase proteins have been studied in various barley plants, *Arabidopsis* and poplar as well as in *Thlaspi caerulescens* J.Presl & C.Presl (Takahashi et al. 2012).

In poplar (*Populus trichocarpa* Torr. & A.Gray ex. Hook), seventeen HMAs are known. PtHMA1 – PtHMA4 belong to the subgroup of metals on cadmium, lead and cobalt. PtHMA5 – PtHMA8 belonging to the silver and copper groups have been identified. Most of these genes are located on chromosome 1 and 2 of poplar. On both sides of the P_{1B}-*ATPase* C and N terminals there is also a metal binding site HMA4 in poplar which produces mature RNA transcripts during alternative splicing of mRNA, containing approximately six hundred and twenty-six amino acids with an amino acid average of ninety-eight. PtHMA in poplar are all in plasma membrane except PtHMA1 and PtHMA5.1 which are located in the cytoplasm. Poplar HMAs have 5 to 16 introns, PtHMA6, 5 introns, 8 PtHMA has 16 introns and 1 PtHMA has 5 introns with the remaining possessing 10 introns (Li et al., 2015). PtHMA1 – PtHMA4 belong to the subgroup of metals consisting of cobalt and cadmium with the rest belonging to lead, silver and copper. There are 10 HMA genes related to silver and copper in poplar that are significantly higher than those in rice and *Arabidopsis*.2 OsHMA plays an important role in transmitting cadmium entry from the root to the stem and especially to rice grains (Li et al., 2015). OsHMA3 transports cadmium to root cell vacuoles. Manipulating and altering the expression of these genes is a useful tool for reducing cadmium concentration in the seeds. AtHMA1 is within the chloroplast and zinc anti-toxic while AtHMA 3 is present in the vacuolar membrane with zinc and cadmium playing a role. The motifs of poplar HMA are very similar to *Arabidopsis* and rice proteins and it seems that family members of these genes may be functionally divergent due to differences in gene organization and existing motifs (Tian et al. 2023). AtHMA 1 and 2 are in the plasma membrane and in zinc and cadmium fluxes. OsHMA 1 is involved in zinc transfer. No HMA 4 type has been reported in rice. The number of HMA genes in the soybean genome is higher than that in *Arabidopsis* and rice, probably due to duplication of the soybean genome. Phylogenetic study of these genes divides them into six groups, based on their divergent gene structure, conserved segments or protein motif patterns. Examination of the cellular location of these proteins indicates that only *GmHMA1* is involved in the secretion pathway while 1, 16, 17, 20, 20 peptides are mitochondrial targets, whereas 1, 2, 2, and 2 GmHMA2 are chloroplast peptides (Fang et al., 2016). Researchers have identified nine *typical* P_{1B}*-ATPase* in barley. HvHMA2, a P (1B)-ATPase is highly conserved among cereal crops with functionality in the transportation of zinc and cadmium. Additionally, HMA4 (Heavy Metal ATPase 4) has a key role in the translocation of cadmium in non-hyperaccumulating dicots, such as *Arabidopsis thaliana* (Mills et al., 2012).

5 CONCLUSION

Chickpea seedlings exposed to cadmium exhibited changes in their morphological features which included changes in plant length, coloration and leaf size. The results indicated that shoot and root length were significantly reduced. With the addition of cadmium (4 µg Cd g^{-1} perlite), stomatal densities on the upper epidermis decreased significantly but subsequently increased while higher concentrations of cadmium. Oxidative enzyme activities were also affected by cadmium stress. Oxidative enzyme activity (peroxidase, superoxide dismutase, catalase, ascorbate peroxidase) increased in the leaves of plants exposed to cadmium suggesting that these enzymes play an integral role in combatting heavy metal contamination. Cadmium content in aerial parts of chickpea increased significantly. The study also revealed that by increasing cadmium concentration there was a significant reduction in the amount of copper and zinc transported to the aerial regions of the plant. Moreover, at low concentrations of cadmium, the amount of manganese increased It has been suggested that there is a competitive mechanism for mineral uptake in plants. One may therefore be able to manage cadmium accumulation by varying the type of fertilizers utilized in cultivating plants. *In silico* analysis led to the identification of 13 Heavy Metal ATPases (HMAs) in chickpea. These proteins contain 130 to 1032 amino acids with 3 to 18 exons. Comparison of the protein sequences of chickpea HMA with *Arabidopsis* indicated that there was great similarity between these proteins. The presence of a variety of genes indicates the various mechanisms utilized by chickpeas to combat heavy metal stress. Genetic engineering could be utilized to create heavy metal resistant chickpea species.

6 CONFLICT OF INTEREST

There is no conflict of interest in the publication of this paper.

7 REFERENCES

Akhter, F., Omelon, Ch., Gordon, R., Moser, D., Macfe, S. (2014). Localization and chemical speciation of cadmium in the roots of barley and lettuce. *Environmental and Experimental Botany, 100*, 10-19. https://doi.org/10.1016/j.envexpbot.2013.12.005

Altaf, MA., Shahid, R., Ren, M. X., Naz, F., Altaf, MM. et al (2022). Melatonin mitigates cadmium toxicity by promoting root architecture and mineral homeostasis of tomato genotypes. *Journal of Soil Science and Plant Nutrition, 22*, 1112-1128. https://doi.org/10.1007/s42729-021-00720-9

Ayangbenro, AS., & Babalola, OO. (2017). A new strategy for heavy metal polluted environments: A review of microbial biosorbents. *International Journal of Environmental Research and Public Health, 14*, 94. https://doi.org/10.3390/ijerph14010094

Bae, W., & Chen, X. (2004). Proteomic study for the cellular responses to Cd^{2+} in *Schizosaccharomyces pombe* through amino acid-coded mass tagging and liquid chromatography tandem mass spectrometry. *Molecular & Cellular Proteomics*, *3*, 596-607. https://doi.org/10.1074/mcp.M300122-MCP200

Benavides, MP., Gallego, SM., Tomaro, ML. (2005). Cadmium toxicity in plants. BJPP, *17*, 21-34. https://doi.org/10.1590/S1677-04202005000100003

Bose, S., Bhattacharyya, A. (2008). Heavy metal accumulation in wheat plant grown in soil amended with industrial sludge. *Chemosphere*, *70*, 1264-1272. https://doi.org/10.1016/j.chemosphere.2007.07.062

Bowler, C., Van, Camp, W., Van Montagu, M., Inzé, D., Asada, K. (1994). Superoxide dismutase in plants. *Critical Reviews in Plant Sciences*, *13*(3), 199-218. https://doi.org/10.1080/07352689409701914

Bradford, MM. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1), 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Brzoska, MM, & Moniuszko-Jakoniuk, J. (2001). Interactions between cadmium and zinc in the organism. *Food and Chemical Toxicology*, *39*(10), 967-980. https://doi.org/10.1016/S0278-6915(01)00048-5

Chellaiah, ER. (2018). Cadmium (heavy metals) bioremediation by *Pseudomonas aeruginosa*: A minireview. *Applied Water Science*, *8*(6), 154. https://doi.org/10.1007/s13201-018-0796-5

Chen, JW., Lu ,YQ., Chen, ZH. (2007). Variations in form of copper, cadmium and lead in rhizosphere soil of corn. *Journal of Hunan Agricultural University*, *33*(5), 626.

Chkadua, G., Nozadze, E., Tsakadze, L., Shioshvili, L., Arutinova, N., Leladze, M., Dzneladze, S., Javakhishvili, M. (2022). Effect of H_2O_2 on Na,K-ATPase. *Journal of Bioenergetics and Biomembranes*, *54*(5-6), 241-249. https://doi.org/10.1007/s10863-022-09948-1

Cregeen, S., Radišek, S., Mandelc, S., Turk, B., Štajner, N., Jakše, J., Javornik, B. (2015). Different gene expressions of resistant and susceptible hop cultivars in response to infection with a highly aggressive strain of *Verticillium albo-atrum*. *Plant Molecular Biology*, *33*, 689-704. https://doi.org/10.1007/s11105-014-0767-4

Dala-Paula, BM., Custódio, FB., Knupp, EAN., Palmieri, HEL., Silva, JBB., Glória, MBA. (2018) . Cadmium, copper and lead levels in different cultivars of lettuce and soil from urban agriculture. *Environmental Pollution*, *242*, 383-389. https://doi.org/10.1016/j.envpol.2018.04.101

Dias, MC., Monteiro, C., Moutinho-Pereira, J., Correia, C., Gonçalves, B., Santos, C. (2013). Cadmium toxicity af-

fects photosynthesis and plant growth at different levels. *Acta Physiologiae Plantarum, 35*(4), 1281-1289. https://doi.org/10.1007/s11738-012-1167-8

Fang, X., Wang, L., Deng, X., Wang, P., Ma, Q., Nian, H., Wang, Y., Yang, C. (2016). Genome-wide characterization of soybean P 1B -ATPases gene family provides functional implications in cadmium responses. *BMC Genomics*, *17*,1-10. https://doi.org/10.1186/s12864-016-2730-2

Faria, JMS., Pinto, AP., Teixeira, D., Brito, I., Carvalho, M. (2022). Diversity of native arbuscular mycorrhiza extraradical mycelium influences antioxidant enzyme activity in wheat grown under Mn toxicity. *Bulletin of Environmental Contamination and Toxicology*, *108*, 451–456. https://doi.org/10.1007/s00128-021-03240-5

Gomes, MP. (2013). Cadmium effects on mineral nutrition of the Cd-hyperaccumulator *Pfaffia glomerata*. *Biologia*, *68*(2), 223-230. https://doi.org/10.2478/s11756-013-0005-9

Guerinot, M. (2000). The ZIP family of transporters. *BBA*, *1465*, 190-198. https://doi.org/10.1016/S0005-2736(00)00138-3

Hasan, MK., Cheng, Y., Kanwar, MK., Chu, XY., Ahammed, GJ., Qi, ZY. (2017). Responses of plant proteins to heavy metal stress. A review. *Frontiers in Plant Science*, *8*. 1492. https://doi.org/10.3389/fpls.2017.01492

Hassan, SA., Hayat, S., Ali, B., Ahmad, A. (2008). 28-Homobrassinolide protects chickpea (*Cicer arietinum*) from cadmium toxicity by stimulating antioxidants. *Environmental Pollution*, *151*, 60–66. https://doi.org/10.1016/j.envpol.2007.03.006

He, L., Su, R., Chen, Y.,…, Zhu, H. 2022). Integration of manganese accumulation, subcellular distribution, chemical forms, and physiological responses to understand manganese tolerance in *Macleaya cordata*. *Environmental Science and Pollution Research*, *29*, 39017–39026. https://doi.org/10.1007/s11356-022-19562-8

Hoagland, D.R., Snyder, W.C. (1933). Nutrition of strawberry plant under controlled conditions. (a) Effects of deficiencies of boron and certain other elements, (b) susceptibility to injury from sodium salts. *The Journal of the American Society for Horticultural Science*, *30*, 288–294.

Irfan, M., Ahmad, A., Hayat, S. (2014), Effect of cadmium on the growth and antioxidant enzymes in two varieties of *Brassica juncea*. *Saudi Journal of Biological Sciences*, *21*(2), 125-131. https://doi.org/10.1016/j.sjbs.2013.08.001

Jibril, S., Hassan, SA., Ishak, F., Megat Wahab, P. (2017). Cadmium toxicity affects phytochemicals and nutrient elements composition of lettuce (*Lactuca sativa* L.). *Advances in Agriculture*, *10*, 1-7. https://doi.org/10.1155/2017/1236830

Jogawat, A., Yadav, B., Chhaya, Narayan, OP. (2021) Metal transporters in organelles and their roles in heavy metal transportation and sequestration mechanisms in plants. *Physiologia Plantarum*, *173*(1), 259-275. https://doi.org/10.1111/ppl.13370

Karcz, W., & Kurtyka, R. (2007). Effect of cadmium on growth, proton extrusion and membrane potential in maize coleoptile segments. *Biologia Plantarum*, *51*(4), 713. https://doi.org/10.1007/s10535-007-0147-0

Kaur, H., Hussain, SJ., Kaur, G., Poor, P., Alamri, S., Iqbal R. Khanm M. (2022). Salicylic acid improves nitrogen fixation, growth, yield and antioxidant defence mechanisms in chickpea genotypes under salt stress. *Journal of Plant Growth Regulation*, *41*, 2034–2047. https://doi.org/10.1007/s00344-022-10592-7

Kim, D., Gustin, J., Lahner, B., Persans, M., Baek, D., Yun, DJ., Salt, D. (2004). The plant CDF family member TgMTP1 from the Ni/Zn hyperaccumulator *Thlaspi goesingense* acts to enhance efflux of Zn at the plasma membrane when expressed in *Saccharomyces cerevisiae*. *The Plant Journal*, *39*, 237-251. https://doi.org/10.1111/j.1365-313X.2004.02126.x

Kisa, D. (2018). The responses of antioxidant system against the heavy metal-induced stress in tomato. *Süleyman Demirel University Journal of Natural and Applied Sciences*, *22*, 105-115. https://doi.org/10.19113/sdufbed.52379

Koroi, SA. (1989). Gel electrophoresis tissue and spectrophotometrscho unter uchungen zomeinfiuss der temperature auf struktur der amylase and peroxidase isoenzyme. *Physiological Reviews*, *20*, 15-23.

Kumar, R., Mishra, RK., Mishra, V., Qidwai, A., Pandey, A., Shukla, SK., Pandey, M., Pathak, A., Dikshit, A. (2016). *Chapter 13 - Detoxification and tolerance of heavy metals in plants*. In: Ahmad, P.B.T.-P.M.I. (Ed.), Elsevier, pp. 335–359. https://doi.org/10.1016/B978-0-12-803158-2.00013-8

Lee, S., Kim,YY., Lee, Y., An, G. (2007). Rice P1B-type heavy-metal ATPase, OsHMA9, is a metal efflux protein. *Plant Physiology*, *145*(3), 831-842. https://doi.org/10.1104/pp.107.102236

Li, D., Xu, X., Hu, X., Liu, Q., Wang, Z., Zhang, H., Wang, H., Wei, M., Wang, H., Liu, H., Li, C. (2015). Genome-wide analysis and heavy metal-induced expression profiling of the HMA gene family in *Populus trichocarpa*. *Frontiers in Plant Science*, *6*, 1149. https://doi.org/10.3389/fpls.2015.01149

Li, FT., Qi, JM., Zhang, G.Y., Lin, LH., Fang, PP., Tao, AF., Xu, JT. (2013). Effect of cadmium stress on the growth, antioxidative enzymes and lipid peroxidation in two Kenaf (*Hibiscus cannabinus* L.) plant seedlings. *Journal of Integrative Agriculture*, *12*(4), 610-620. https://doi.org/10.1016/S2095-3119(13)60279-8

Linger, P., Ostwald, A., Haensler, J. (2005). *Cannabis sativa* L. growing on heavy metal contaminated soil: growth, cadmium uptake and photosynthesis. *Plant Biology*, *49*(4), 567-576. https://doi.org/10.1007/s10535-005-0051-4

Liu, L., Zhang, Q., Hu, L., Tang, J., Xu, L., Yang, X., Yong, JWH., Chen, X. (2012). Legumes can increase cadmium contamination in neighboring crops. PLoS One, *7*(8), e42944-e42944. https://doi.org/10.1371/journal.pone.0042944

Long, A., Zhang, J., Yang, LT., Ye, X., Lai, NW., Tan, LL., Lin, D., Chen, LS. (2017). Effects of low pH on photosynthesis, related physiological parameters, and nutrient profiles of *Citrus*. *Frontiers in Plant Science, 8*, 185. https://doi.org/10.3389/fpls.2017.00185

Marques, AP., Moreira, H., Franco, AR., Rangel, AO., Castro, PM. (2013). Inoculating *Helianthus annuus* (sunflower) grown in zinc and cadmium contaminated soils with plant growth promoting bacteria–Effects on phytoremediation strategies. Chemosphere, *92*(1), 74-83. https://doi.org/10.1016/j.chemosphere.2013.02.055

Mills, RF. (2012). HvHMA2, a P1B-ATPase from barley, is highly conserved among cereals and functions in Zn and Cd

transport. PLoS One, *7*(8), e42640. https://doi.org/10.1371/journal.pone.0042640

Mohanty, JK., Jha, UC., Dixit, GP., Parida, SK. (2022). Harnessing the hidden allelic diversity of wild *Cicer* to accelerate genomics-assisted chickpea crop improvement. *Molecular Biology Reports*, *49*, 5697–5715. https://doi.org/10.1007/s11033-022-07613-9

Morel, M., Crouzet, J., Gravot, A., Auroy, P., Leonhardt, N., Vavasseur, A., Richaud, P. (2009). AtHMA3, a P1B-ATPase allowing Cd/Zn/Co/Pb vacuolar storage in *Arabidopsis*. *Plant Physiology, 149*(2), 894-904. https://doi.org/10.1104/pp.108.130294

Nakano, Y., & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant Cell Physiolology*, *22*, 867–880.

Page, V., Feller, U. (2005). Selective transport of zinc, manganese, nickel, cobalt and cadmium in the root system and transfer to the leaves in young wheat plants. *Annals of Botany*, *96*(3), 425-434. https://doi.org/10.1093/aob/mci189

Pence, N., Larsen, P., Ebbs, S., Letham, D., Lasat, M., Garvin, D., Eide, D., Kochian, L. (2000). The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*. *Proceedings of the National Academy of Sciences*, *97*,4956-4960. https://doi.org/10.1073/pnas.97.9.4956

Pereira, G., Molina, S., Lea, P., Azevedo, R. (2002). Activity of antioxidant enzymes in response to cadmium in *Crotalaria juncea*. *Plant Nutrients in Soil*, *239*, 123-132. https://doi.org/10.1023/A:1014951524286

Rietra, R., Heinen, M., Dimkpa, C., Bindraban, P. (2017). Effects of nutrient antagonism and synergism on yield and fertilizer use efficiency. *Communications in Soil Science and Plant Analysis*, *48*(16), 1895-1920. https://doi.org/10.1080/00103624.2017.1407429

Santos, R., Schmidt, É., Marthiellen, R., Polo, L., Kreusch, M., Pereira, D., Costa, G., Simioni, C., Chow, F., Ramlov, F. (2014). Bioabsorption of cadmium, copper and lead by the red macroalga *Gelidium floridanum*: Physiological responses and ultrastructure features. *Ecotoxicology and Environmental Safety*, *105*, 80-89. https://doi.org/10.1016/j.ecoenv.2014.02.021

Sarwar, N., Malhi, S., Zia, M., Naeem, A., Bibi, S., Farid, G. (2010). Role of mineral nutrition in minimizing cadmium accumulation by plants. *Journal of the Science of Food and Agriculture*, *90*(6), 925-937. https://doi.org/10.1002/jsfa.3916

Satoh-Nagasawa, N., Mori, M., Nakazawa, N., Kawamoto, T., Nagato, Y., Sakurai, K., Takahashi, H., Watanabe, A., Akagi, H. (2012). Mutations in rice (*Oryza sativa*) heavy metal ATPase 2 (OsHMA2) restrict the translocation of zinc and cadmium. *Plant Cell Physiology*, *53*(1), 213-224. https://doi.org/10.1093/pcp/pcr166

Schmidt, T., Bergner, A., Schwede, T. (2014). Modelling three-dimensional protein structures for applications in drug design. *Drug Discovery Today*, *19*, 890-897. https://doi.org/10.1016/j.drudis.2013.10.027

Schutzendubel, A., & Polle, A. (2002). Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. *Journal of Experimental Botany, 53*(372), 1351-1365. https://doi.org/10.1093/jexbot/53.372.1351

Schutzendubel, A., Schwanz, P., Teichmann, T., Gross, K., Langenfeld-Heyser, R., Godbold, D., Polle, A. (2001). Cadmium-induced changes in antioxidative systems, hydrogen peroxide content, and differentiation in *Scots pine* roots. *Plant Physiology*, *127*(3), 887-898. https://doi.org/10.1104/pp.010318

Singh, S., Parihar, P., Singh, R., Singh, V., Prasad, S. (2016). Heavy metal tolerance in plants: Role of transcriptomics, proteomics, metabolomics, and ionomics. *Frontiers in Plant Science*, *6*, 1143. https://doi.org/10.3389/fpls.2015.01143

Socha, A., & Guerinot, M. (2014). Mn-euvering manganese: the role of transporter gene family members in manganese uptake and mobilization in plants. *Frontiers in Plant Science*, *5*, 106-106. https://doi.org/10.3389/fpls.2014.00106

Sun, J., Shen, Z. (2007). Effects of Cd stress on photosynthetic characteristics and nutrient uptake of cabbages with different Cd-tolerance. *Ying Yong Sheng Tai Xue Bao*, *18*(11), 2605-2610.

