ACTA AGRICULTURAE SLOVENICA

105•2
2015

Biotehniška fakulteta Univerze v Ljubljani
Biotechnical Faculty University of Ljubljana

Acta agriculturae Slovenica • ISSN 1581-9175 • 105 – 2 • Ljubljana, september 2015

Acta agriculturae Slovenica
Volume / Letnik **105** · Number / Številka **2** · **2015**

VSEBINA / CONTENTS

Članki / Articles

COBISS Code 1.01

DOI: 10.14720/aas.2015.105.2.01
Agrovoc descriptors: drought stress, drought, photosynthesis, slenium, water supply, wheats, organic compounds, pigments, field experimentation, protected cultivation

Agris category code: f06,f60

Influence of selenium in drought-stressed wheat plants under greenhouse and field conditions

Roghieh HAJIBOLAND[1], Noushin SADEGHZADEH[2], Nashmin EBRAHIMI[2], Behzad SADEGHZADEH[3] and Seyed Abolgasem MOHAMMADI[4]

Received February 08, 2015; accepted May 19, 2015.
Delo je prispelo 08. februarja 2015, sprejeto 19. maja 2015.

ABSTRACT

Effects of selenium (Na_2SeO_4) was studied in two wheat genotypes under well-watered and drought conditions in greenhouse (15 µg Se L^{-1}) and field (20-60 60 g ha^{-1}) experiments. Application of Se improved dry matter and grain yield under both well-watered and drought conditions. Se increased leaf concentration of pigments and photosynthesis rate under both well-watered and drought conditions. Our results indicated that Se alleviates drought stress via increased photosynthesis rate, protection of leaf photochemical events, accumulation of organic osmolytes and improvement of water use efficiency. Under well-watered condition, Se-mediated growth improvement was associated with higher photosynthesis rate and water use efficiency, greater root length and diameter, and higher leaf water content.

Key words: Drought, Organic osmolytes, Photosynthesis rate, Selenium, Water relations, Wheat

IZVLEČEK

VPLIV SELENA NA PŠENICO V SUŠNEM STRESU V RASTLINJAKU IN NA POLJU

Raziskovan je bil vpliv selena (Na_2SeO_4) na rastline dveh genotipov navadne pšenice, v sušnih razmerah oziroma pri dobri oskrbi z vodo, v rastlinjaku (15 µg Se L^{-1}) in na polju (20-60 60 g ha^{-1}). Dodatek Se je povečal sušino rastlin in pridelek zrnja pri obeh načinih oskrbe z vodo. Se je vplival na povečano koncentracijo pigmentov in na povečanje fotosinteze listov pri obeh oskrbah z vodo. Rezultati kažejo, da dodatek Se omili vpliv sušnega stresa s povečanjem fotosinteze, zaščito lista s fotokemičnimi procesi, akumulacijo organskih ozmotikov in povečano učinkovitostjo porabe vode. V razmerah dobre oskrbe z vodo je bila povečana rast, omogočena z dostopnostjo selena, povezana z intenzivnejšo fotosintezo in večjo učinkovitostjo uporabo vode, daljšimi in debelejšimi koreninami in večjo vsebnostjo vode.

Ključne besede: suša, organski ozmotiki, fotosinteza, selen, vodna oskrba, pšenica

1 INTRODUCTION

Plants often encounter unfavorable conditions, which interrupts their growth and productivity. Among the various abiotic stresses, drought is the major factor that limits crop productivity worldwide (Tardieu et al., 2014). Inadequate water availability during the life cycle of a crop species restricts the expression of its full genetic potential. Most of the crops are sensitive to water deficits, particularly during flowering to seed development stages. Even drought-tolerant crops are adversely

[1] Associate Professor of Plant Physiology, Plant Science Department, University of Tabriz, 51666-14779 Tabriz, Iran

[2] M.Sc. of Plant Physiology, Plant Science Department, University of Tabriz, 51666-14779 Tabriz, Iran

[3] Assistant Professor of Crop Molecular Breeding, Dryland Agricultural Research Institute (DARI), Maragheh, P.O. Box 119, Iran

[4] Professor of Plant Breeding and Biotechnology, Faculty of Agriculture, University of Tabriz, 51666-14779 Tabriz, Iran

This paper is a part of the PhD thesis of N.E. under supervision of R.H.

affected by water scarcity at reproductive stage (Mitra, 2001).

One of the initial responses to water deficiency is stomatal closure that depresses in turn photosynthesis and ability of plants for dry matter production (Chaves et al., 2009; Farooq et al., 2009). Drought-induced reduction of photosynthetic activity, however, is linked also to non-stomatal mechanisms, i.e. reduction of enzyme activities (Chaves et al., 2009). Down-regulation of photosynthetic carbon metabolism leads, in turn, to generation of excess excitation energy and formation of reactive oxygen species that induce damages to photosystems (Hajiboland, 2014). The relative contribution of stomatal and non-stomatal limitations of photosynthesis depends on the severity of water stress and plants susceptibility to desiccation (Chaves et al., 2009).