Tabarzad, A., Ayoubi, B., Riasat, M., Saed-Moucheshi, A., Pessarakli, M. (2017). Perusing biochemical antioxidant enzymes as selection criteria under drought stress in wheat varieties. *Journal of Plant Nutritoin*, *40*(17), 2413-2420. https://doi.org/10.1080/01904167.2017.1346679

Takahashi, R., Bashir, K., Ishimaru, Y., Nishizawa, N., Nakanishi, H. (2012). The role of heavy-metal ATPases, HMAs, in zinc and cadmium transport in rice. *Plant Signal Behavior*, *7*(12), 1605-1607. https://doi.org/10.4161/psb.22454

Tamura, K., Dudley, J., Nei, M., Kumar, S. (2007). MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, *24*(8), 1596-1599. https://doi.org/10.1093/molbev/msm092

Tang, R., Nkrumah, P., Erskine, P., van der Ent, A. (2022). Polymetallic (zinc and cadmium) hyperaccumulation in the Australian legume *Crotalaria novae-hollandiae* compared to *Crotalaria cunninghamii*. *Plant Nutrition and Soil*, *479*, 589–606. https://doi.org/10.1007/s11104-022-05547-6

Thomine, S., Wang, R., Ward, J., Crawford, N., Schroeder, J. (2000). Cadmium and iron transport by members of a plant metal transporter family in *Arabidopsis* with homology to Nramp genes. *Proceedings of the National Academy of Sciences*, *97*(9), 4991-4996. https://doi.org/10.1073/pnas.97.9.4991

Tian, P., Feng, Y., Li, C., Zhang, P., Yu, X. (2023) Transcriptional analysis of heavy metal P1B-ATPases (HMAs) elucidates competitive interaction in metal transport between cadmium and mineral elements in rice plants. *Environmental Science and Pollution Research*, *30*, 287-297. https://doi.org/10.1007/s11356-022-22243-1

Tuver, G., Ekinci M., Yildirim, E. (2022). Morphological, physiological and biochemical responses to combined cadmium and drought stress in radish (*Raphanus sativus* L.). *Rendiconti Lincei. Scienze Fisiche e Natural*, *33*,419-429. https://doi.org/10.1007/s12210-022-01062-z

Ulusu, Y., Öztürk, L., Elmastaş, M. (2017). Antioxidant capacity and cadmium accumulation in parsley seedlings exposed to cadmium stress. *Russian Journal of Plant Physiology*, *64*(6), 883-888. https://doi.org/10.1134/S1021443717060139

Vijendra, P., Huchappa, K., Lingappa, R., Basappa, G., Jayanna, S., Kumar, V. (2016). Physiological and biochemical changes in moth bean (*Vigna aconitifolia* L.) under cadmium stress. *Journal of Botany*, *10*, 1-13. https://doi.org/10.1155/2016/6403938

Zhang, L., Wu, M., Teng, Y., Jia, S., Yu, D., Wei, T., Chen, C., Song, W. (2019). Overexpression of the glutathione peroxidase 5 (RcGPX5) gene from *Rhodiola crenulata* increases drought tolerance in *Salvia miltiorrhiza*. *Frontiers in Plant Science*, *9*, (1950). https://doi.org/10.3389/fpls.2018.01950

Zhou, Z., Wei, C., Liu, H., Qiujuan, J., Gezi, L., Jingjing, Z., …, Yang, S. (2022). Exogenous ascorbic acid application alleviates cadmium toxicity in seedlings of two wheat (*Triticum aestivum* L.) varieties by reducing cadmium uptake and enhancing antioxidative capacity. *Environmental Science and Pollution Research*, *29*, 21739–21750. https://doi.org/10.1007/s11356-021-17371-z

Zulfiqar, U., Ayub, A., Hussain, S., Waraich, E., El-Esawi, M., Ishfaq, M., Ahmad, M., Ali, N., Faisal Maqsood, M. (2022). Cadmium Toxicity in Plants: Recent Progress on Morpho-physiological Effects and Remediation Strategies. *Journal of Soil Science and Plant Nutrition*, 22, 212–269. https://doi.org/10.1007/s42729-021-00645-3

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Gamma irradiation of eggplant seeds influences plant growth, yield and nutritional profile in M_1 generation

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Gamma irradiation of eggplant seeds influences plant growth, yield and nutritional profile in M_1 generation

Abstract: The study examines agromorphological traits and nutrient compositions in three genotypes of eggplants (*Solanum melongena* 'African Beauty F_1' and 'Melina F_1' and *S. aethiopicum* 'Kotobi') grown from seeds irradiated by gamma rays (γ-ray) with 100 Gy. Experiments were carried out in the screenhouse and experimental field of Crop Science Department, University of Calabar, Nigeria. Completely randomised design with four replications and randomised complete block design with three replications was used in the screenhouse and field experiments respectively. Eggplant × γ-ray effect reduced ($p \leq 0.05$) seedling emergence, plant height and number of leaves in the nursery at 2 and 4 weeks after sowing. In the field, these traits were consistently lower for irradiated Melina F_1 and Kotobi ($p > 0.05$) at ten weeks after transplanting. Irradiated African Beauty F_1 had the highest ($p \leq 0.05$) upper canopy leaf area (429.54 cm^2), higher ($p > 0.05$) plant height and stem width; lower ($p > 0.05$) number of branches and leaves. Un-irradiated and irradiated Kotobi had the highest ($p \leq 0.05$) fruit load, lower ($p \leq 0.05$) fruit volume, weight and yields over four harvest intervals. Carbohydrate and energy contents of Kotobi fruits grown from 100 Gy gamma-ray irradiated seeds were concurrently improved ($p \leq 0.05$). Gamma ray irradiation had both positive and negative influences on the agromorphological traits, mineral composition and nutrient profile of eggplants. However, 100 *Gy* dose of irradiation had a negative effect on fruit characteristics in general. From the results of this study, inconsistent variations in the agromorphological traits of the irradiated eggplants of the three varieties were reported. Therefore, the goal of mutation breeding in eggplant should not undermine the importance of the eggplant genotype as well as the actual radiation dose.

Key words: γ-ray, eggplant, fruits, induced mutation, irradiation, Solanaceae

Obsevanje semen jajčevca z γ-žarki vpliva na rast rastlin, pridelek in prehransko vrednost plodov v M_1 generaciji

Izvleček: Raziskava preučuje agromorfološke lastnosti in prehransko sestavo treh sort jajčevca (*Solanum melongena* 'African Beauty F_1' and 'Melina F_1' in *S. aethiopicum* 'Kotobi') vzgojenih iz semen obsevanih z gama žarki, jakosti 100 Gy. Poskusi so bili izvedeni v rastlinjaku in na poskusnem polju ustanove Crop Science Department, University of Calabar, Nigeria. V obeh primerih je bil poskus zasnovan kot popolni naključni bločni poskus s štirimi ponovitvami. Obsevanje semen jajčevca z gama žarki je zmanjšalo vznik sejank ($p \leq 0,05$), višino rastlin in število listov v rastlinjaku dva in štiri tedne po setvi. V poljskem poskusu so bile vrednosti teh parametrov vedno manjše pri obsevanih sortah Melina F_1 in Kotobi ($p > 0,05$) deset tednov po presaditvi. Rastline obsevane sorte African Beauty F_1 so imele največjo listno površino ($p \leq 0,05$; 429,54 cm^2), večjo višino ($p > 0,05$) in večjo debelino stebla, a manjšo število stranskih poganjkov in listov ($p > 0,05$). Neobsevane in obsevane rastline sorte Kotobi so imele največ plodov ($p \leq 0,05$), manjši volume plodov ($p \leq 0,05$), manjšo maso in pridelek v vseh štirjih obdobjih pobiranja plodov. Vsebnosti ogljikovih hidratov in energetska vrednost plodov sorte Kotobi, zrasle iz semen obsevanih z 100 Gy gama žarki sta se izboljšali ($p \leq 0,05$). Obsevanje semen jajčevca z gama žarki je imelo pozitivne in negativne učinke na agromorfološke lastnosti, mineralno sestavo in na prehranski profil plodov jajčevca. Doza obsevanja 100 Gy je imela nasplošno negativni učinek na lastnosti plodov. Iz rezultatov raziskave je razvidno, da so spremembe agromorfoloških lastnosti jajčevca vseh treh obravnavanih sort, vzgojenih iz obsevanih semen nekonsistetne. Iz tega sledi, da cilji žlahtnenja z mutacijami ne smejo prezreti pomena genotipa jajčevca kot tudi ne dejanskih doz obsevanja.

Ključne besede: γ-*žarki*, jajčevec, plodovi, inducirane mutacije, obsevanje, Solanaceae

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1 INTRODUCTION

Eggplant is a vegetable crop mostly cultivated in tropical and subtropical regions of the world. It belongs to the Solanaceae family and the genus *Solanum* with more than 90 genera comprising nearly 3,000 species (Melissa, 2017; Singh et al., 2006). Eggplant has been recognized as the fifth most economically important Solanaceous crop after potato (*Solanum tuberosum* L.), tomato (*Solanum lycopersicum* L.), pepper (*Capsicum annuum* L.) and tobacco (*Nicotiana tabacum* L.) (FAO, 2014). Eggplant has a very low caloric value and is considered among the healthiest vegetables with high vitamin, mineral and bioactive compounds (Raigon et al., 2008; Plazas et al., 2013; Docimo et al., 2016). It is very common in rich dishes such as stews and soups (Edem et al., 2009; Chinedu et al., 2008). The need for improved eggplant varieties for sustainable production and adaptation to climate change challenges cannot be overemphasized. The low yielding ability of the crop has been attributed to lack of varietal replacement through development of hybrid and persistent use of traditional practices coupled with the influence of environmental degradation (Chinedu et al., 2008). Increasing crop yields is a major demand for assuring food security and as such mutagenesis is an important tool to improve crops (Beyaz et al., 2017). As an alternative to natural mutation, which can take years, inducing mutations with different mutagens has greatly aided breeding projects in a variety of ways. Many studies have reported that genetic variability for numerous desired traits may be successfully created through mutations, and its application in plant development programmes is well known (Chopra, 2005). Because of its penetrating capabilities, gamma irradiation is one of the most successful techniques of creating genetic diversity in plants when compared to other ionising radiations (Moussa, 2006), as well as in the production of new varieties (Animasaun, 2014; Mohamad et al., 2006). Gamma-ray photons have the shortest wavelength in the electromagnetic spectrum, and therefore possess more energy which gives them the ability to penetrate deeper into the plant tissues (Amano, 2006). Accordingly, gamma irradiation has been used to induce mutation and still shows great potential for improving vegetative plants (Predieri, 2001). Mutation breeding is utilised in addition to traditional plant breeding because it has a stronger potential for enhancing plant architecture and resulting in improved crop development (Khin, 2006). Gamma rays are used in inducing mutations in seeds, and other planting materials such as cuttings, pollens or callus cultures (Ali et al., 2015). Gamma rays are also being widely used as mutation techniques in an attempt to improve morphological and plant growth characteristics. For example, gamma ray irradiation was used to extend the shelf life of tomatoes (Antaryami et al., 2016) and to improve potato storage capacity (Nouani et al., 1987) as well as the morphological traits in pepper (Abu et al., 2020). This can also be of great value and benefit for the improvement of eggplant. The improvement of eggplants through creation of variability using gamma rays would enable the selection of high yielding genotypes with improved agromorphological characters and increase the crop's agricultural productivity. Thus, the objective of this study was to assess the effect of gamma irradiation on the growth and yield traits of three varieties of eggplants.

2 MATERIALS AND METHODS

2.1 SOURCE OF SEEDS

Seeds of three varieties of eggplants, African Beauty F_1 (*Solanum melongena* L.), Kotobi (*Solanum aethiopicum* L.) and Melina F_1 (*Solanum melongena* L.), were purchased from Technisem® (Longue-Jumelles, France).

2.2 IRRADIATION OF SEEDS

Protocol for the irradiation of eggplant seeds was followed according to the National Institute of Radiation Protection and Research at the University of Ibadan, Nigeria. The irradiator used was a Gamma-Photon Irradiator with model GammaBeam™ X200 (Best Theratronics Ltd., Canada). The samples were irradiated with 100 Gy of gamma rays.

2.3 EXPERIMENTAL SITE AND DESIGN

The study was conducted at the University of Calabar Teaching and Research Farm, Calabar in two phases – the screenhouse and the field. Completely randomised design with four replications was used for the potted experiment in the screenhouse comprising while randomised complete block design with three replications was used in the field.

2.3.1 Screenhouse experiment

The top-soil (15–20 cm depth) used for the screenhouse experiment was obtained from the earmarked experimental field site. The friable, humus-rich topsoil was properly sieved, uniformly mixed, weighed and then transferred into conically shaped base-perforated plastic

pots with the following dimensions: 20 cm – height, 8 cm – base radius and 10 cm – rim radius. The total volume of each pot was 5108 cm^3. Three-quarters of the total volume of each pot was filled with the prepared topsoil (i.e., 3831 cm^3 of topsoil). The potted soil was sufficiently and uniformly wetted with 500 ml of irrigation water the after preparation of the seed bed. The seeds were primed in distilled water for 24 h prior to sowing on 2 March 2020. A total range of 30 seeds were sown in each pot. The total number of pots was 24. Seedlings of irradiated and unirradiated seeds were raised indoors in potted nursery in the screenhouse. Subsequent irrigation was done for 22 days: at germination (thrice, 50 ml at three days interval) and emergence (thrice, 100 ml at two days interval). At full emergence and growth (≥ 22 days after sowing), 500 ml of the irrigation water was applied at two days interval for three weeks. Transplanting of vigorous seedlings was done at six weeks after sowing (WAS). The seedlings were transplanted at a height of 10–15 cm on 13 April 2020 using the ball-of-earth method. The plant spacing was 0.6 m × 0.6 m and the gross treatment plot size was 2.4 m × 3.0 m (for 20 seedlings) giving a total plant population of 27,777 stands per hectare.

2.3.2 Field experiment

The net plot size of 1.2 m × 1.8 m, comprising of six tagged stands of eggplants, was earmarked for growth and yield data collection. Organic fertilizer, poultry manure (15 t ha^{-1}), was applied by broadcasting to the soil at two weeks before transplanting. Inorganic fertilizer, NPK 15:15:15 (120 kg ha^{-1}), was applied by ring method at 10 cm away from the base of the plants at two weeks after transplanting (WAT). Pest was controlled using a systematic pyrethroid insecticide, Fighter 35 EC (Lambda-Cyhalothrin 15 g l^{-1} + Acetamiprid 20 g l^{-1}). Foliar application at the rate of 640 ml ha^{-1} was done at two and four WAT; manual weeding (hand-hoeing and hand-rouging) was done concurrently. Manual harvesting of mature fruits was done between 65 and 95 days after transplanting (DAT) (Mahanta and Kalita, 2020). The fruits were hand-picked four times at 10 days intervals.

2.4 DATA COLLECTION

Growth and yield data were collected on the number of germinated seedlings that emerged in the screenhouse, plant height, number of fully-opened leaves per plant, number of branches (primary and secondary), leaf area according to Rivera et al. (2007), stem width, fruit load (i.e., number of mature fruits per plant), fruit volume based on water displacement method, fruit mass, and fruit yield (per plant and per hectare).

2.5 PROXIMATE ANALYSIS

Moisture, crude protein (Kjeldahl method), fat (Soxhlet method), crude fibre and ash contents of the harvested fruits (mean of the four harvests) were determined according to the standard procedures of Association of Official Analytical Chemists (AOAC) (2010). Content of carbohydrates was calculated by percentage difference between 100 % (accepted total value of nutritional status) and the sum of the moisture, fat, ash, crude protein and crude fibre (Ovenuga, 1986). Calorific value (Kcal 100 g^{-1}) was determined from crude protein, crude fat and carbohydrate values accordingly:

[(Crude protein × 4.0) + (Crude fibre × 9.0) + (Carbohydrate × 3.75)] (FAO, 2003).

2.6 DETERMINATION OF ESSENTIAL MINERALS

Analysis of essential minerals in fruit samples were performed in three replicates, and data are presented as mean ± SD. Iron was determined following the method of Pearson (1976). Phosphorus was determined by molybdate method as described by Onwuka (2005). Flame photometer was used to determine potassium by the procedure described by Osborne & Voogt (1978). Calcium (extracted by the titrimetric method with EDTA) and Zinc were determined by atomic-absorption spectrophotometry (David, 1958; David, 1959). Magnesium was determined with disodium ethylenediaminetetra-acetate (Smith & McCallcum, 1956) and sodium was determined using ion chromatography (Basta & Tabatabai, 1985).

2.7 DATA ANALYSIS

Treatments and replicates mean values of all the nursery and field data obtained were subjected to a two-way analysis of variance (ANOVA) using software GenStat 16th Edition (VSN International, 2013). Turkey's

Honest Significant Difference test (HSD) was used for significant treatment means separation at 95 % confidence limit.

3 RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 Soil physical and chemical properties

Soil properties for the two experiments are presented in Table 1. The screenhouse soil texture was sandy loam while the field had a loamy sand soil texture. Soil pH ranged from strongly acidic (4.9) to moderately acidic (5.9). Overall, screenhouse soil had higher cation-exchange capacity (CEC) and base saturation (BS) compared to the soil in the field. Both soils were suitable for the cultivation of eggplant.

3.1.2 Effects of irradiation (γ-ray) on growth of eggplants in the nursery

The effects of γ-ray radiation, eggplant variety and their interactions on seedling emergence, height and number of leaves were examined at two and four weeks after sowing (Table 2). At 2 weeks after sowing (WAS), the eggplant variety, Melina F_1 had the highest seedling emergence ($p \leq 0.05$) followed by Kotobi and African Beauty F_1 varieties. 'Kotobi' was shorter in height ($p \leq 0.05$) than 'Melina F_1' and 'African Beauty F_1'. There was no significant difference ($p > 0.05$) in the average number of leaves for the three eggplant varieties.

In general, control (no irradiation) had significantly ($p \leq 0.05$) higher effect on seedling emergence and plant height, but no significant ($p > 0.05$) influenced on the number of leaves borne by each of the eggplant varieties. Following the interaction effect, un-irradiated Melina F_1 eggplant variety had a 100 % seedling emergence while irradiated 'African Beauty F_1' had the lowest seedling emergence (31.1 %). In terms of plant height, irradiated Melina F_1 eggplant variety was the tallest (4.85 cm) and significantly ($p > 0.05$) different from irradiated 'Kotobi', the shortest (2.98 cm) eggplant variety. There was no significant difference in the number of leaves for eggplant × γ-ray interaction effect at 2 WAS. At 4 WAS, single effects of eggplant and γ-ray radiation were significant ($p \leq 0.05$) for plant height. γ-ray radiation did not lead to a significant ($p > 0.05$) variation in the number of leaves. However, the eggplant × γ-ray interaction effect showed that un-irradiated and irradiated 'Melina F_1' plants were the tallest, similar to irradiated and un-irradiated 'African Beauty F_1', but significantly different ($p > 0.05$) from irradiated 'Kotobi'. Meanwhile, the number of leaves ranged from 3.72 (irradiated 'Kotobi') to 4.78 (un-irradiated 'Melina F_1'). All other eggplant × γ-ray effects, except 'Melina F_1', were similar ($p > 0.05$) to irradiated 'Kotobi' in terms of the average number of leaves per plant at 4WAS.

3.1.3 Effects of irradiation (γ-ray) on growth of eggplants in the field

The single effects of γ-ray radiation, eggplant variety and their interactions on plant height, stem width, number of branches, number of leaves and leaf area (upper, middle and lower canopies) were assessed at ten weeks after transplanting to field (Table 3). Varietal effect was only significant ($p \leq 0.05$) for leaf area of the upper canopy while radiation effect was significant ($p \leq 0.05$) for stem width and number of branches. 'African Beauty F_1' had the largest leaves (382.85 cm^2) and was not significantly ($p > 0.05$) different from those of 'Melina F_1' (339.75 cm^2). Plants from un-irradiated eggplant seeds had thicker stems and more leaves than its counterparts from irradiated seeds. The eggplant × γ-ray interaction only had significant influence on leaf area of the upper canopy of the eggplants. The largest upper canopy leaf area was obtained from irradiated 'African Beauty F_1' (429.54 cm^2) while irradiated 'Kotobi' had the lowest upper canopy leaf area (267.77 cm^2). The general observation was that all growth traits of un-irradiated 'Melina F_1' were consistently higher than its irradiated group. A similar trend was observed for un-irradiated 'Kotobi', except for leaf area of the middle canopy where the irradiated group led with larger leaves.

3.1.4 Effects of irradiation (γ-ray) on yield of eggplants at harvests

Four harvests were made and at each of these harvests, records were taken on several yield and yield-related characters of the three eggplants varieties obtained from their irradiated and un-irradiated seeds. There were highly significant ($p \leq 0.05$) variations observed for all the characters (Table 4). Fruit load (number of mature whole fruits per plant) ranged from 5.5 ('African Beauty F_1' and 'Melina F_1') to 51.5 ('Kotobi'). Up to the third harvest, with the exception of Melina F_1, all eggplant varieties from un-irradiated seeds had either similar or higher fruit load in comparison with the irradiated group. At fourth harvest, all irradiated varieties had higher fruit load, 'African Beauty F_1' recorded its highest. Volume and mass

Table 1: Soil physical and chemical properties

	pH in H_2O (1:25)	Sand	Silt	Clay	OC	TN	Available P	K^+	Ca^{2+}	Mg^{2+}	Na^+	Al^{3+}	H^+	CEC	BS
			(g kg^{-1})		(%)			(mg kg^{-1})				(cmol (+) kg^{-1})			(%)
Screenhouse	5.9	690	150	160	2.89	0.24	82.50	0.13	6.2	2.2	0.10	1.16	0.56	10.15	83.00
Field	4.9	843	54	103	1.10	0.14	23.17	0.10	1.4	1.2	0.09	0.56	1.76	5.11	54.59

Table 2: Single and interaction effects of radiation (γ-ray) and variety on growth of eggplants at two and four weeks after sowing in the nursery

	Emergence (%)	Plant Height (cm)	Number of Leaves	Plant Height (cm)	Number of Leaves
Treatment	Two Weeks After Sowing			Four Weeks After Sowing	
Eggplant					
African Beauty F_1	46.4 b	4.64 a	2.28 a	7.66 a	4.03 b
Kotobi	54.2 b	3.41 b	2.09 a	5.34 b	3.97 b
Melina F_1	77.9 a	4.67 a	2.17 a	8.33 a	4.72 a
$HSD_{0.05}$	<0.01	<0.01	0.14	<0.01	<0.01
γ-ray					
Irradiation	36.67 b	4.00 b	2.13 a	6.44 b	4.15 a
No irradiation	82.32 a	4.49 a	2.22 a	7.78 a	4.33 a
$HSD_{0.05}$	<0.01	0.03	0.25	<0.01	0.18
Eggplant × γ-ray					
Irradiated African Beauty F_1	31.1 d	4.52 a	2.22 a	7.16 ab	4.06 bc
Un-irradiated African Beauty F_1	61.7 c	4.77 a	2.33 a	8.17 ab	4.00 bc
Irradiated Kotobi	25.6 d	2.98 b	2.07 a	4.44 c	3.72 c
Un-irradiated Kotobi	82.8 b	3.84 ab	2.11 a	6.24 bc	4.22 abc
Irradiated Melina F_1	53.3 c	4.49 a	2.11 a	7.72 ab	4.67 ab
Un-irradiated Melina F_1	100.0 a	4.85 a	2.22 a	8.93 a	4.78 a
$HSD_{0.05}$	0.01	<0.01	0.92	<0.01	<0.01

$HSD_{0.05}$ = Tukey's honestly significant difference test at 95 % confidence level

Table 3: Single and interaction effects of radiation (γ-ray) and variety on growth of eggplants at ten weeks after transplanting in the field

Treatment	Plant Height (cm)	Stem Width (mm)	Number of Branches	Number of Leaves	Leaf Area (Upper Canopy)	Leaf Area (Middle Canopy)	Leaf Area (Bottom Canopy)
					(cm^2)		
Eggplant							
African Beauty F_1	63.72 a	17.85 a	16.26 a	42.23 a	382.85 a	300.34 a	327.08 a
Kotobi	63.00 a	16.95 a	16.51 a	44.56 a	278.65 b	241.82 a	266.44 a
Melina F_1	61.93 a	15.00 a	12.53 a	30.56 a	339.75 ab	262.79 a	256.35 a
$HSD_{0.05}$	0.94	0.11	0.12	0.14	0.02	0.24	0.19
γ-ray							
Irradiation	60.23 a	15.31 b	13.17 b	33.97 a	339.08 a	275.06 a	277.98 a
No irradiation	65.53 a	17.89 a	17.04 a	44.26 a	328.42 a	261.57 a	288.60 a
$HSD_{0.05}$	0.22	0.03	0.04	0.10	0.67	0.62	0.74
Eggplant × γ-ray							
Irradiated African Beauty F_1	65.35 a	18.02 a	12.50 a	34.46 a	429.54 a	336.85 a	324.50 a
Un-irradiated African Beauty F_1	62.08 a	17.69 a	20.02 a	50.00 a	336.15 ab	263.84 a	329.67 a
Irradiated Kotobi	56.16 a	14.38 a	15.06 a	38.43 a	267.77 b	251.25 a	256.61 a
Un-irradiated Kotobi	69.85 a	19.51 a	17.95 a	50.68 a	289.53 ab	232.38 a	276.27 a
Irradiated Melina F_1	59.19 a	13.53 a	11.94 a	29.03 a	319.93 ab	237.08 a	252.84 a
Un-irradiated Melina F_1	64.67 a	16.46 a	13.13 a	32.08 a	359.57 ab	288.50 a	259.85 a
$HSD_{0.05}$	0.28	0.14	0.29	0.65	0.01	0.21	0.98

$HSD_{0.05}$ = Tukey's honestly significant difference test at 95 % confidence level

Table 4: Effects of radiation (γ-ray) × variety × harvest interval on yield of eggplants

Treatment Combination		Fruit Load	Fruit Volume (ml^3)	Fruit Mass (g)	Fruit Yield (kg $plant^{-1}$)	Fruit Yield (t ha^{-1})
First Harvest	Irradiated African Beauty F_1	5.50 i	9.35 ab	505.05 a	2.30 cd	62.55 e
	Un-irradiated African Beauty F_1	5.50 i	8.15 d	320.05 f	1.55 e	41.72 i
	Irradiated Kotobi	23.50 d	3.85 j	17.41 r	0.42 ijkl	10.23 q
	Un-irradiated Kotobi	46.64 b	3.68 jkl	18.23 q	0.69 hi	17.91 n
	Irradiated Melina F_1	5.50 i	4.92 gh	241.72 n	1.05 f	27.83 k
	Un-irradiated Melina F_1	7.90 hi	5.31 ef	296.48 k	1.79 e	48.38 g
Second Harvest	Irradiated African Beauty F_1	5.50 i	9.25 ab	490.05 b	2.45 c	66.71 c
	Un-irradiated African Beauty F_1	5.50 i	8.85 c	450.05 d	2.15 d	58.38 f
	Irradiated Kotobi	13.30 f	3.85 j	17.22 r	0.23 l	5.05 t
	Un-irradiated Kotobi	51.50 a	3.78 jk	15.76 u	0.75 gh	19.49 m
	Irradiated Melina F_1	11.50 fg	5.05 fg	277.55 l	2.15 d	58.38 f
	Un-irradiated Melina F_1	5.50 i	4.65 hi	271.30 m	1.03 fg	27.13 l
Third Harvest	Irradiated African Beauty F_1	5.50 i	9.15 b	450.05 d	2.15 d	58.38 f
	Un-irradiated African Beauty F_1	9.50 gh	8.81 c	384.05 e	2.95 b	80.60 b
	Irradiated Kotobi	18.00 e	3.45 l	15.46 v	0.28 kl	6.30 s
	Un-irradiated Kotobi	36.83 c	3.45 l	16.12 t	0.55 hijk	13.94 p
	Irradiated Melina F_1	5.50 i	4.95 g	305.05 h	1.15 f	30.60 j
	Un-irradiated Melina F_1	11.50 fg	4.53 i	186.72 o	1.75 e	47.27 h
Fourth Harvest	Irradiated African Beauty F_1	17.50 e	8.32 d	318.38 g	4.55 a	125.05 a
	Un-irradiated African Beauty F_1	8.50 h	9.47 a	480.05 c	4.55 a	125.05 a
	Irradiated Kotobi	36.17 c	3.51 kl	16.41 s	0.58 hij	14.86 o
	Un-irradiated Kotobi	21.32 d	3.71 jkl	21.30 p	0.40 jkl	9.90 r
	Irradiated Melina F_1	11.50 fg	5.37 e	298.18 j	2.38 cd	64.63 d
	Un-irradiated Melina F_1	10.00 gh	5.52 e	303.26 i	2.15 d	58.38 f
	$HSD_{0.05}$	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

$HSD_{0.05}$ = Tukey's honestly significant difference test at 95% confidence level

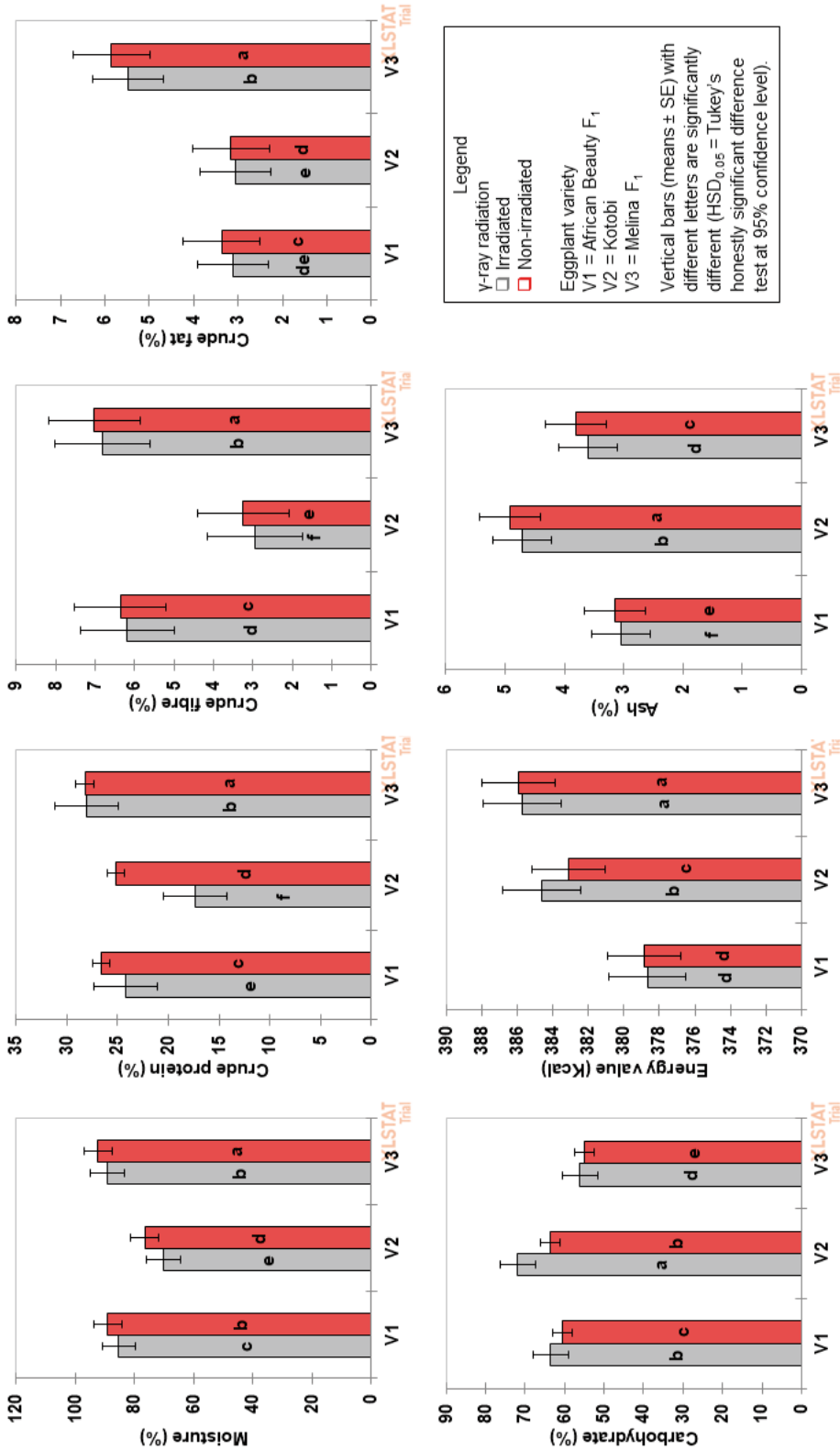

Figure 1: Comparative proximate compositions of γ-ray irradiated and un-irradiated eggplants

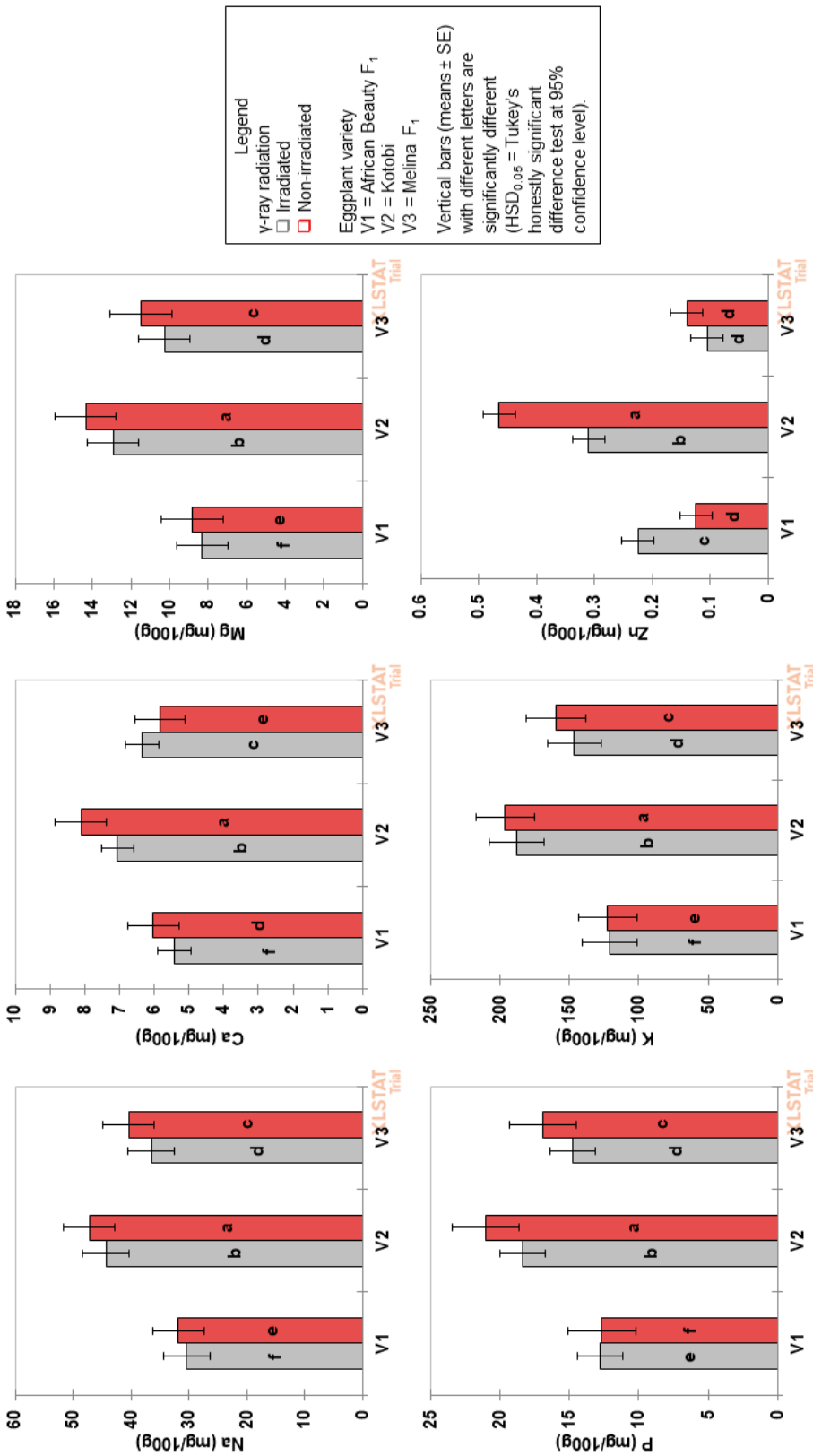

Figure 2: Comparative micronutrient profiles of γ-ray irradiated and un-irradiated eggplants

of eggplant fruits from irradiated seeds were the highest for 'African Beauty F_1' up to the third harvest. Fruits of 'Melina F_1', from both irradiated and un-irradiated seeds, were significantly ($p \leq 0.05$) lighter in mass and smaller in volume than 'African Beauty F_1'. Characteristically, 'Kotobi' had higher fruit bearing attribute indicated by its high fruit load but with smaller fruit volume and lighter mass. The average fruit yield per plant ranged from 0.23 kg to 4.55 kg.

The highest fruit yield on plant basis was recorded from 'African Beauty F_1' (irradiated and un-irradiated seeds) at fourth harvest. Fruit yield (plant^{-1} and hectare^{-1}) of 'Kotobi' was generally lower than other eggplant varieties across the four harvest intervals. 'African Beauty F_1' maintained the same trend for fruit yield on per hectare basis.

3.1.5 Effects of irradiation (γ-ray) on comparative proximate composition of eggplants

Proximate analysis of freshly harvested fruits of the three eggplants varieties in our study showed that fruits obtained from plants grown from γ-ray irradiated seeds had significantly ($p \leq 0.05$) lower moisture content, crude protein, crude fibre, crude fat and ash content (Figure 1). In the other hand, all eggplants fruits from un-irradiated seeds had lower carbohydrate across the three varieties. Fruits of Kotobi variety had the lowest moisture content (70–77 %) followed by African Beauty F_1 (85–89 %) and Melina F_1 (89 – 92 %). Crude protein ranged from 17 % (irradiated 'Kotobi') to 28 % (un-irradiated 'Melina F_1'). Irradiated 'Kotobi' also had the lowest crude fibre (2.95 %) and crude fat (3.06 %), but had a significantly higher carbohydrate content (71.88 %). Although un-irradiated 'Melina F_1' had the lowest carbohydrate content (55.09 %), its energy value was the highest (385.9 Kcal 100 g^{-1}) which was not significantly ($p > 0.05$) different from the un-irradiated 'Melina F_1' (385.72 Kcal 100 g^{-1}). Irradiated (378.68 Kcal 100 g^{-1}) and un-irradiated (378.88 Kcal 100 g^{-1}) fruits of 'African Beauty F_1' had the lowest energy values ($p > 0.05$). Overall, 'Kotobi' had the highest ash content (4.72–4.92 %) followed by 'Melina F_1' (3.61–3.81 %) and 'African Beauty F_1' (3.04–3.15 %).

3.1.6 Effects of irradiation (γ-ray) on macro- and micro-nutrients profile of eggplants

There were significant ($p \leq 0.05$) differences among the three eggplant varieties in micronutrient profiles of fruits obtained from γ-ray irradiated and un-irradiated seeds (Figure 2). In general, un-irradiated Kotobi eggplant variety had the richest nutrient contents: sodium (47.18 mg 100 g^{-1}), calcium (8.11 mg 100 g^{-1}), magnesium (14.36 mg 100 g^{-1}), phosphorus (21.02 mg 100 g^{-1}), potassium (196.15 mg 100 g^{-1}) and zinc (0.47 mg 100 g^{-1}). With exception of zinc (varieties: Kotobi > African Beauty F_1 ≥ Melina F_1), the micronutrient profile richness followed the varietis order: Kotobi > Melina F_1 > African Beauty F_1. It was observed that Na, Mg and K contents followed the same trend in the eggplant fruits for grown from both irradiated and un-irradiated seeds.