Plants respond and adapt to water deficit by the accumulation of osmolytes and proteins specifically involved in stress tolerance (Krasensky and Jonak, 2012). These molecules are accumulated in plant cells in response to drought stress and act as osmotic balancing agents. In addition of roles in osmotic homeostasis, these organic solutes are free radical scavengers and protect cell structures and membranes against desiccation damages (Krasensky and Jonak, 2012). Synthesis of osmoprotectants, osmolytes or compatible solutes including amino acids particularly proline and soluble carbohydrates is one of the mechanisms for adaptation to water deficit (Verbruggen and Hermans, 2008). Stress tolerance are controlled also by developmental and morphological traits such as root thickness, the ability of roots to penetrate compacted soil layers, and root depth and mass (Valliyodan and Nguyen, 2006).

To improve production efficiency of crop plants under drought conditions, development of more tolerant genotypes using breeding strategies are necessary (Mitra, 2001; Valliyodan and Nguyen, 2006). Alternatively, exogenous application of various growth regulators (jasmonate and salicylate) and some osmoprotectants (e.g. glycine betaine) has proven worldwide in inducing drought tolerance at various growth stages (Farooq et al., 2009). Among mineral compounds, application of silicon (Liang et al., 2007) and selenium (Se) (Feng et al., 2013) attracted much more attention.

Influence of low concentrations of Se in the amelioration of various abiotic stresses such as salt, UV radiation and drought stresses has been reported for various plant species (Hajiboland, 2012; Feng et al., 2013). Mechanisms for alleviating effect of Se on drought have mainly focused on the Se-mediated activation of antioxidative defense (Wang, 2011; Hasanuzzaman and Fujita, 2011; Hajiboland, 2012; Feng et al., 2013). However, Se-mediated changes in water relation parameters, accumulation of osmoticums and water uptake capacity under drought remain largely unknown and the obtained results are contradictory. Se application was reported to increase the accumulation of proline (Yao et al., 2009) while did not affect water uptake capacity (Habibi, 2013) and did not influence plant biomass under drought (Yao et al., 2009; Habibi, 2013).

Wheat (*Triticum aestivum* L.) is one of the most important staple food crops and its productivity is adversely affected by drought particularly in arid and semi arid regions of the world. It has been estimated that about 50 % of the 237 million ha area in the world under wheat cultivation is affected by periodic drought (Ashraf, 2010). Effect of Se on the mitigation of drought stress in wheat plants has not been studied under field conditions. On the other hand, considering Se as a necessary element for animals and humans, fortification of grains with Se may contribute to an increase in Se intake for humans particularly in the countries where soil Se is low (Lyons et al., 2003).

This work was aimed at studying the effect of Se application on the amelioration of drought stress in wheat genotypes. Some physiological parameters such as gas exchange, water relations and osmolyte accumulation were studied in this crop species in the pot and field experiments in order to compare Se effect as related to growth conditions and developmental stages.

2 MATERIALS AND METHODS

2.1 Plants materials

Two common winter wheat (*Triticum aestivum* L.) genotypes 'Homa' (drought-tolerant) and 'Sara-BW-F6-06-85-86-29-1' (drought-sensitive) were used in both pot experiment and field study. Seeds were provided by Dryland Agricultural Research Institute (DARI) (Maragheh, Iran).

2.2 Pot experiment

Seeds were surface-sterilized and germinated on perlite. Five-days-old seedlings were transferred into 1.5 L pots (40 plants per pot) filled with acid-washed perilte irrigated with 200 ml week^{-1} of 50 % wheat nutrient solution (Hajiboland et al., 2003). Se and irrigation treatments were started 9 days after transplanting, treatments were assigned randomly to the pots. Se treatments at two levels including without and with (15 µg L^{-1}) Se (as Na_2SeO_4) were applied gradually during 4 weeks. Simultaneously, irrigation treatments included well-watered (irrigation at field capacity) and drought stress (irrigation at 20 % FC) were started by omitting watering from drought treatments. One week after withholding watering, pots reached the 20 % FC. Throughout the experiment, pots were irrigated daily after weighing with nutrient solution or water as interval. Control and water-stressed plants received the same amount of nutrient solution and the respective FC was achieved by different volumes of water. Plants were grown under greenhouse conditions with a day/night temperature regime of 25-28/15-17°C, a relative humidity of 70/80 % and a photoperiod of 17/7 h at a photon flux density of about 300 µmol m^{-2}s^{-1} provided by natural light supplemented with fluorescent lamps.