4 DISCUSSION

The use of gamma ray irradiation on eggplant varieties has helped in recent years to induce favourable mutation and improve agronomic attributes of the crop. There are reports that gamma ray could affect the growth and yield of eggplant. Contrary to Zanzibar and Sudrajat (2016) report that gamma ray irradiation could improve seed metabolism and stimulate seed germination, our results consistently showed that seedling emergence in the nursery was however higher from un-irradiated (100 Gy) eggplant seeds of African Beauty F_1, Kotobi and Melina F_1 varieties. In comparison, Rozman (2014) found that the percentage of germination of barley (*Hordeum vulgare* L.) seeds irradiated with 100 Gy did not differ from the un-irradiated in the first year, it was significantly higher in the fifth year. Also, Suparno (2018) conducted a study on the phenotypic diversity of eggplant (*S. melongena* L.) resulting from various doses of gamma-ray irradiation (0, 100, 150 and 200 *Gy*). The results showed that gamma ray irradiation resulted in high significant differences in seedling growth, 100 Gy giving the highest percentage of seedlings emergence (77.5 %) and contrary to our report where we had a range of 31.1 to 53.3 %. However, our results on the effect of gamma ray on plant height of eggplant (14 and 28 days after planting) grown from irradiated seeds was in consonant with Suparno (2018) who reported taller plants from un-irradiated seeds (4.19–10.45 cm) over 100 Gy irradiated seeds (3.99–10.38 cm). Another study conducted by David et al. (2018) on the effects of gamma irradiation on the agromorphological traits of two eggplant (*S. aethiopicum* L.) accessions conforms with our findings which showed reductions in germination percentage (in the nursery) and plant height (nursery and field) at irradiation dose of 100 Gy when compared with plants grown from un-irradiated seeds (control). Although David et al. (2018) had reported that irradiation doses of 40 Gy and 60 Gy were appropriate in creating beneficial agronomic traits in *S. aethiopicum* L. accessions, these were not consistent between the eggplant accessions. In support of our findings, Muhammad et al. (2021) reported that the growth, development, and survival rate of Bambara groundnut (*Vigna subterranea*

(L.) Verdc.) increased with a decrease in gamma-irradiation. In our study, we also observed similar varietal differences for 'Kotobi' (*S. aethiopicum* L.) in terms of stem width, number of branches, number of leaves and leaf area at different canopy heights in the field. Eggplant fruits have high contents of carbohydrates, proteins and some minerals such as Ca, Mg, and P (Kowalski et al., 2003; El-Nemr et al., 2012) and have low calories (25 kcal 100 g^{-1}) (Aly et al., 2019). We reported higher amount of mineral composition in the un-irradiated eggplants group over the irradiated ones. Aly et al. (2019) reported that eggplant growth increased when using a dose of 50 Gy gamma rays while increasing irradiation dose level to 100 Gy reduced phenyl alanine ammonia-lyase (PAL) enzyme and polyphenol oxidase enzyme activities which influences plant growth and invariably its accumulation of some active compounds. Additionally, these enzymes could also have a role in mineral accumulation. Gamma rays are one type of ionising radiation that interact with atoms or molecules to produce free radicals in cells, according to Aly et al. (2019). These radicals can change essential constituents of plant cells. In contrast to Hussein et al. (2012) that treating seeds before sowing by gamma radiation (40–80 Gy) generally increased Na and K in growing damsisa plant (*Ambrosia maritima* L.) compared by its corresponding un-irradiated control, our Na and K contents were higher in un-irradiated eggplants. Also, our results on Ca content in fruits of plants produced from irradiated seeds (except for 'Melina F_1') was similar to Hussein et al. (2012) for Ca in *A. maritima* at fruiting even under salinity stress. It was reported that exposure of red radish (*Raphanus sativus* L.) seeds to gamma irradiation before cultivation improved the root contents of the elements (N, K, S, P, Ca, and Mg) (El-Beltagi et al., 2022). In the study, it was clear that the dose of 100 Gy had different effects on the fruit characteristics of eggplants according to the genotypes. For example, while 100 Gy increased the amount of carbohydrate and energy in 'Kotobi' genotype, Ca content increased in Melina F_1 genotype, Zn content increased in African Beauty F_1 genotype. Our research clearly shows that gamma-ray irradiation of eggplant is genotype dependent as a technique of producing variation for the generation of new genotypes. Our findings correspond with those of Ulukapi et al. (2015), who discovered inconsistencies in determining optimal gamma radiation dose in eggplant mutation breeding. The preservation, decrease, or increase in agromorphological traits, mineral and nutrient compositions of plants grown from irradiated eggplant seeds compared to the control plants made it a problematic task to clearly highlight the behaviour of eggplants in response to gamma ray irradiation. Consistent with our study, studies in different plants show that variations in growth and yield traits in response to gamma ray irradiation is dependent on the crop variety as well as the radiation dose (Rozman, 2014; Majeed et al., 2018; Aparecida Costa Nobre et al., 2022; Puripunyavanich et al., 2022; Saibari et al., 2023) and as such it is difficult to establish a standard dose for mutation breeding in eggplants.

5 CONCLUSIONS

Though variations were created, gamma ray irradiation dose of 100 Gy had inconsistent influences on the agromorphological traits and nutritional profile M_1 generation of 'African Beauty F_1' (*Solanum melongena* L.), 'Kotobi' (*Solanum aethiopicum* L.) and 'Melina F_1' (*Solanum melongena* L.) eggplant varieties. Plants grown from irradiated eggplant seeds were negatively affected in terms of the following fruit characteristics: fruit load, fruit volume, fruit mass and fruit yield compared to eggplants grown form un-irradiated seeds. These differences could be partly attributed to their genetic make-up and gamma radiation dose. Hence, either higher or lower dose of gamma ray treatment could be suggested for use in subsequent studies depending on the following: (1) eggplant genotype and (2) the goal of the mutation breeding programme in view.

6 REFERENCES

Abu, N., Ojua, E., & Udensi, O. (2020). Induction of variability in three Nigerian pepper varieties using gamma irradiation. *Journal of Experimental Agriculture International, 42*(4), 111–119. https://doi.org/10.9734/jeai/2020/v42i430505

Ali, H., Ghori, Z., Sheikh, S. & Gul, A. (2015). Effects of gamma radiation on crop production. In: Hakeem, K. (Ed.), *Crop Production & Global Environmental Issues* (pp. 27–78). Cham, Denmark: Springer. https://doi.org/10.1007/978-3-319-23162-4_2

Aly, A. A., Eliwa, N. E., & AbdEl-Megid, M. H. (2019). Stimulating effect of gamma radiation on some active compounds in eggplant fruits. *Egyptian Journal of Radiation Sciences & Applications, 32*(1), 61–73. http://doi.org/10.21608/ejrsa.2019.10024.1066

Amano, E. (2006). Use of induced mutants in rice breeding in Japan. *Plant Mutation Report, 1*(1), 21–24. https://www-pub.iaea.org/MTCD/Publications/PDF/Newsletters/PMR-01-01.pdf

Animasaun, D. A., Morakinyo, J. A. & Mustapha, O. T. (2014). Assessment of the effects of gamma irradiation on the growth and yield of *Digitaria exilis* [Haller]. *Journal of Ap-*

plied Biosciences, 75, 6164–6172. https://doi.org/10.4314/jab.v75i1.1

Antaryami, S., Durgeshwer, S. & Rita, S. (2016). Shelf-life extension of tomatoes by gamma radiation. *Radiation Science and Technology, 2*(2), 17–24 .https://doi.org/10.11648/j.rst.20160202.12

AOAC. (2010). *Official methods of analysis of Association of Official Analytical Chemists* (18th ed.). Washington, DC: AOAC.

Aparecida Costa Nobre, D., Salezzi Bonfá, C., Ferreira da Silva, A., Arthur, V., & Sigueyuki Sediyama, C. (2022). Soybean generations under gamma rays and effects on seed quality. *Chilean Journal of Agricultural and Animal Sciences*, *38*(3), 287-296. https://doi.org/10.29393/CHJAA38-27KSRD10027

Basta, N. T. & Tabatabai, M. A. (1985). Determination of total potassium, sodium, calcium, and magnesium in plant materials by ion chromatography. *Soil Science Society of America Journal*, *49,* 76–81. https://doi.org/10.2136/sssaj1985.03615995004900010015x

Beyaz, R. & Yildiz, M. (2017). The use of gamma irradiation in plant mutation breeding. In S. Jurić (Ed.), *Plant Engineering.* London, UK: IntechOpen Limited. https://doi.org/10.5772/intechopen.69974

Chinedu, S. N., Olasumbo, A. C., Eboji, O. K., Emiloju, O. C., Arinola, O. K. & Schippers R. R.(2008).Proximate and phytochemical analysis of *Solanum aethiopicum* L. and *Solanum macrocarpa* L. fruits. *Research Journal of Chemical Sciences, 1*(3), 63–71. https://core.ac.uk/download/pdf/12356262.pdf

Chopra, V. L. (2005). Mutagenesis: investigating the process and processing the outcome for crop improvement. *Current Science*, *89*(2), 353–359. https://www.currentscience.ac.in/Volumes/89/02/0353.pdf

David, D. J. (1958). Determination of zinc and other elements in plants by atomic-absorption spectroscopy. *Analyst*, *83*, 655–661. https://doi.org/10.1039/AN9588300655

David, D. J. (1959). Determination of calcium in plant material by atomic-absorption spectrophotometry. *Analyst*, *84*, 536–545. https://doi.org/10.1039/AN9598400536

David, T. S., Olamide, F., Yusuf, D. O. A., Abdulhakeem, A. & Muhammad, M. L. (2018). Effects of gamma irradiation on the agro-morphological traits of selected Nigerian eggplant (*Solanum aethiopicum* L.) accessions. *GSC Biological and Pharmaceutical Sciences, 2*(3), 23–30. https://doi.org/10.30574/gscbps.2018.2.3.0014

Docimo, T., Francese, G., Ruggiero, A., Batelli, G., De Palma, M. & Bassolino, L. (2016). Phenylpropanoids accumulation in eggplant fruit: characterization of biosynthetic genes and regulation by MYB transcription factor. *Frontiers in Plant Science, 6*, 1233–3389. https://doi.org/10.3389/fpls.2015.01233

Edem, C. A., Dounmu, M. I., Bassey, F. I., Wilson, C. & Umoren, P. (2009). A comparative assessment of the proximate composition, ascorbic acid and heavy metal content of two species of garden egg (*Solanum gilo* and *Solanum aubergine). Pakistan Journal of Nutrition*, *8*(5), 582–584. https://dx.doi.org/10.3923/pjn.2009.582.584

El-Beltagi, H. S., Maraei, R. W., Shalaby, T. A., & Aly, A. A. (2022). Metabolites, nutritional quality and antioxidant activity of red radish roots affected by gamma rays. *Agronomy*, *12*(8), 1916. https://doi.org/10.3390/agronomy12081916

El-Nemr, M. A., El-Desuki, M. & Fawzy, Z. F. (2012). Yield and fruit quality of eggplant as affected by NPK sources and micronutrient application. *Journal of Applied Sciences Research, 8*(3), 1351-1357. http://www.aensiweb.com/old/jasr/jasr/2012/1351-1357.pdf

FAO. (2003). Chapter 3 - Calculation of the energy content of foods – energy conversion factors. In: *Food energy – methods of analysis and conversion factors. Food and Nutrition Paper 77*. Rome, Italy: Food and Agriculture Organization of the United Nations.

FAO. (2014). *FAOSTAT: Production databases*. Retrieved from: http://www.faostat.fao.org

Hussein, O. S., Hanafy Ahmed, A. H., Ghalab, A. R. & El-Hefny, A. M. (2012). Some active ingredients, total protein and amino acids in plants produced from irradiated *Ambrosia maritima* seeds growing under different soil salinity levels. *American Journal of Plant Physiology, 7,* 70–83. https://dx.doi.org/10.3923/ajpp.2012.70.83

Khin, T. N. (2006). Rice mutation breeding for varietal improvement in Myanmar. *Plant Mutation Report, 1*, 34–36. http://www-pub.iaea.org/MTCD/publications/PDF/Newsletters/PMR-01-01.pdf

Kowalski, R., Kowalska, G. & Wierciński, J. (2003). Chemical composition of fruits of tree `eggplant' (*Solanum melongena* L.) cultivars. *Folia Horticulturae*, *15*(2), 89–95. https://www.researchgate.net/publication/332427598_Chemical_composition_of_fruits_of_tree_eggplant'_Solanum_melongena_L_cultivars#:~:text=The%20eggplant%20fruits%20contained%20on,g%20%E2%80%931%20f.m.%20of%20polyphenols

Mahanta, C. L., & Kalita, D. (2020). Chapter 16 - Eggplant. In A. K. Jaiswal (Ed.), *Nutritional composition and antioxidant properties of fruits and vegetables* (pp. 273-287). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-812780-3.00016-7

Majeed, A., Muhammad, Z., Ullah, R., & Ali, H. (2018). Gamma irradiation I: effect on germination and general growth characteristics of plants – a review. *Pakistan Journal of Botany, 50*(6), 449-2453.

Melissa, P. (2017). *List of plants in the family Solanaceae* (*Encyclopedia Britannica*). Retrieved from https://en.m.wikipedia.org/wiki/Eggplant

Mohamad, O., Mohd-Nazir, B., Alias, I., Azlan, S., Abdul-Rahim, H., Abdullah, M. Z.,.Golam, F. (2006). Development of improved rice varieties through the use of induced mutations in Malaysia. *Plant Mutation Reports, 1*(1), 27–34. http://www-pub.iaea.org/MTCD/publications/PDF/Newsletters/PMR-01-01.pdf

Moussa, H. R. (2006). Gamma irradiation regulation of nitrate level in rocket (*Eruca vesicaria* subsp. *sativa*) plants. *Journal of New Seeds*, *8*(1), 91–100. https://doi.org/10.1300/J153v08n01_08

Muhammad, I., Rafii, M. Y., Nazli, M. H., Ramlee, S. I., Harun, A. R., & Oladosu, Y. (2021). Determination of lethal (LD)

and growth reduction (GR) doses on acute and chronic gamma- irradiated Bambara groundnut [*Vigna subterranea* (L.) Verdc.] varieties. *Journal of Radiation Research & Applied Sciences*, *14* (1), 133–145. https://doi.org/10.1080/16878507.2021.1883320

Nouani, A., Boussaha, B. & Azzout, B. (1987). *Preservation of potato by irradiation*. *Food Irradiation Newsletter, 11*(1), 48. https://inis.iaea.org/collection/NCLCollectionStore/_Public/19/004/19004963.pdf

Onwuka, G. I. (2005). *Food analysis and instrumentation (theory and practice)* (1st edn). Surulere, Lagos: Napthali Prints.

Osborne, D. R. & Voogt, P. (1978). *Analysis of nutrients in foods*. London, UK: Academic Press.

Oyenuga, V. A. (1968). *Nigeria's foods and feeding stuffs their chemistry and nutritive values*. Ibadan, NG: University Press.

Pearson, D. (1976). *Chemical analysis of foods* (7th ed.). Edinburgh; New York: Churchill

Plazas, M., Lopez-Gresa, M. P., Vilanova, S., Torres, C., Hurtado, M., Gramazio, P.,.Prohens, J. (2013). Diversity and relationships in key traits for functional and apparent quality in a collection of eggplant: fruit phenolic content, antioxidant activity, polyphenol oxidase activity and browning. *Journal of Agriculture & Food Chemistry, 61*(37), 8871–8879. https://doi.org/10.1021/jf402429k

Predieri, S. (2001). Mutation induction and tissue culture in improving fruits. *Journal of Plant Cell Tissue & Organ Culture, 64*, 185–219. https://doi.org/10.1023/A:1010623203554

Puripunyavanich, V., Maikaeo, L., Limtiyayothin, M., & Orpong, P. (2022). New Frontier of Plant Breeding Using Gamma Irradiation and Biotechnology. IntechOpen. doi: 10.5772/intechopen.104667

Raigon, M. D., Prohens, J., Muñoz-Falcónm, J. E. & Nuez, F. (2008). Comparison of eggplant landraces and commercial varieties for fruit content of phenolics, minerals, dry matter and protein. *Journal of Food Composition & Analysis*, *21*(5), 370–376. https://doi.org/10.1016/j.jfca.2008.03.006

Rivera, C. M., Rouphael, Y., Cardarelli, M. & Colla, G. (2007). A simple and accurate equation for estimating individual leaf area of eggplant from linear measurements. *European Journal of Horticultural Sci*ence, *72*(5), 228–230. https://www.pubhort.org/ejhs/2007/file_449535.pdf

Rozman, L. (2014). The effect of gamma radiation on seed germination of barley (*Hordeum vulgare* L.). *Acta Agriculturae Slovenica, 103*(2), 307–311. https://doi.org/10.14720/aas.2014.103.2.15

Saibari, I., Barrijal, S., Mouhib, M., Belkadi, N. & Hamim, A. (2023) Gamma irradiation-induced genetic variability and its effects on the phenotypic and agronomic traits of groundnut (*Arachis hypogaea* L.). *Frontiers in Genetics, 14,* 1124632. https://doi.org/10.3389/fgene.2023.1124632

Singh, A. K., Singh, M., Singh, A. K., Singh, R, Kumar, S. & Kalloo, G. (2006). Genetic diversity within the genus *Solanum* (Solanaceae) as revealed by RAPD markers. *Current Science, 90*(5), 711–716. https://www.currentscience.ac.in/Volumes/90/05/0711.pdf

Smith, A. M. & McCallcum, E. S. R. (1956). The determination of calcium and magnesium in plant material with disodium ethylenediaminetetra-acetate. *Analyst*, *81*, 160–163. https://doi.org/10.1039/AN9568100160

Suparno, S. (2018). Characterization of phenotypic diversity of eggplant (*Solanum melongena* L.) result of gamma radiation irradiation on growth and production. *International Journal of Applied Environmental Sciences*, *13*(3), 275–286. https://www.ripublication.com/ijaes18/ijaesv13n3_04.pdf

Ulukapi, K., Ozdemir, B., & Onus, A. N. (2015). Determination of proper gamma radiation dose in mutation breeding in eggplant (*Solanum melongena* L.). In: *Advances in Environmental and Agricultural Science.* Proceedings of the 4th International Conference on Energy Systems, Environment, Entrepreneurship and Innovation. Athens: WSEAS Press.

VSN International (2013). *GenStat for Windows* 16th Edition. VSN International, Hemel Hempstead, UK.

Zanzibar, M. & Sudrajat, D. (2016). Effect of gamma irradiation on seed germination, storage, and seedling growth of *Magnolia champaca* L. *Indonesian Journal of Forestry Research*, *3*, 95–106. https://doi.org/10.20886/ijfr.2016.3.2.95-106

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Relationship between laboratory and field assessments of common bean (*Phaseolus vulgaris* L.) seed quality indicators

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Relationship between laboratory and field assessments of common bean (*Phaseolus vulgaris* L.) seed quality indicators

Abstract: The objective of this study was to extend the measure of seed quality beyond seed germination using three common bean (*Phaseolus vulgaris*. L) cultivars. Under laboratory conditions, total seed germination was included in calculation of other seed performance measures, mean germination rate and germination vigour index. These parameters were used to produce a new parameter, total potential value for germination. The laboratory measures were duplicated under field conditions over two seasons to produce comparable data for seedling emergence, mean emergence rate and emergence vigour index. Consequently, total potential value for emergence was derived. The crop was grown under field conditions at three seeding rates (177 000 plants ha^{-1}, 150 000 plants ha^{-1} and 115 000 plants ha^{-1}). Prediction of seed performance under field conditions was extended by measuring plant size from the first trifoliate to initiation of reproductive stage. During this period, new measures comparable to those of laboratory seed vigour and emergence vigour were derived on the basis of vegetative growth vigour, resulting in total potential value of plant growth. The study revealed that germination and plant growth can be correlated using vigour indices.

Key words: emergence, germination, growth index, seed vigour

Razmerje med laboratorijskimi in poljskimi indikatorji kakovosti semen navadnega fižola (*Phaseolus vulgaris* L.)

Izvleček: Namen raziskave je bil določiti poleg kalitve še dodatne kakovostne indikatorje semen treh sort navadnega fižola (*Phaseolus vulgaris*. L). V laboratorijskih razmerah je bila celokupna kalivost semen vključena v izračun dodatnih meril določanja kakovosti semen kot sta poprečna kalivost in indeks kalitvenega vigorja. Ti parametri so bili uporabljeni za izdelavo novega parametra, imenovanega celukopni potencial kalitve. Laboratorijski postopki so bili podvojeni v razmerah poljskega poskusa v dveh rastnih sezonah za pridobitev primerljivih podatkov za vznik kalic, poprečno vrednost vznika in indeks vigorja vznika. Iz teh podatkov je bila izračunana poprečna celokupne vrednost vznika. Posevek je rastel v treh gostotah (177 000 rastlin ha^{-1}, 150 000 rastlin ha^{-1} in 115 000 rastlin ha^{-1}). Napoved uspešnosti rasti v poljskih razmerah je bila narejena na meritvah velikosti rastlin of prvega trojnatega lista do začetka reproduktivne faze razvoja. V tem obdobju so bila pridobljena merila za vigor rasti in celokupno potencialno vrednost rasti podobna tistim v laboratoriju, ki so določala vigor semen in vznika. Raziskava je pokazala, da bi kalitev in rast rastlin lahko korelirali z uporabo indeksov vigorja.

Ključne besede: vznik, kalitev, indeks rasti, vigor semen

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1 INTRODUCTION

Seed quality is an important determinant of plant genetic material performance under a wide range of environmental conditions. Its assessment can be done to fit the purpose of the researcher in the laboratory where quick physiological responses can be adequate. However, for purposes of commercial crop production and long-term genetic preservation, it is necessary to use a reliable method whose results can be interpreted meaningfully and linked to crop or germplasm performance to protect the plant breeder, the seed market, and the farmer (Bishaw & Turner, 2008; Fajardo et al., 2010; Francki et al., 2021). Seed germination has been tested and accepted as a reliable method to test seed quality. To accommodate its limitations, which can be linked to the effects of environment e.g., temperature, moisture as well as pre-and post-harvest growth and management conditions (Louwaars & Manicad, 2022), international standards accept seminal root protrusion as a baseline indicator of quality in the context of laboratory seed germination.

However, good seed quality is expected to provide a significant genotypic contribution of crop resilience to environmental conditions associated with soil, weather and management conditions of the farm. Seed germination is the basic measure of seed quality recognised by scientists and producers. Previous studies have shown that seed germination response can be expanded to determine other laboratory related parameters, mainly seed vigour, which is based on germination rate and seedling size (Farshid et al., 2019; Hassani et al., 2019). Hence, germination and vigour are commonly used together for quality determination because they are linked. However, seed germination can be used independently to recommend crop potential performance under field conditions (Beveridge, 2020). For example, it is estimated that rapid seminal root protrusion under laboratory conditions must be a minimum of 90 % for it to be considered for optimum production, but 100 % seed germination is required (Allen & Meyer 1990, 1998; Rajendndra, 2023). However, there is no conclusive evidence that seed germination parameters are always linked to crop establishment, growth and final yield (Ellis, 1992). It is generally accepted that relating seed quality directly with plant performance parameters is difficult to achieve. This relationship may be implicitly indicated by special definition of vigour. Previous studies have indicated that this relationship can be shown in theory. It was suggested that seed germination, vigour and size are three aspects of quality that may indirectly influence percentage emergence and time from sowing to emergence (Ellis, 1992). These factors may implicitly influence yield by altering plant population density, spatial arrangement, and crop performance. Seed vigour has become a reliable seed quality measure to confirm results of seed quality, including genetics, for both cultivated and other plant species (Priyanka et al., 2019). Previous studies have shown that plant population affects growth and yield (Ihsanullah et al., 2002). In view of rapidly changing environmental rigours for crop production, due to climate change, it is important to trace and relate seed quality aspects from the laboratory to a wide range during crop growth and development (Akinci et al. 2008; Singh, 2014). Hence, the objective of this study was to provide a practical method of explaining the concept of seed quality based on laboratory and field-based methods in order to produce new indices that have not been shown in seed science studies before.

2 MATERIALS AND METHODS

2.1 LABORATORY SEED QUALITY DETERMINATION

Fresh seeds of three common bean cultivars, Ukulinga, Gadra and Mthatha were donated by Pro-seed cc (https://www.africanadvice.com) from a plant breeding stock. Seed germination percent (G), shown as seminal root protrusion, was determined according to International Seed Testing Association guidelines (ISTA, 2013) for a total period of seven (7) days. The paper towel method was used at 25 °C and replicated four times, with 25 seeds per replication. In addition, mean germination rate (MGR) and germination vigour index (GVI) were determined according to modification of Thanuja et al. (2019).

For mean germination rate:

$$MGR = (\Sigma\, D\, n)/ (\Sigma\, n) \qquad \text{(Equation 1)}$$

Where, MGR is mean germination rate, D is the number of days from the beginning of germination, and n is the number of seeds that have germinated on day D. This value indicates the average rate of seed germination as indicated by seminal root protrusion.

For germination vigour index (GVI):

$$GVI = G1/N1 + G2/N2 + \ldots + Gn/Nn \qquad \text{(Equation 2)}$$

Where, G1, G2…Gn = number of germinated seeds in the 1st, 2nd… last count (n), and N1, N2…Nn = number of germination days at the 1st, 2nd… last count (n). This value indicates the rate of germination daily. Laboratory seed quality was taken to have different aspects from minimum (G), moderate (MGR) and high (GVI)

indicator, respectively. The reason for this was that the value of germination is incrementally improved by considering the rate (MGR) and then the rate combined with potential impression of physiological factors (Taiz et al., 2018, Takahashi et al. 2018) that affect robustness (GVI). To complete that seed quality view of aspects, the study calculated a new parameter, total potential value for germination (TPVg).

$$TPVg = GVI/N \quad \text{(Equation 3)}$$

Where, GVI is germination vigour index (see equation 2 above) and N is the total germination period (7 days). This value indicates the number of seeds in relation to vigour.

2.2 FIELD TRIAL FOR SEED QUALITY DETERMINATION AND CROP GROWTH

A field trial was undertaken (29°37'45 S 30° 24' 17" E) and repeated during the normal planting season. The experimental design was a split-plot in a randomised complete block, replicated three times. There were two factors used, namely three intermediate growth cultivars (Ukulinga, Gadra and Mthatha) and three plant densities [high (177 000 plants/ha), medium (150 000 plants/ha) and low (115 000 plants/ha)]. The variables were emergence and above ground plant size (mm). Prior to planting, soil analysis was performed to determine suitable fertiliser application for common bean (Liebenberg, 2010).

2.3 SEED QUALITY DETERMINATION UNDER FIELD CONDITIONS

Seedling emergence was monitored daily for a period of seven (7) days from planting. Total emergence percent (E), mean emergence rate (MER), daily rate of emergence EVI and total potential value for seedling emergence (TPVe) were determined using the same formulae as for laboratory seed quality.

2.4 PLANT GROWTH PARAMETERS

From emergence, non-destructive evaluation of plant growth (cm) was determined. An average of five randomly selected plants per plot (two middle rows of a 2 m^2 area used as one replicate) was used to measure plant growth weekly, between VE (emergence) and R1 (initiation of flowering) (Rahman et al., 2011). Accordingly, plant growth (P) was monitored for seven (7) growth stages from the first trifoliate (V1) to initiation of reproductive stage (R1). Plant size (MPI) and plant growth vigour index (PVI) were used to calculate potential value for plant growth (TPVp) using the same formulae as for laboratory seed and emergence quality.

2.5 STATISTICAL ANALYSIS

Data were subjected to analysis of variance using GenStat® Version 18 (VSN International, United Kingdom) at the 5 % probability level ($p \leq 0.05$). Duncan's Multiple Range test was used to compare means. There were no significant differences between seasons. Therefore, no data to compare the two growth seasons are shown.

3 RESULTS AND DISCUSSION

Cultivar differences with respect to germination were significant (p = 0.03), with 'Ukulinga' showing complete germination by the fourth day. 'Gadra' was better than 'Mthatha', but both of these cultivars did not rich 100 % germination. The trend of differences between cultivars was consistent throughout the germination period (Table 1). With respect to germination rate, differences between cultivars diminished over time, so that by the fourth day there were no significant differences (Table 1). High germination was associated with a steady germination rate, whereas delayed germination continued to have a high germination rate until the end of incubation period (Table 1). Cultivar differences with respect to emergence followed a similar trend to that of germination ($p = 0.01$). 'Ukulinga' showed complete emergence five days after planting, but all cultivars emerged completely seven days after planting (Table 1). Rate of emergence also showed a similar trend to that of germination (Table 1). Seeding rate had no effect on emergence.

For all cultivars germination was highly significantly correlated with germination rate index (Figure 1A). Emergence was highly significantly correlated with emergence rate index (Figure 1B). Plant growth was highly significantly correlated with plant growth index (Figure 1C).

Plant growth from the first plant trifoliate to initial reproduction stage showed no significant differences between seeding rates and cultivars, overall (Figure 2).
When the total potential seed value for germination was compared with that for emergence and plant growth to flowering, it was clear that both emergence and plant growth are highly correlated with seed quality (Figure 3).

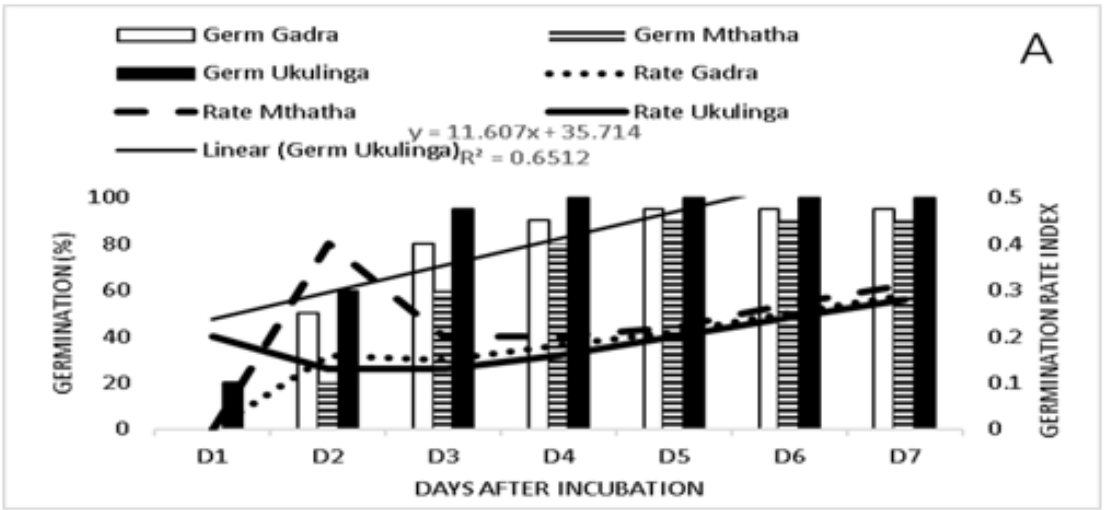
Table 1: Comparison common bean cultivars (Gadra, Mthatha and Ukulinga) with respect to germination (G) and emergence (E) as well as their respective daily rates (MGR and MER) over a period of seven days (Day 1 to Day 7) of laboratory incubation and field planting, respectively. Values sharing the same letters are not significantly different ($p \leq 0.05$)

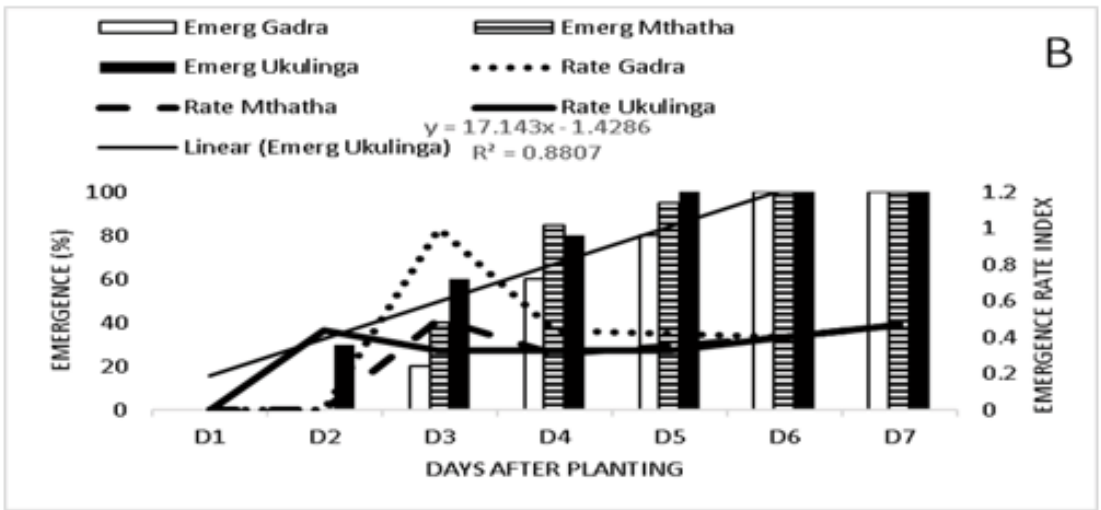
Variable	Cultivar	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
G	Gadra	0a	50b	80b	90b	95b	95b	95b
	Mthatha	0a	20a	60a	80a	90a	90a	90a
	Ukulinga	20b	60c	95c	100c	100c	100c	100c
MGR	Gadra	0a	0.16a	0.15a	0.18ab	0.21a	0.25a	0.29a
	Mthatha	0a	0.4b	0.2b	0.2b	0.22a	0.27a	0.31a
	Ukulinga	0.2b	0.13a	0.13a	0.16a	0.2a	0.24a	0.28a
Variable		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
E	Gadra	0a	0a	20a	60a	80a	100a	100a
	Mthatha	0a	0a	40b	85c	95b	100a	100a
	Ukulinga	0a	30b	60c	80b	100c	100a	100a
MER	Gadra	0a	0a	1c	0.44b	0.42b	0.4a	0.47a
	Mthatha	0a	0a	0.5b	0.31a	0.35a	0.4a	0.47a
	Ukulinga	0a	0.44b	0.33a	0.33a	0.33a	0.4a	0.47a

Seed germination has been a reliable measure of seed quality in science and for agricultural production regardless of system (Sako et al., 2001). Both controlled environment nursery production and widely variable field conditions rely mainly on seed germination percent as the primary indicator of seed quality (Rahman et al., 2011). Over time, science has developed other measures of seed quality to test seed response to factors associated with harsh conditions for growth, including imbibition, high mineral content and suboptimal temperature and water conditions (Priyanka et al., 2019). This led to seed vigour being an additional seed quality measure closely associated with seed germination (ISTA, 2013). The usefulness of other seed quality measures associated with germination is generally limited to laboratory experiments and decisions for micro-level interpretation of seed quality (Ellis, 1992; Farshid et al., 2019). This study attempted to expand the meaning of seed quality beyond relying on seed germination as the most important measure (Beveridge, 2020; Kildisheva et al., 2019). Advantage was taken of seed vigour determined by seed germination rate, which can also be linked to seedling size in the laboratory (Grafton et al., 1988). Seed germination was found to be directly linked to emergence, but it does not guarantee a perfect match in that emergence can be overestimated if one uses germination alone (Ambika et al., 2014). However, it was found useful to continue to use both germination (a clear indicator of seed viability in terms of seminal root protrusion under favourable conditions) and emergence (a clear indicator of the ability of seed to produce a seedling under optimum soil and climate conditions). A comparison of data in Table 1 with Figures 1A and 1 B clearly confirms this argument. Further, when plant size was related to growth rate (Figure 1C), there was a consistent comparison with what happened when germination and emergence were similarity related to what would be a direct physiological response to them, mean germination rate and mean emergence rate, respectively. This comparison allowed the basic measure of seed quality, germination to be indirectly linked to measures of plant performance under field conditions, regardless of cultivar or seeding rate (Figure 2).

Further consideration of all known laboratory seed quality indicators, germination, rate of germination and germination vigour index led to a new index of seed quality, total potential value for germination (TPVg). This index was directly comparable to those for field emergence (TPVe) (Figure 3). From these results, it can be assumed that the direct relationship between laboratory seed quality index with plant performance in the field is possible.

The study showed that it is possible to replicate seed vigour measures during the early stages of crop establishment. This was shown by the significant similarity of mean germination test results to mean emergence test, as well as germination vigour index and emergence vigour index. The reliability of these new comparisons between simple measures of seed quality (germination and emergence) led to the interest in producing more indirect measures of seed value that may be implicitly related to seed quality beyond germination and emergence. This study expanded the concept of emergence to crop establishment as well as growth and development under field





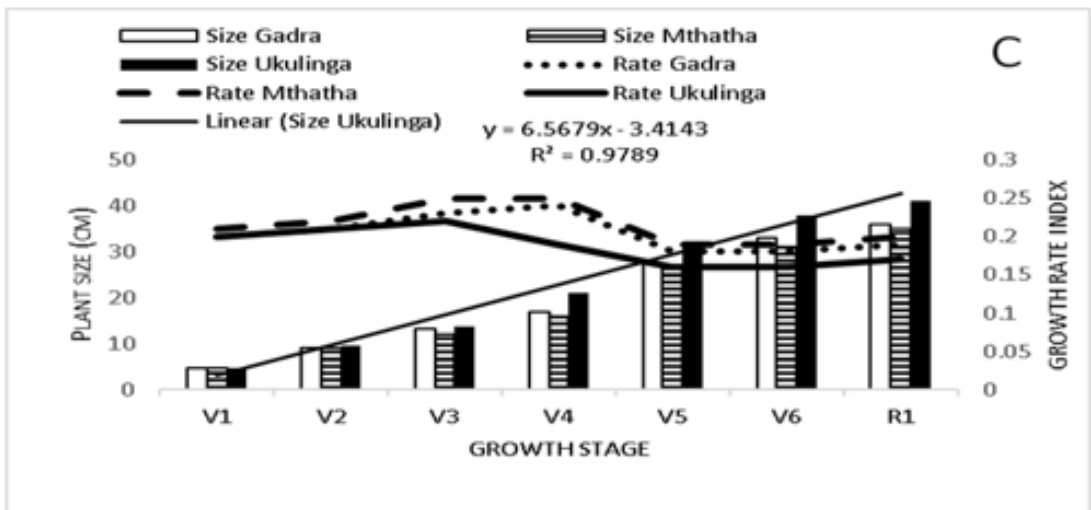


Figure 1: Comparison of germination (A) (Note: Germ = germination; Rate = germination rate), emergence (B) and plant size (C) with rates of seed germination, seedling emergence and plant growth, respectively. Germination and emergence occurred over a period of seven days (D1 to D7). Growth occurred over seven stages from the first trifoliate (V1) to initial reproduction (R1). Correlation across all parameters was indicated using cultivar Ukulinga mean values to represent the general trend

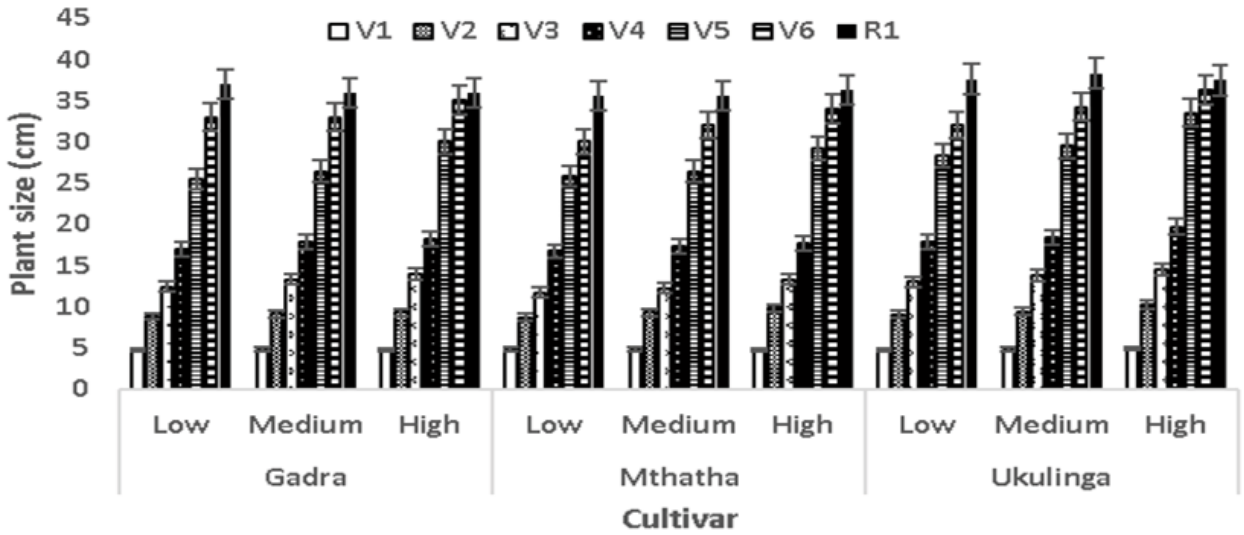


Figure 2: Plant size of three common bean cultivars (Gadra, Mthatha and Ukulinga) from first trifoliate (V1) to initiation of reproductive phase (R1) under different seeding rates (Low = 115 000 plants ha^{-1}, Medium = 150 000 plants ha^{-1} and High = 177 000 plants ha^{-1})