Eight weeks after starting Se treatments (7 weeks after reaching the respective FC, 10 weeks after sowing) plants were harvested. Shoot and roots were separated, roots were washed with distilled water and blotted dry on filter paper and their fresh weight (FW) were determined. Plants dry weight (DW) was determined after drying in 70°C for 48 h. Subsamples from leaves and roots were taken for biochemical analyses before drying. Change in the root morphology in Se-treated plants was visually observed at harvest. For its quantification, the root system of each pot was spread out in a tray filled with distilled water. Thereafter, root length was determined according to the line intersect method (Tennant, 1975) and the root diameter was determined using a micrometer in 50 randomly-selected parts of root system of each replicate pot and the average of obtained data was calculated.

2.3 Field experiment

Field experiment was conducted during the 2012-2013 growing season at the Research Station of Faculty of Agriculture, University of Tabriz. At the beginning of the season (fall of 2012), the experimental area was prepared and soil samples were taken at a depth of 30 cm and analyzed for main soil properties. Soil properties before planting were 76 % sand, 18 % silt, and 6 % clay; 2.1 % organic matter; pH 8.7, EC 33.3 (soil:water, 1:1); 5.0 NO_3-N, 36 P and 480 K in mg kg^{-1} soil. Plot dimensions were 1.5 m×3.0 m containing 5 rows. Seeds of two genotypes were planted by hand in rows and covered with soil. Approximately 60 seeds per row and 300 seeds per plot were planted on 20 October 2012. Weeds were controlled by hand as required. Nitrogen was added at a rate of 60 kg N ha^{-1} as urea in 08 April 2013.

Control and drought-exposed plots were grown under rainfed conditions and supplemental irrigation applied for three times: one day and two weeks after planting and on 08 April 2013. Thereafter, drought was imposed by water withholding while control plots were irrigated weekly up to two weeks before harvest. Soil humidity (%) was estimated in samples collected weekly from drought-imposed plots at 10 cm simultaneous with irrigation of control plots. Soil relative humidity was 12.79±1.2 at the start of withholding irrigation and decreased to 3.52±0.21 at two weeks before harvest without significant difference among plots.

Selenium (as Na_2SeO_4) was sprayed on the leaves at the concentrations of 1 and 3 µg L^{-1} with the final amounts of 20 µg ha^{-1} (Se_1) and 60 µg ha^{-1} (Se_2) respectively, in the mornings before sunrise. Control plots (–Se) were sprayed with distilled water. The first foliar Se treatment was applied 6 weeks after planting. In spring, four subsequent Se

treatments each with 3 weeks interval starting on 09 April 2013 were applied.

At maturity (15 July 2013), plants of each experimental plot were harvested. The harvested material was sun-dried, threshed manually, and weighed for total biomass, grain yield and elemental analyses. Yield components (heads per plant, grains per head, and grain weight) were measured on ten plants that were sampled randomly from the two middle rows of each plot at maturity and added to the total. For determination of K, Ca and P, oven dried leaf samples were ashed in a muffle furnace at 550ºC for 8 h, resolved in HCl, and made up to volume by distilled water. Concentrations of K and Ca were determined by flame photometry (PFP7, Jenway, UK) and P by a spectrophotometer (Specord 200, Analytical *Jena,* Germany) (Jaiswal, 2004).

2.4 Measurement of chlorophyll fluorescence and gas exchange parameters

Chlorophyll (Chl) fluorescence parameters were measured in the pot experiment at harvest using a portable fluorometer (OSF1, ADC Bioscientific Ltd., UK). Leaves were acclimated to dark for 30 min using leaf clips before taking the measurements for dark-adapted leaves. Maximum quantum yield of PSII (F_v/F_m) was calculated using initial (F_0), maximum (F_m) and variable ($F_v=F_m–F_0$) fluorescence parameters. Calculations for light-adapted leaves were undertaken using initial (F_t), steady-state (F_s), maximum (F'_m), variable ($F'_v=F'_m–F_t$), ΦPSII ($F'_m–Ft/F'_m$) and F'_0 [$F'_0=F_0/(F_v/F_m)+(F_0/F'_m)$] fluorescence for excitation capture efficiency of open PSII (F'_v/F'_m), photochemical quenching (qP) [$(F'_m–F_s)/(F'_m–F'_0)$], non-photochemical quenching (qN) [$1–(F'_m–F'_0)/(F_m–F_0)$] ΦPSII ($F'_m–Ft/F'_m$) and electron transport rate (ETR) [ΦPSII×PFD×0.84×0.5] (Krall and Edwards, 1992).