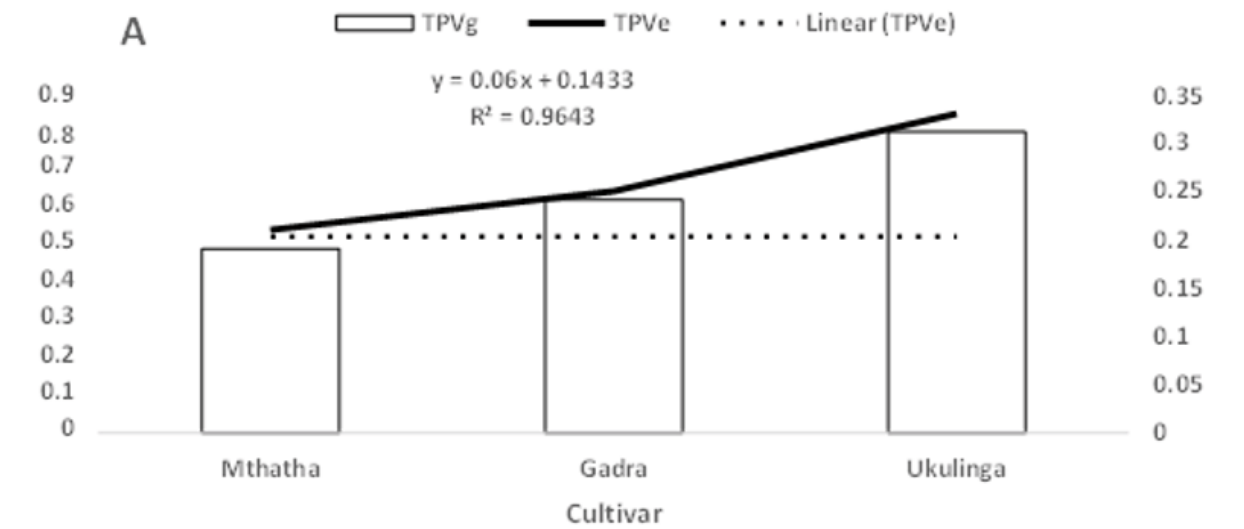


Figure 3: Relationship of total potential value of seed germination (TPVg; y-axis, 0 to 0.35) with those of emergence (TPVe; y-axis, 0 to 0.9) for three common bean cultivars

conditions using different cultivars and seeding rates (Grafton et al., 1988). Hence, the potential value of seed performance beyond germination was shown when a new measure of total potential value under germination (TPVg) was comparable to that derived for emergence (TPVe) and plant growth (TPVp). Both indicators were comparable to germination and emergence performances of the crop. Further, the study was able to show that following the monitoring of vegetative growth stages, a new index of perceived seed potential can be determined based on crop growth vigour sub-indices. The vegetative growth rate index and growth range index were derived simply from measurement of plant size in the field. In combination, they were useable to predict growth potential index.

4 CONCLUSION

The results of this study confirm the parameters for seed quality determination which are already accepted by the International Institute for Seed Testing Association

(ISSTA) based on the general parameters of germination and vigour. The combination of existing parameters using a simple model suggests that there may be three options to relate seed quality to potential seed performance. These indices have different values, but TPVe and TPVg are not very different in terms of linkage to basic seed quality, germination and emergence. The index associated with field growth and development indicated greater differences between cultivars. It may be less effective than the indices done closer to early stages of biological activity for seed. This study showed that there is consistency of the relationship between laboratory seed quality determination, seedling establishment, shown as emergence, and plant growth following emergence. It is a simple study that was designed to minimise variation in that related common bean genotypes and one site were used under a limited range of management conditions. The relative control of variation is necessary in experiments where new findings are a focus, instead of testing existing knowledge. The study concludes that there is a potential to expand seed quality determination beyond the simple germination and vigour indices under laboratory conditions. Total value potential (TPV) under laboratory and field conditions could be a new way of increasing knowledge about seed vigour. Expansion of this seed quality determinant to link it to field performance of the crop was encouraging. More research is needed to confirm the results under a wide range of genotypes and environmental or management conditions. Future studies should also determine the relationship between relevant crop performances that can be directly linked to interaction of plant physiology and yield.

5 REFERENCES

Akinci C., Yildirim M. & Bahar B. (2008). The effect of seed size on emergence and yield of durum wheat. *Journal of Food Agriculture and Environment, 6*(6), 234-237.

Allen P.S. & Meyer, S.E. (1990). Temperature requirements for reed germination of three *Penstemon* species. *HortScience, 25,* 191–193. https://doi.org/10.21273/HORTSCI.25.2.191

Allen P.S. & Meyer, S.E. (1998). Ecological aspects of seed dormancy loss. *Seed Science Research, 8,* 183–192. https://doi.org/10.1017/S0960258500004098

Ambika S., Manonmani V. & Somasundaram, G. (2014). Review on effect of seed size on seedling vigour and seed yield. *Research Journal of Seed Science, 7,* 31-38. https://doi.org/10.3923/rjss.2014.31.38

Beveridge F.C. (2020). Seed enhancement technologies to improve germination and emergence of Australian native Poaceae. *Seed Science Research, 30*(4), *293-303*. https://doi.org/10.1017/S0960258520000276

Bishaw Z. & Turner, M. (2008). Linking participatory plant breeding to the seed supply system. *Euphytica, 163,* 31- 44. https://doi.org/10.1007/s10681-007-9572-6

Ellis R. (1992). Seed and seedling vigour in relation to growth and yield. *Plant Growth Regulation, 11*(3), 249-255. https://doi.org/10.1007/BF00024563

Fajardo J., Lutaladio N., Larinde M., Rosell, C., Roca W. & Chujoy, E. (2010). Quality declared planting material: protocols and standards for vegetatively propagated crops. FAO, Rome (Italia). https://www.sweetpotatoknowledge.org/files/quality-declared-planting-material-protocols-and-standards-for-vegetatively-propagated-crops/

Francki MG, Stainer GS, Walker E, Rebetzke GJ, Stefanova KT & French RJ (2021). Phenotypic evaluation and genetic analysis of seedling emergence in a global collection of wheat genotypes (*Triticum aestivum* L.) under limited water availability. *Frontiers in. Plant Science, 12*, 796176. https://doi.org/10.3389/fpls.2021.796176

Grafton K., Schneiter, A. & Nagle, B. (1988). Row spacing, plant population, and genotype× row spacing interaction effects on yield and yield components of dry bean. *Agronomy, 80*(4), 631-634. https://doi.org/10.2134/agronj1988.00021962008000040017x

Hassani F., Zare L. & Khaledi, N. (2019). Evaluation of germination and vigor indices associated with *Fusarum*-infected seeds in pre-basic seeds wheat fields. *Journal of Plant Protection Research, 29*(1), 69-85. https://doi.org/10.24425/jppr.2019.126037

Ihsanullah A.J., Taj F.H., Amanulla J. & Ahmad I. (2002). Effect of sowing dates on yield and yield components of Mashbean varieties. *Asian Journal of Plant Science, 1*(6), 622-624. https://doi.org/10.3923/ajps.2002.622.624

ISTA (International Seed Testing Association) (2013). The germination test. In: *International Rules for Seed Testing*. Bassersdorf, Switzerland, 56 pp. https://doi.org/10.15258/istarules.2017.05

Kildisheva O.A., Erickson T.E., Madsen M.D., Nixon K.W. & Merritt D.J. (2019). Seed germination and dormancy traits of forbs and shrubs important for restoration of North American dryland ecosystems. *Plant Biology, 21,* 458-469. https://doi.org/10.1111/plb.12892

Liebenberg A.J. (2010). *Drybean production*. Department of Agriculture, Republic of South Africa. https://www.nda.agric.za/docs/drybean/drybean.htm

Louwaars N.P. & Manicad, G. (2022). Seeds Systems Resilience – An Overview. *Seeds, 1(4): 340-356* https://doi.org/10.3390/seeds1040028

Priyanka N., Geetha N., Mansour G. & Perumal, V. (2019). Role of engineered zinc and copper oxide nanoparticles in promoting plant growth and yield: Present status and future prospects. *Advances in Phytonanotechnology,* 183-201. https://doi.org/10.1016/B978-0-12-815322-2.00007-9

Rahman M., Hossain M., & Bell R. (2011). Plant density effects on growth, yield and yield components of two soybean varieties under equidistant planting arrangement. *Asian Journal of Plant Science, 10*(5), 278-286. https://doi.org/10.3923/ajps.2011.278.286

Rajendra Prasad, S. (2023). Testing Seed for Quality. In: Dadlani, M., Yadava, D.K. (eds) Seed *Science and Technology*.

Springer, Singapore. https://doi.org/10.1007/978-981-19-5888-5_13

Sako Y., McDonald M.B., Fujimura K., Evans A.F. & Bennett, M.A. (2001). A system for automated seed vigour assessment. *Seed Science and Technology 29(3):625-636.* https://www.researchgate.net/publication/279571809_A_system_for_automated_seed_vigour_assessment

Singh, M., Bisht, I.S. & Dutta, M. eds. (2014). *Broadening the genetic base of grain legumes*. Springer. https://doi.org/10.1007/978-81-322-2023-7

Taiz L., Zeiger E., Møller I.M. & Murphy A. (2018). *Fundamentals of Plant Physiology*. ISBN: 9781605357904. 561 pp. Oxford University Press.

Takahashi F., Suzuki T., Osakabe Y., Betsuyaku S., Kondo Y., & Dohmae N. (2018). A small peptide modulates stomatal control via abscisic acid in long-distance signaling. *Nature, 556(*7700), 235. https://doi.org/10.1038/s41586-018-0009-2

Thanuja P.C., Sadashiv N. & Shashikala S.K. (2019). Effect of pre-sowing seed treatments on seed germination and seedling growth in Rakta Chandana (*Pterocarpus santalinus* L.): An endangered medicinal plant. *International Journal of Chemical Studies, 7*(3), 1577-1580.

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Do mutations modifying the leaf area (*nr3*) and the number of potential seeds (*dfc*) influence photosynthetic gas exchange characteristics in common buckwheat *Fagopyrum esculentum* Moench?

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Do mutations modifying the leaf area (*nr3*) and the number of potential seeds (*dfc*) influence photosynthetic gas exchange characteristics in common buckwheat *Fagopyrum esculentum* Moench?

Abstract: Contemporary buckwheat breeding in Russia is based mainly on a Mendelian mutation *det*. Some additional mutations are being considered for inclusion in buckwheat breeding programs. Among them are the *nr3* (narrow leaf 3) and *dfc* (determinate floret cluster). We evaluated the effects of the mutations on both the characteristics of photosynthetic gas exchange and the number of seeds per plant. The *nr3* reduces the leaf surface area by 1.4 times. The mutant plants show some compensatory increase in photosynthesis rate, which, however, is not enough to reach the level of the source ability as in the wild type since the number of seeds per plant is significantly decreased. The possibility of using this mutation in buckwheat breeding depends on the accumulation of modifiers that increase either leaf size or photosynthesis rate. The reduced number of flowers of the *dfs* mutation is compensated by an increase in flower fertility, and the number of seeds per plant does not change compared to the wild type. It explains the absence of differences between the *dfs* and wild type in terms of the photosynthesis rate. This experiment did not reveal any problems for using the *dfc* mutation in breeding. In general, the results of the work support the photosynthesis rate in buckwheat is regulated based on the source-sink ratio.

Key words: common buckwheat, photosynthesis, leaf area, source-sink ratio, breeding

Ali mutaciji, ki spreminajata listno površino (*nr3*) in število potencialnih semen (*dfc*) vplivata na značilnosti fotosintezne izmenjave plinov pri navadni ajdi (*Fagopyrum esculentum* Moench)?

Izvleček: Sodobno žlahtnjenje ajde v Rusiji temelji v glavnem na Mendlovi mutaciji *det* a so bile za vključitev v žlahtniteljske programme predlagane še dodatne mutacije. Med njimi sta mutaciji *nr3* (ozki listi 3) in *dfc* (determinantno socvetje). V raziskavi smo ovrednotili vplive obeh mutacij na značilnosti fotosintezne izmenjave plinov in na število semen na rastlino. Mutacija *nr3* zmanjša listno površino za 1,4 krat. Mutantne rastline kažejo nekatere kompenzacijske mehanizme v velikosti fotosinteze, ki pa ne zadoščajo za doseganje ravni pri divjem tipu, kar kaže značilno zmajšanje števila semen na rastlino. Možnost uporabe te mutacije v žlahtniteljskih programih ajde je odvisna na kopičenju sprememb, ki povečujejo listno površino ali velikost fotosinteze. Zmanjšano število cvetov pri mutaciji *dfs* je kompenzirano s povečanjem plodnosti cvetov, pri čemer število semen na rastlino ni spremenjeno v primerjavi z divjim tipom. To razloži tudi odsotnost razlike v velikosti fotosinteze med *dfs* in divjim tipom. V poskusu tudi ni bilo ugotovljenih nobenih problemov v uporabi *fc* mutacije pri žlahtnenju. Na splošno rezultati raziskave kažejo, da je velikost fotosinteze pri navadni ajdi uravnavana z razmerjem vir : ponor.

Ključne besede: navadna ajda, fotosinteza, listna površina, razmerje vir-ponor, žlahtnenje

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1 INTRODUCTION

Common buckwheat, *Fagopyrum esculentum* Moench, is a grain and groats crop widespread throughout Eurasia (Kreft et al., 2003; Fesenko et al., 2006). Since the 1960s, buckwheat breeding in Russia has been based on mutations that are sometimes accumulated in populations not affected by scientifically based selection, i.e. approved by natural selection (Fesenko, 1983; Fesenko et al., 2006). For example, *det*-mutation causing determinate growth habit (Fesenko, 1968; Ohnishi, 1990) is a core of most contemporary Russian buckwheat varieties (Fesenko & Fesenko, 2019). The use of the *det* mutation made a local green revolution since the mass distribution of the determinate varieties doubled the average yield of buckwheat in Russia (Fesenko & Fesenko, 2019). An assessment of the groups of both determinate and indeterminate buckwheat varieties according to the intensity of photosynthesis revealed the advantage of the determinate ones at a stage of mass seed filling (Amelin et al., 2020). However, analysis of the effect of *det*-mutation *per se* using a segregated hybrid population did not reveal a significant difference with the wild type, i.e. indeterminate one. On the one hand, it clarifies the role of the *det*-mutation in control of the photosynthesis intensity is not entirely clear. But it is evident that on its background, some other complexes of genes are formed, including ones determining the photosynthesis characteristics (Amelin et al., 2020).

At present, some additional mutations are being considered for inclusion in buckwheat breeding programs. Two of them are *dfc* and *nr3* mutations (Figure 1). One of the significant aspects of the effects of the mutations on plants in the context of their breeding application is their influence on the characteristics of photosynthesis. The *nr3* reduces the leaves area surface, and is considered as the basis for creating varieties with reduced self-shading. At the same time, reducing leaf area changes the source potential (using the terminology of photosynthesis researchers). The *dfc* mutation drastically, by 4-5 times, reduces the number of flowers in an inflorescence (Fesenko et al., 2010). It can change the sink potential (i.e. demand for assimilates), firstly by reducing the assimilates demand for flower production, and secondly by reducing the fruiting potential. However, the latter can be leveled by increasing the fertility of flowers. So, the mutations can affect the source-sink ratio, which is the key to regulating the intensity of photosynthesis (Paul et al., 2001; McCormick et al., 2008; Katoh et al., 2015).

An objective of this work was to evaluate both the characteristics of photosynthetic gas exchange (which reveal the source ability) and the number of seeds per plant (which shows the sinking ability) of *dfc* and *nr3* mutants vs wild type.

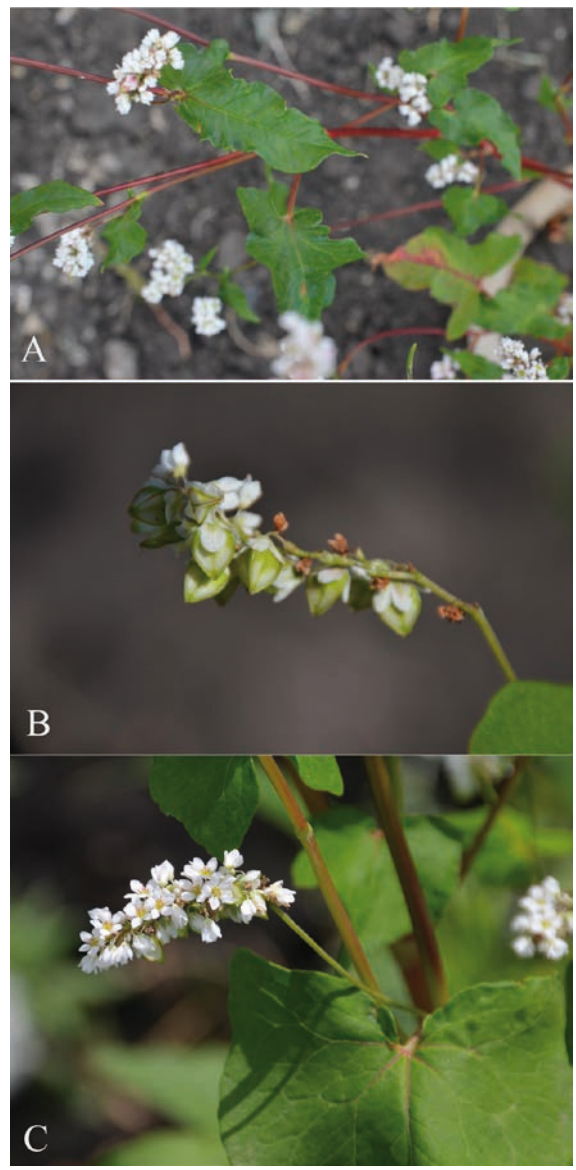

Figure 1: A) Mutant *nr3*; B) Mutant *dfc* at a stage of almost mature seeds; C) Wild type (both normal leaves and normal flower number)

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

Mutants analized were next:

- Mutant *dfs* (determinate floret cluster) leads to a sharp, 4-5 times reduction in the number of flowers in the inflorescence. The dfc plants participating in crosses were determinate (genotype *det det*).

- Mutant *nr3* (narrow leaf 3) causes a change in leaf geometry due to a decrease in its width and significantly reduces the leaves' surface area of a plant. It reduces the plant's photosynthetic potential in morphological terms. The nr3 plants participating in the work were determinate (genotype *det det*).

Both *dfc* and *nr3* mutants were isolated in the lab of buckwheat breeding, Federal Scientific Center of Legumes and Groats Crops.

To level the possible influence of some unidentified genes on the parameters of photosynthetic gas exchange F_2 hybrids 'mutant × wild type' were used for the analyses.

As a wild type the next varieties were used:

- Dikul, a determinate common buckwheat variety bred in Federal Scientific Center of Legumes and Groats Crops. The variety was registered in 1999.

- Bogatyr, an indeterminate common buckwheat variety bred on Shatilovskaya Experimental Station (Orel region, Russia). It is the first commercial buckwheat variety in Russia which was registered in 1938.

F_2 hybrids analized were next:

(1) '*dfc dfc*/*det det* × Dikul'.

(2) '*nr3 nr3*/*det det* × Dikul'.

(3) '*nr3 nr3* / *det det* × Bogatyr'.

Since Dikul is a determinate variety, the F_2 hybrids (1) and (2) manifest segregation only according to *dfc* and *nr3* alterations, respectively. F_2 hybrids with indeterminate variety Bogatyr shown expected segregation comprising four phenotypical classes (Table 1).

2.2 EXPERIMENTAL APPROACHES

The photosynthesis and transpiration intensities were evaluated on intact plants in real-time regime with a portable gas analyzer Li–COR – 6400 using the original methodology of the company Li–COR. The WUE (water use efficiency) was calculated for each plant analyzed using the formula *WUE = photosynthesis rate/transpiration intensity*.

The evaluations of photosynthesis and transpiration intensities were conducted in 2017, 2018 and 2021. All experimental plants were labeled and numbered. The measurements within single mutant segregations (*dfc* or *nr3*) were made in order "mutant - wild type - mutant - etc" with alternation on each plant. The measurements within a segregation for the two recessives (*nr3* and *det*) were conducted in order "nr3 (non-det) - det (non-nr3) - wild type (both non-nr3 and non-det) - nr3+det - etc" with regular alternation in such order.

To measure the leaf size of F_2 hybrids 'nr3 × Dikul' with both narrow leaves and normal leaves the largest leaf from each plant was photographed with a scale in 2021. Leaves sizes were measured on the photos using Axio Vision Software. 25 plants of both types were taken randomly.

Sowing dates were June 1in 2017, May 23 in 2018 and May 27 in 2021. Blossom beginning dates were July 6-7 in 2017, June 26-27 in 2018 and July 1-2 in 2021. The dates of photosynthesis assessment (which are mentioned in the Table 1) fell on the period of mass filling of seeds.

The number of filled grains per plant was evaluated on August 15 in 2017, on August 14 in 2018, and on August 10 in 2021.

The significance of differences was assessed using ANOVA (Software Statistica 7).

3 RESULTS

3.1 ANALYSES OF PHOTOSYNTHETIC GAS EXCHANGE CHARACTERISTICS AND SEED PRODUCTIVITY OF INDIVIDUAL PLANTS WITHIN POPULATIONS SEGREGATED ACCORDING TO *DFC*, *NR3*, AND *NR3+DET* ALTERATIONS

3.1.1 dfc-mutant

The experiment revealed no differences between the mutant and non-mutant plants in photosynthesis and transpiration intensities (Table 1). Also, there were no significant differences in the number of seeds per plant between the groups of plants with normal (wt) and reduced (dfc) number of flowers. Apparently, it points out that at this ontogenesis stage, the demand for assimilates is mainly formed by developing seeds.

This mutation is considered the basis for creating varieties both with more simultaneous maturation and more early ripening. According to the results of the work *dfc*- mutation does not disturb the system of regulation of physiological processes associated with photosynthesis. It simplifies any application of the mutant for buckwheat breeding.

Table 1: Characteristics of mutant and non-mutant (wt) classes in segregated populations in terms of photosynthetic gas exchange and seed productivity (Mean ± SD)

Hybrid combination (date of analysis)	Phenotype class	N	t (leaf),°C	Photosynthesis (μmol m^{-2} s^{-1})	Transpiration (μmol m^{-2} s^{-1})	Water use efficiency	Seeds per plant
F_2(Dikul × dfc) (July 28, 2017)	wt	42	24.7 ± 0.62	8.32 ± 3.85	5.84 ± 1.73	1.40 ± 0.56	44.87 ± 10.31
	dfc	42		8.69 ± 3.63	6.12 ± 1.92	1.40 ± 0.49	45.80 ± 6.84
One-way ANOVA				NS	NS	NS	NS
F_2(Dikul × nr3) (July 26, 2021)	wt	40	30.6 ± 3.97	8.88 ± 4.44	2.51 ± 1.18	4.06 ± 2.40	51.20 ± 5.14
	nr3	40		11.11 ± 4.93	2.81 ± 1.34	4.76 ± 3.00	46.53 ± 6.82
One-way ANOVA				$p < 0.05$	NS	NS	$p < 0.001$
F_2(Dikul × nr3) (July 12, 2018)	wt	50	24.8 ± 0.63	12.58 ± 4.17	2.14 ± 0.57	6.38 ± 4.42	42.92 ± 4.36
	nr3	50		13.75 ± 4.88	2.16 ± 0.35	6.20 ± 2.71	38.96 ± 5.24
One-way ANOVA				NS	NS	NS	$p < 0.001$
F_2(Bogatyr× nr3) (July 13, 2018)	wt	30	25.0 ± 0.35	11.48 ± 3.84	2.70 ± 1.10	5.13 ± 3.22	35.17 ± 2.96
	nr3	30		10.37 ± 3.43	2.08 ± 0.77	6.03 ± 4.35	35.73 ± 2.77
	det	30		9.73 ± 4.45	2.47 ± 0.73	4.13 ± 2.06	38.03 ± 3.13
	nr3 + det	30		10.38 ± 3.81	2.21 ± 0.88	5.17 ± 2.37	37.23 ± 3.02
Two-way ANOVA	nr3			NS	$p < 0.02$	NS	NS
	det			NS	NS	NS	$p < 0.001$
	nr3 × det			NS	NS	NS	NS

3.1.2 nr3-mutant

This single-gene mutation makes leaves narrow and reduces their area by 1.4 times, 38.4 ± 12.2 vs 27.1 ± 7.4 (mean ± SD). Apparently, it should cause a shift within the "source-sink" balance toward a source deficiency. A priori, there can be two ways to compensate for such a shift. The first is a decrease in the number of seeds, i.e. a decrease in sink strength. The second is an increase in the intensity of photosynthesis per leaf area unit. In the experiments, we observed both types of effects (Table 1).

In two experiments carried out in different years on the same hybrid material, the same patterns were obtained in terms of both the changes in the number of seeds per plant and the gas exchange parameters. In both cases, the number of seeds on narrow-leaved plants was significantly less compared to plants with normal leaves. Photosynthesis rate in both cases was higher for the narrow-leaved plants, on average, and in one of the two experiments, the difference was significant. Water use efficiency (WUE) have tends to slight growth when photosynthesis rate is significantly higher. Thus, this mutation can be considered as a model in which the sink potential is not fully realized due to the source insufficiency.

Since this work shows the *nr3*-mutant has insufficient leaf area resulting in the source deficiency, an essential aspect of its use for breeding commercial varieties should most likely be an increase in leaf size or/and an additional increase in photosynthesis rate due to the selection of some hypothetical modifiers that can be able to compensate the effect of the mutation.

3.1.3 nr3 + det

We have previously reported that determinate varieties manifest higher photosynthesis rates at the seed-filling stage than indeterminate varieties. However, the *det* mutation itself does not affect the gas exchange intensity. An experiment was conducted to evaluate the joint effect of *det* and *nr3* mutations on photosynthesis parameters.

The less number of grains per plant in the experiment compared to other ones in this work is due to the old low-yielding variety with normal leaves and indeterminate growth habit Bogatyr has been used in crosses with determinate plants with narrow leaves (genotype *det det*/*nr3 nr3*). The *det* mutation did not affect the rates of photosynthetic gas exchange and had a certain effect on the number of seeds. However, the difference revealed was at a low level of significance (Table 1). The photosynthesis rate did not differ significantly also between *nr3* mutants and plants with normal leaves. It should be noted that *nr3* homozygotes, both determinate and indeterminate, showed a significantly lower transpiration intensity. However, it could not be considered as a trend since in the experiments with F_2 hybrids 'nr3 × 'Dikul' the differences between mutants and non-mutants in transpiration intensity were not significant (Table 1). Water use efficiency also was not significantly affected by the mutations across all the experiments.

4 DISCUSSION

There are many evidences suggesting the photosynthetic gas exchange intensity is a function of the source-sink interaction. The source potential is not realized at full capacity, and the value of photosynthesis rate per unit of leaf area can be increased if the leaves area of the plant is reduces by any way. Thus, soybean having leaves with smaller surface areas manifests a higher photosynthesis rate per unit of leaf area than those with larger leaves (Sung, Chen, 1989). Similar effect can be observed on a rice mutant *NAL1* with more narrow leaves compared wild type (Takai et al., 2013). In addition, the excision of several leaves from trees of *Eucalyptus globulus* Labill. resulted in the growth of photosynthesis rate in the remained leaves (Eyles et al., 2013).

Since photosynthesis rate usually does not reach the maximum possible values, there are some factors restricting it. Matsuda et al. (2011) discussed the hypothesis for sink-limitation suggesting the photosynthesis rate is limited by the demand for assimilates. This hypothesis was tested on two varieties of tomato and was not fully confirmed (Matsuda et al., 2011).Thus, when several fruits at the early developmental stage were removed from plants, the remaining ones became larger. On the other hand, on intact plants all the fruits were smaller. It suggests rather a lack of source ability in this case. The examples when assimilate demand was increased due to experimental manipulation also are known. So, nitrogen application can provide higher sink strength (Pissolato et al., 2019; Chen et al., 2022). Inoculation with nodule bacteria also resulted in a higher photosynthesis rate which could be explained in two ways: 1) it is able to provide additional nitrogen and 2) growing nodules requires additional assimilates (Kaschuk et al., 2012).

On potatoes it was shown the possibility to increase both source and sink ability using transgenic manipulations: it sufficiently increased the yield of starch in tubers (Jonik et al., 2012). Also, it was revealed the genetically controlled mechanisms influencing the translocation of assimilates toward developing seeds (Phung et al., 2019). In addition, it was hypothesized the buckwheat varieties with determinate growth habit manifest higher pho-

tosynthesis due to optimizing the assimilates logistics (Amelin et al., 2020).

We have analyzed the effect of well-distinguishable morphological mutations of two types on the photosynthetic gas exchange parameters. One of them, the *dfc* mutation, reduces the number of flowers within cyme (i.e., partial inflorescence) by 4-5 times. It can be assumed it forms some tendency to reduce sink potential. However, our experiment with this mutation shows the demand for assimilates is not different between non-mutant and *dfc*-mutant plants. The number of formed seeds on mutant and non-mutant plants also did not differ significantly. Thus, the increasing proportion of flowers setting seeds compensates for reducing flowers number on *dfc*-plants and allows them to form sufficient number of seeds and maintain the demand for assimilates.

Mutation *nr3* reduces leaf area by 1.4 times and increases photosynthesis rate, sometimes significantly. It can be interpreted as compensation for the decrease in leaf area. Also, it allows us to understand that in buckwheat, the physiological processes associated with photosynthesis are not at full capacity, and there are some possibilities to increase its intensity. Since the mutants typically produced fewer seeds compared to the wild type, it can be concluded that the compensatory increase in photosynthesis rate is unsufficient to reach the wild type source levels.

Our experiments to assess the effects of mutations on the intensity of photosynthesis, both presented in this article and previously published, make it clear that buckwheat plants have a particular source limit per unit of leaf area which, however, is usually not reached, i.e. the source ability is not fully realized. It is due to certain limitations in the sink ability, an example of overcoming which, however, exists. This is a significant increase in the photosynthesis intensity within varieties with determinate growth habit at the stage of seed filling. The *det* mutation *per se* does not affect photosynthesis characteristics. Therefore, the higher photosynthesis rate of the determinate varieties is due to the accumulation of some additional genes, the role of which in physiology we do not yet understand (Amelin et al., 2020).

There are no commercial varieties based on the *nr3* and *dfc* mutations yet. If (or when) such varieties appear, it will be possible to evaluate their difference from varieties that do not carry these mutations, and, accordingly, it is possible to obtain additional cases of modifying the regulation of photosynthesis. This is especially true for the *nr3* mutation, which application for buckwheat breeding can be successful only with the accumulation of certain modifiers.

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6 REFERENCES

Amelin, A.V., Fesenko, A.N., Chekalin, E.I., Fesenko, I.N., & Zaikin, V.V. (2020). Higher yielding varieties of common buckwheat (*Fagopyrum esculentum* Moench) with determinate growth habit (single mutation *det*) manifest higher photosynthesis rate at stage of grain filling. *Acta agriculturae Slovenica, 115*, 59-65.https://doi:10.14720/aas.2020.115.1.1316

Chen C.-C., Huang, M.-Y., Lin, K.-H., & Hsueh, M.-T. (2022). The effects of nitrogen application on the growth, photosynthesis, and antioxidant activity of *Amaranthus viridis*. *Photosynthetica, 60*, 420-429. https://doi: 10.32615/ps.2022.034

Eyles, A., Pinkard, E.A., Davies, N.W., Corkrey, R., Churchill, K., O'Grady, A.P., ... Mohammed, C. (2013) Whole-plant- versus leaf-level regulation of photosynthetic responses after partial defoliation in *Eucalyptus globulus* saplings. *Journal of Experimental Botany, 64*, 1625-1636. https://doi:10.1093/jxb/ert017

Fesenko, A.N., Biryukova, O.V., Shipulin, O.A., & Fesenko, I.N. (2010). A new mutation of buckwheat – "*determinant floret cluster*". In *Proceedings of 11th International Symposium on Buckwheat* (pp. 386-388). Orel, Russia.

Fesenko, A.N., & Fesenko, I.N. (2019). Buckwheat breeding and production in Russia during the past 100 years. *Proceedings on applied botany, genetics and breeding 180*, 113-117. https://doi.org/10.30901/2227-8834-2019-1-113-117 (in Russian with English summary)

Fesenko, N.V. (1968). A genetic factor responsible for the determinant type of plants in buckwheat. *Genetika, 4*, 165-166. (in Russian)

Fesenko, N.V. (1983). *Breeding and seed farming of buckwheat*. Moscow: Kolos. (in Russian)

Fesenko, N.V., Fesenko, N.N., Romanova, O.I., Alexeeva, E.S., & Suvorova, G.N. (2006). *Buckwheat (Theoretical basis of plant breeding)*. St.-Petersburg: Vavilov's Institute of Plant Industry. (in Russian)

Jonik, C., Sonnewald, U., Hajirezaei, M.-R., Flügge, U.-I., & Ludewig, F. (2012). Simultaneous boosting of source and sink capacities doubles tuber starch yield of potato plants. *Plant Biotechnology Journal, 10*, 1088-1098. https://doi: 10.1111/j.1467-7652.2012.00736.x

Kaschuk, G., Yind, X., Hungriae, M., Leffelaar, P.A., Giller, K.E., & Kuyper, T.W. (2012). Photosynthetic adaptation of soybean due to varying effectiveness of N_2 fixation by two distinct *Bradyrhizobium japonicum* strains. *Environmental and Experimental Botany, 76*, 1-6. https://doi:10.1016/j.envexpbot.2011.10.002

Katoh, A., Ashida, H., Kasajima, I., Shigeoka, S., & Yokota, A. (2015). Potato yield enhancement through intensification of sink and source performances. *Breeding Science, 65*, 77-84. https://doi.org/10.1270/jsbbs.65.77

Kreft, I., Chang, K.J., Choi, Y.S., & Park, C.H. (2003). *Ethnobotany of buckwheat*. Seoul: Jinsol Publishing Co.

Matsuda, R., Suzuki, K., Nakano, A., Higashide, T., & Takaichi, M. (2011). Responses of leaf photosynthesis and plant growth to altered source–sink balance in a Japanese and a Dutch tomato cultivar. *Scientia Horticulturae, 127*, 520-527. https://doi:10.1016/j.scienta.2010.12.008

McCormick, A.J., Cramer, M.D., & Watt, D.A. (2008). Changes in photosynthetic rates and gene expression of leaves during a source-sink perturbation in sugarcane. *Annals of Botany, 101*, 89-102. https://doi: 10.1093/aob/mcm258.

Ohnishi, O. (1990). Analyses of genetic variants in common buckwheat, *Fagopyrum esculentum* Moench: A review. *Fagopyrum, 10*, 12-22.

Paul, M.J., & Foyer, C.H. (2001). Sink regulation of photosynthesis. *Journal of Experimental Botany, 52*, 1383–1400. https://doi.org/10.1093/jexbot/52.360.1383

Phung, H.D., Sugiura, D., Sunohara, H., Makihara, D., Kondo, M., Nishiuchi, S., & Doi, K. (2019). QTL analysis for carbon assimilate translocation-related traits during maturity in rice (*Oryza sativa* L.). *Breeding Science, 69*, 289-296. https://doi:10.1270/jsbbs.18203

Pissolato, M.D., Silveira, N.M., Machado, E.C., Zambrosi, F.C.B., Sodek, L., & Ribeiro, R.V. (2019). Photosynthesis and biomass accumulation in young sugarcane plants grown under increasing ammonium supply in nutrient solution. *Theoretical and Experimental Plant Physiology, 31*, 401-411. https://doi:10.1007/s40626-019-00154-w

Sung, F.J., & Chen, J.J. (1989). Changes in photosynthesis and other chloroplast traits in lanceolate leaflet isoline of soybean. *Plant Physiology, 90*, 773-777. https://doi: 10.1104/pp.90.2.773

Takai, T., Adachi, S., Taguchi-Shiobara, F., Sanoh-Arai, Y., Iwasawa, N., Yoshinaga, S,...Yamamoto, T. (2013). A natural variant of *NAL1*, selected in high-yield rice breeding programs, pleiotropically increases photosynthesis rate. *Scientific Reports, 3*, 2149. https://doi.org/10.1038/srep02149

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Results of testing of the efficacy of sublethal concentrations of bacterial-chemical insecticides combinations against cabbage moth larvae

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The experiments of sublethal concentrations of bacterial-chemical insecticides against cabbage moth larvae

Abstract: Using chemical pesticides has adverse effects on the environment and humans. Bacterial preparations may provide an alternative to chemical pesticides. The study aims to test different combinations of sublethal concentrations of bacterial and chemical preparations against cabbage moth larvae.

During 2020-2022 different combinations of sublethal concentrations of bacterial (Lepidocide) and chemical (Arrivo, Voliam Flexi, Proclaim Fit) preparations were tested in laboratory and field conditions, against cabbage moth young larvae (stage I-II).

The combinations of insecticides with bacterial and chemical sublethal concentrations show high biological efficiency against the cabbage moth larvae. No statistical difference was found between the efficiency indicators of the combined and standard chemical (Arrivo, Voliam Flexi, Proclaim Fit) options and the significance level was generally between 2.0 and 5.9 %, showing that the results of the scientific experiments are reliable.

Key words: biological effectiveness, cabbage moth larvae, insecticides, laboratory and field tests, statistical analysis

Poskusi s subletalnimi koncentracijami bakterijsko-kemijskih insekticidov na gosenice kapusnega molja

Izvleček: Uporaba kemijskih insekticidov ima škodljive učinke na okolje in ljudi. Pripravki iz bakterij so lahko alternativa kemijskim pesticidom. Namen raziskave je bil preiskusiti različne kombinacije subletalnih koncentracij bakterijskih in kemijskih pripravkov proti gosenicam kapusnega molja.

V letih 2020-2022 so bile v laboratoriju in poljskih razmerah preiskušene različne kombinacije subletalnih koncentracij bakterijskih (Lepidocide) in kemijskih (Arrivo, Voliam Flexi, Proclaim Fit) pripravkov za zatiranje mladih gosenic kapusnega molja (razvojni štadij I-II).

Kombinacije bakterijskih in kemijskih insekticidov v subletalnih koncentracijah so pokazale veliko biološko učinkovitost na tretiranih gosenicah. V kazalnikih učinkovitosti ni bilo statistično značilne razlike med kombiniranimi pripravki in standardnimi kemijskimi insekticidi (Arrivo, Voliam Flexi, Proclaim Fit). Raven značilnosti je bila nasplošno med 2,0 in 5,9 %, kar kaže, da so izsledki poskusov zanesljivi.

Ključne besede: biološka učinkovitost, gosenica kapusnega molja, insekticidi, laboratorijski in poljski poskusi, statistična analiza

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1 INTRODUCTION

The soil and climatic conditions of Armavir region of the Republic of Armenia are favorable for the cultivation of white-head cabbage (*Brassica oleracea* L. ssp. *oleracea* convar. *capitata* (L.) Alef f. *alba*). The increase of the yield of this plant is often hindered by the cabbage moth *Plutella maculipennis* (Curtis, 1832) which belongs to the Plutellidae family of the insect order Lepidoptera. The damage caused by its larvae reduces the yield and lowers the quality of the crop.

Hatched larvae eat the parenchyma of the leaves, leaving the epidermis intact, resulting in the formation of areas covered with a thin membrane, called "windows" (Avetyan & Marjanyan, 1976), and the more mature larvae open through holes on the leaves. The damage becomes more dangerous when they feed on the young leaves forming the head of the plant (Safaryan, 1968; Terlemezyan, 1996; Philips et al., 2014; Andreeva et al., 2021).

Besides the white-head cabbage, phytophagous larvae also damage other economic importance cruciferous plants, for example cauliflower, broccoli, rapeseed, etc. (Tsedeler, 1931; Harcourt, 1957; Terlemezyan, 1996; Churikova & Silaev, 2010; Shpanev, 2015; Kholod & Korenyuk, 2016; Tuleeva & Sarmanova, 2019). Therefore, it is extremely important to implement effective, environmentally safe control measures against harmful larvae.

In the integrated pest control system, the preference is given to the use of bacterial preparations based on *Bacillus thuringiensis* Berliner, 1915 (Bt) species, which have high biological efficiency against leaf-eating harmful insects and, unlike chemical preparations, are safe for humans, warm-blooded animals, entomophages and fish. (Talekar & Shelton, 1993; Belyaev & Nozdrenko, 2004; Ivantsova, 2004; Sarantseva & Bobreshova, 2006; Sargsyan, 2013; Fathipour & Mirhosseini, 2017; Semerenko, 2019; Zakharova et al, 2022).

Currently, the implementation of economically justified control of phytophagous larvae through combinations of sublethal concentrations (used in small quantities) of bacterial and chemical insecticides is also emphasized (Mesropyan, 2011; Avagyan, 2012; Chapanyan, 2022).

Based on the above, we aimed to test different combinations of sublethal concentrations of bacterial and chemical preparations against cabbage moth larvae in laboratory and field conditions.

2 MATERIALS AND METHODS

The scientific experiments were carried out during 2020-2022, in the laboratory conditions at the Scientific Center for Risk Assessment and Analysis of Food Safety and cabbage plantations of Nalbandian community of Armavir region.

The research materials were: the young cabbage moth larvae (stage I-II), the cabbage plant (variety: Slava), the commercial bacterial lepidocide preparations KA 3000 IU mg^{-1} in the powder for liquid suspension: the usage rate is 1.0 kg ha^{-1} (Russian Federation), chemical preparations: 25 % concentrated emulsion Arrivo: the usage rate is 0.3 l ha^{-1} (FMC, USA), 30 % concentrated suspension Voliam Flexi: the usage rate is 0.3 l ha^{-1}, and 45 % water-soluble granules of Proclaim fit: the usage rate is 0.1 l ha^{-1} (Syngenta, Switzerland).

All the above-mentioned preparations are allowed to be used against harmful insects in the Republic of Armenia.

Cabbage plantations, where the number of moth larvae was at the threshold of economic damage of a specified pest (that is: 2-5 larvae per plant), when 10 % or more of the plants in the experimental site are occupied by them (Polyakov, 1984), were selected as experimental sites.

The biological effectiveness of insecticides combined with sublethal concentrations (Lepidocide + Arrivo, Lepidocide + Voliam Flexi, Lepidocide + Proclaim Fit) was determined according to the methodological manual (Methodological guidelines for testing biological products for plant protection from pests' diseases and weeds, 1973). The lethal concentrations of 3 (in case of lepiocide: 0.33 kg ha^{-1}) and 10 dilutions (in case of Arrivo and Voliam Flexi: 0.03 l ha^{-1}, in case of Proclaim Fit: 0.01 l ha^{-1}) of bacterial (lepidocide) and chemical (Arrivo, Voliam Flexi and Proclaim Fit) insecticides, respectively, were combined.

The samples sprayed with different solvents (Lepidocide: the usage rate is 1.0 kg ha^{-1}, Arrivo: the usage rate is 0.3 l ha^{-1}, Voliam Flexi: the usage rate is 0.3 l ha^{-1}, Proclaim Fit: the usage rate is 0.1 l ha^{-1}) were taken from the plantations naturally inhabited by moth larvae.

During small-scale and production experiments, cabbage plants grown under laboratory conditions (in camps) and artificially inhabited by moths were sprayed with a hand-held sprayer full of working fluid, using backpack AO - 2 and motorized K-14 sprayers. The working fluid consumption was 400 l ha^{-1}. In small-scale experiments, the size of the experimental area for each (sampled separately and experimentally combined) option was 100 m^2, as for large-scale spraying, it was 0.2 ha.

Each option included in the experiments had 3 replicates.

In laboratory conditions, 30 larvae were included in each option (10 larvae in each replicate), and

in two-year small-scale and production experiments, the number of phytophagous larvae was generally between 51 and 70, and 55 and 77, in certain cases.

The numbers of alive and dead larvae in the experimental plots were counted before spraying (baseline), and 3, 5 and 7 days after spraying, also before the mating phase.

In laboratory conditions when experimenting the options with the sub-threshold concentrations, the microbiological isolation of *Bacillus thuringinsis* var. *kurstaki* Bulla et al. 1979 pathogens which are the basis for the production of the sprayed commercial lepidocide bacterial preparations, were isolated according to the methodological manual (Netrusov et al., 2005).

The statistical analysis of the results of the scientific experiments was carried out according to the protocol presented by (Ashmarin and Vorobyev, 1962; Bernstein, 1968).

3 RESULTS AND DISCUSSION

According to the results of scientific experiments carried out in laboratory conditions in 2020, it was proved that the combinations of standard lepidocide bacterial (3 dilutions of the lethal concentration) and diluted lethal chemical (Arrivo, Voliam Flexi and Proclaim Fit) concentrations (10 dilutions of the lethal concentration) have shown a high biological efficiency against phytophagous larvae (stage I-II) just in 7 days after spraying, generally ranging from 93.3 % to 96.7 %. The indicators of biological efficiency of the sample options for the same recording period were also high, ranging from 93.3 to 100 %.

No mortality of phytophagous larvae was observed on the sprayed sample, during the observation period.

The high rates of biological efficiency recorded in laboratory conditions made it possible, as well, to test the insecticides individually (standard/sample options) and in combination with sublethal concentrations (experimental options) against the cabbage moth larvae under field conditions (field and production experiments).

According to the results of the partial (small-scale) research, it was demonstrated that even 7 days after spraying, the indicators of biological efficiency of combined options, such as Lepidocide + Arrivo, Lepidocide + Voliam Flexi, Lepidocide + Proclaim Fit, were still high, generally ranging from 91.5 % to 94.3 % (Table 1).

As it is presented in Table 1, Lepidocide (sample), Arrivo (sample), Voliam Flexi (sample) and Proclaim Fit (sample) options have also demonstrated high biological efficacy (overall 85.7 % - 96.1 %).

The pattern of high biological efficiency in small-scale experiments demonstrated by individual and sublethal concentrations of insecticides against the cabbage moth larvae was maintained during production experiments conducted in 2021-2022 (Table 2).

According to the two-year data from Table 2, the indicators of biological efficiency (7 days after spraying) of the standard lepidocide were 82.4 % and 85.4 %. As for the combined options of sublethal concentrations, such as Lepidocide + Arrivo, Lepidocide + Voliam Flexi, and Lepidocide + Proclaim Fit, those were 89.1 % and 90.1 %, 92.2% and 93.0 %, and 91.3 % and 91.5 %, respectively.

The indicators of biological efficiency recorded for all standard (sample) chemical insecticides were between 86.3 % and 95.2 % for the same period of observation. Moreover, the above-mentioned indicators recorded on the 7th day were constant for all tested options before the mating period.

From the data in Tables 1 and 2, it is clear that the indicators of biological efficiency recorded in the experiments conducted 3 and 5 days after spraying were relatively low compared to those recorded on the 7th day,

Table 1: The indicators of biological effectiveness of standard (sample) and combined insecticides against cabbage moth larvae (stage I-II) (small-scale experiments, Nalbandyan, 2020)

Options	The number of larvae on the plant 20 option, quantity	Biological efficiency according to accounting days, %		
		3	5	7
Lepidocide + Arrivo	59	57.6	78.0	91.5
Lepidocide +Voliam Flexi	53	62.3	84.9	94.3
Lepidocide + Proclaim Fit	67	56.7	80.6	92.5
Lepidocide (Sample)	70	51.4	77.1	85.7
Arrivo (Sample)	58	67.2	84.5	89.7
Voliam Flexi (Sample)	51	78.4	88.2	96.1
Proclaim Fit (Sample)	62	62.9	87.1	93.5

Table 2: The indicators of biological effectiveness of standard (sample) and combined insecticides against cabbage moth larvae (stage I-II) by years (production experiments, Nalbandian, 2020)

Options	The number of larvae on the plant 20 option, quantity	Biological efficiency according to accounting days, %		
		3	5	7
During 2021				
Lepidocide + Arrivo	55	56.4	76.4	89.1
Lepidocide + Voliam Flexi	64	60.9	84.4	92.2
Lepidocide + Proclaim Fit	59	50.8	78.0	91.5
Lepidocide (Sample)	55	49.1	76.4	85.4
Arrivo (Sample)	73	64.4	83.6	86.3
Voliam Flexi (Sample)	61	77.0	85.2	95.1
Proclaim Fit (Sample)	62	61.3	85.5	93.5
During 2022				
Lepidocide + Arrivo	71	54.9	77.5	90.1
Lepidocide + Voliam Flexi	57	61.4	82.4	93.0
Lepidocide + Proclaim Fit	69	53.6	79.7	91.3
Lepidocide (Sample)	74	50.0	75.7	82.4
Arrivo (Sample)	77	66.2	81.8	88.3
Voliam Flexi (Sample)	63	76.2	87.3	95.2
Proclaim Fit (Sample)	70	60.0	84.3	91.4

which is apparently due to the specificity of the mechanism of action of insecticides on larvae.

Compared to the water-spraying practices, when the experimental options, i.e., the combined bacterial and chemical sublethal concentrations, were applied, the cabbage larvae gradually refused to feed on plants. Moreover, no response to contact or any other mechanical stimuli was observed, which, eventually, contributed and led to larval death. The bodies of dead larvae, compared to healthy larvae, have become grey and have reduced in size.

Microbiological studies have confirmed that the gut cavity and decayed tissues of dead moth larvae were full of vegetative cells of the *Bacillus thuringiensis* pathogen, as well as insecticidal spore-crystal components.

Using the Student's t-test criteria, it was proved (Table 3) that the two-year indicators of biological efficiency of the experimental options, when the lepidocide was combined with sublethal concentrations of insecticides, significantly exceeded those of the standard lepidocide samples, because in the first case with $p = 0.95$ and n = 3, the student's t-test scores, generally ranging from 3.601 to 7.095, were higher than the tabulated Student's t-test score of 3.182. It was also statistically confirmed that there was no significant difference between the indicators of biological effectiveness recorded in the combined versions, on the one hand, and in the standard individual options of Arrivo, Voliam Flexi and Proclaim Fit, on the other hand (with $p = 0.95$ and n = 3, the calculated two-year average scores of the student's t-tests were between 0.056 and 1.756, which were less than its table (3.182) index).

In the two-year production studies, the statistical error was generally ranging from 2.0 % to 5.9 %, confirming that the results of the scientific experiments are reliable (Table 4).

4 CONCLUSIONS

Based on the results of the experiments, we came to a conclusion that the combinations of insecticides with bacterial and chemical sublethal concentrations show high biological efficiency against the cabbage moth larvae. The efficiency indicators for the latter are statistically different from those of the standard bacterial lepidocide. However, no statistical difference was found between the efficiency indicators of the combined and standard chemical (Arrivo, Voliam Flexi, Proclaim Fit) options.

The statistical error indicators prove that the results of the scientific experiments are accurate.

Table 3: The comparative assessment of indicators of biological efficiency recorded in experimental and standard (sample) options during production experiments verified by Student's t test criteria (by years)

Options	Indicators of biological efficiency 7 days after spraying, %	Student's t test scores	Indicators of biological efficiency 7 days after spraying, %	Student's t test scores
	During 2021		During 2022	
Lepidocide + Arrivo	89.1	3.601*1.756	90.1	4.424*1.208
Lepidocide + Voliam Flexi	92.2	4.687**1.756	93.0	7.095**1.535
Lepidocide + Proclaim Fit	91.5	4.643***1.628	91.3	4.537***0.056
Lepidocide (Sample)	85.4	-	82.4	-
Arrivo (Sample)	86.3	-	88.3	-
Voliam Flexi (Sample)	95.1	-	95.2	-
Proclaim Fit (Sample)	93.5	-	91.4	-

Note. * in the numerator: the combined experimental and lepidocide (sample) options, in the denominator: the comparative indicators of biological efficiency recorded in the experimental and Arrivo (sample) options, ** in the numerator: experimental and lepidocide (sample) options, in the denominator: the comparative indicators of the biological efficiency recorded in the experimental and Voliam Flexi (sample) options and *** in the numerator: the comparative indicators of biological efficiency recorded in the experimental and lepidocide (sample) options, in the denominator: the experimental and Proclaim Fit (sample) options

Table 4: The statistical indicators of the average number of dead cabbage moth larvae (stages I-II) per replicate, 7 days after spraying, by years (production experiments)

Options	The average number of dead larvae per replicate, quantity	Statistical indicators			
		The squared deviation	The coefficient of variation, %	The average error	The statistical error, %
During 2021					
Lepidocide + Arrivo	16.33	0.575	3.52	0.332	2.0
Lepidocide + Voliam Flexi	19.67	1.661	8.44	0.959	4.9
Lepidocide + Proclaim Fit	18.00	1.414	7.85	0.816	4.5
Lepidocide (Sample)	15.67	1.205	7.69	0.696	4.4
Arrivo (Sample)	21.00	1.633	7.78	0.943	4.5
Voliam Flexi (Sample)	19.33	1.737	8.99	1.003	5.2
Proclaim Fit (Sample)	19.33	1.009	5.22	0.583	3.0
During 2022					
Lepidocide + Arrivo	21.33	1.741	8.16	1.005	4.7
Lepidocide + Voliam Flexi	17.67	1.199	6.79	0.692	3.9
Lepidocide + Proclaim Fit	21.00	2.160	10.29	1.247	5.9
Lepidocide (Sample)	20.33	1.739	8.55	1.004	4.9
Arrivo (Sample)	22.67	1.185	5.23	0.684	3.0
Voliam Flexi (Sample)	20.00	1.633	8.17	0.943	4.7
Proclaim Fit (Sample)	21.33	1.303	6.11	0.752	3.5

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6 REFERENCES

Andreeva, I.A., Shatalova, E.I. and Khodakova, A.V. (2021). Cabbage moth *Plutella xylostella*: ecological and biological aspects, harmfulness, population control. *Bulletin of Plant Protection, 104*(1), 28–39. [In Russian]. https://doi.org/10.31993/2308-6459-2021-104-1-14947

Ashmarin, I.P., &d Vorobyev, A.A. (1962). *Statistical methods in microbiological research*. Medgiz, 180 p. [In Russian].

Avagyan, A.M. (2012). *Development of integrated control on ecological basis against main pests – Horse radish leaf beetle in conditions of Lori marz*. Abstr., PhD. Biol. Thesis. Erevan. 23 p. [In Armenian].

Avetyan, H.S. and G.M. Marjanyan (1976). *Pests of agricultural crops, forests and warehouses of Armenia*. Publishing House of the Academy of Sciences of the Armenian SSR, Yerevan. 832 pp. [In Armenian].

Belyaev, O.V., & Nozdrenko, Ya.V. (2004). Lepidocide against the unpaired silkworm, *Protection and Quarantine of Plants, 6*, 42-43. [In Russian].

Bernstein, A. (1968). *Handbook of Statistical solutions*. M.: Statistics, 162 p. [In Russian].

Chapanyan, E.N. (2022). *Developing integrated pest management measures against the main forest leaf beetles with the ecologically-based crystal-forming local bacterial insecticides of Aragatsotn region*. Abstr. PhD. Biol. Thesis. Erevan. 27 p. [In Armenian].

Churikova, V.G., & Silaev, A.I. (2010). Pests of spring rape in the Lower Volga region, *Agro, XXI*, (4-6), 24–27. [In Russian]. https://doi.org/10.28983/asj.y2020i11pp71-77

Fathipour, Y., & Mirhosseini, M.A. (2017). Diamondback moth (Plutella xylostella) management. In: G.V.P. Reddy (ed.). *Integrated Management of Insect Pests on Canola and Other Brassica Oilseed Crops* (pp.13–43). Oxford; Boston, MA: CAB International. https://doi.org/10.1079/9781780648200.0013

Harcourt, D.G. (1957). Biology of the diamondback Moth, *Plutella maculipennis* (Curt.) (Lepidoptera: Plutellidae), in Eastern Ontario. *Canadian Entomologist, 89*(12), 554–564. https://doi.org/10.4039/Ent89554-12

Ivantsova, E.A. (2004). Biopreparation against mustard pests, *Protection and Quarantine of Plants, 6*, 36. [In Russian]. DOI: https://dx.doi.org/10.15688/jvolsiu 11.2015.2.6

Kholod, A.S., & Korenyuk, E.F. (2016). Cabbage moth – a threat to rapeseed crops in the Omsk region, *Protection and Quarantine of Plants, 5*, 32–33. [In Russian]. https://doi.org/10.28983/asj.y2020i11pp71-77

Mesropyan, H.R. (2011). *Effect of BTB and Lepidocide on the microflora and enzymatic activity of afforestation typical chernozem*. Abstr. PhD. Biol. Thesis. Erevan. 24 p. [In Armenian].

Methodological guidelines for testing biological products for plant protection from pests, diseases and weeds. 1973. Moscow, Kolos, 112 p. [In Russian].

Netrusov, A.I., Egorov, M. A., & Zakharchuk L.M. (2005). *Workshop on microbiology*. Moscow: IC Academy, 608 p. [In Russian].

Philips, C.R., Fu, Z., Kuhar, T.P., Shelton, A.M., & Corder,o R.J. (2014). Natural history, ecology, and management of diamondback moth (Lepidoptera: Plutellidae), with emphasis on the United States. *Journal of Integrated Pest Management, 5*(3), D1–D11. https://doi.org/10.1603/IPM14012

Polyakov, I.Ya. (1984). *Forecast of the development of pests and diseases of agricultural crops (with a workshop)*. L.: "Ear", Leningrad branch, 318 p. [In Russian].

Safaryan, S.E. (1968). *The harmful fauna of cabbage in Armenia and the biological basis for the development of control measures*. Abstract of the dis. candidate of agr. sciences. Yerevan, 29 p. [In Russian].

Sarantseva, N.A., & Bobreshova, I. Yu. (2006). Biopreparations against the Colorado potato beetle, *Protection and Quarantine of Plants, 7*, 27–28. [In Russian].

Sargsyan, A.M. (2013). *The biological efficiency of bacterial insecticides isolated from biocenosis against the major leaf beetle pests of the forest and their influence on biological activity of forest brown soils*. Abstract of the thesis candidate of biol. sciences. Yerevan, 26 p. [In Armenian].

Semerenko, S.A. (2019). Pheromonitoring of cabbage moth in rapeseed crops and a search of effective chemicals for pest control in the conditions of the Western Ciscaucasia. Oil Crops. *Scientific and Technical Bulletin of VNIIMK, 4*(180), 143–151. [In Russian]. https://doi.org/10.25230/2412-608X-2019-4-180-143-151

Shpanev, A.M. (2015). Mass reproduction of cabbage moth, *Protection and Quarantine of Plants, 9,* 40-42. (In Russian). DOI: 10.31857/S0002188122080130.

Talekar, N.S., & Shelton, A.M. (1993). Biology, ecology, and management of the diamondback moth. *Annual Review of Entomology, 38*(1), 275–301. https://doi.org/10.1146/annurev.en.38.010193.001423

Terlemezyan, H.L. (1996). *Harmful fauna of vegetable crops of the Ararat Plain: bioecological features of the main species and integrated control against them*. Ph. D. Thesis, Yerevan University, Armenia. 301 pp. [In Russian].

Tsedeler, O.E. (1931). Cabbage moth (*Plutella maculipennis* Curt.) in connection with cultural mustard, *Journal of Experimental Agrochemistry of the South-East, 9*(2), 165–195. [In Russian]. https://doi.org/10.28983/asj.y2020i11pp71-77

Tuleeva, A.K., & Sarmanova, R.S. (2019). Pests of spring rape in Akmola region. *Protection and Quarantine of Plants, 12*, 20–23. [In Russian]. https://doi.org/10.28983/asj.y2020i11pp71-77

Zakharova, Yu.A., Frolov, A.N., & Artemyeva, A.M. (2022). Monitoring of the diamondback moth (*Plutella xylostella* L.) on the *Brassica oleracea* L. collection in the vicinity of St. Petersburg. *Proceedings on Applied Botany, Genetics and Breeding*, *183*(4), 219–228. https://doi.org/10.30901/2227-8834-2022-4-219-228