Gas exchange parameters were measured with a calibrated portable gas exchange system (LCA-4, ADC Bioscientific Ltd., UK) between 10:00 and 13:00. Measurements in the field experiment were undertaken one day after the last Se application (28 May 2013) in the young and flag leaves. In the greenhouse experiment, gas exchange parameters were measured at three time intervals: 4, 6 and 8 weeks after starting treatments. The measurements were conducted with photosynthetically active radiation (PAR) intensity at the leaf surface of 300-400 μmol m^{-2} s^{-1} in the pot experiment and 800-900 μmol m^{-2} s^{-1} in the field experiment. The net photosynthetic rate by unit of leaf area (A, μmol CO_2 m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}) and the stomatal conductance to water vapor (g_s, mol m^{-2} s^{-1}) were calculated using the values of CO_2 and humidity variation inside the chamber, both measured by the infrared gas analyzer of the photosynthesis system. Instant water use efficiency ($iWUE$) was calculated as the ratio of photosynthesis: transpiration (μmol $mmol^{-1}$).

2.5 Determination of osmotic potential and relative water content

Osmotic potential was determined in the leaf and root samples using an osmometer (Heman Roebling Messtechnik, Germany). Relative water content (RWC%) was measured in the leaves and calculated according to the formula: (FW-DW)/(TW-DW)×100. For determination of turgid weight (TW), leaf disks (5mm diameter) were submerged for 18 h in distilled water, thereafter, they were blotted dry gently on a paper towel and weighed.

2.6 Biochemical determinations

Leaf concentration of Chl a, b and carotenoids (Car) were determined according to Lichtenthaler and Wellburn (1985). Determination of anthocyanins was performed using a pH differential method at pH 1 and pH 4.5 in the methanol/HCl (98:2, v/v) extract and was expressed as mg of cyanidine-3-glucoside g^{-1} FW (Giusti and Wrolstad, 2001). Total flavonoids content was determined in the methanol extract of leaves using $AlCl_3$-metanol (2 %, w/v) as indicator at 510 nm and quercetin (Sigma) as standard (Grayer, 1989).

For determination of non-structural carbohydrates, samples were homogenized in 100 mM phosphate buffer (pH 7.5) at 4 ^oC, after centrifugation at 12000 g for 15 min, supernatant was used for determination of total soluble sugars whereas the pellets were kept for starch analysis (Yemm and Willism, 1954). Total soluble proteins were determined using a commercial reagent (Bradford reagent, Sigma) and bovine albumin serum (BSA) as standard. Content of total free α-amino acids

was assayed using a ninhydrin colorimetric method (Yemm and Cocking, 1955). Glycine was used for standard curve. For determination of proline, samples were homogenized with 3% sulfosalicylic acid and the homogenate was centrifuged at 3000 g for 20 min. The supernatant was treated with acetic acid and acid ninhydrin, boiled for 1 h, and then absorbance at 520 nm was determined. Proline (Sigma) was used for production of a standard curve (Bates et al., 1973).

2.7 Experimental design and statistical analyses

Pot experiment was undertaken in randomized block design with four replications as four independent pots. Field experiment was arranged in a split-plot-factorial design with four replicates. Watering treatments were the main-plots with Se treatments (–Se, Se_1 and Se_2) and genotypes combinations as sub-plots. Differences between the means were detected according to Tukey's test (p<0.05) using Sigma Stat 2.03 software.

3 RESULTS

Plants fresh and dry matter production was adversely affected by drought under greenhouse conditions (Table 1). Reduction of shoot and root dry weight was 50 % and 29 % in drought tolerant variety 'Homa' and 68 % and 17 % in drought sensitive line 'Sara', respectively. Root length and diameter were also lower in drought-stressed plants. Se application improved all the growth parameters in control plants of both genotypes. In drought-stressed plants, however, effect of Se was significant only for shoot DW and root diameter in 'Homa'. Contrastingly, Se-treated plants tended to have slightly lower root length under drought conditions in both genotypes (Table 1).

Under field conditions, drought stress declined all yield components. Reduction of whole shoot and straw biomass under drought was 40% and 44% in 'Homa', respectively, whereas it was more in 'Sara' (53 % and 56 %, respectively). For reproductive plant parts, yield depression was 23 % and 39 % for spike and seed in 'Homa' respectively, whereas the corresponding values for 'Sara' were 51 % and 54 % (Table 2). Se treatment increased plants growth parameters mainly under well-watered conditions without significant difference between two levels of applied Se. However, these effects were mainly in tendency, and significant changes were observed only for whole shoot and straw weight in 'Homa' (Table 2).

Table 1: Fresh and dry weight (mg plant^{-1}) of shoot and root, root length (mm plant^{-1}) and diameter (mm) in two wheat genotypes grown under control (100 % FC) and drought (20 % FC) conditions in the absence (–Se) or presence (+Se) of Se (15 µg Se L^{-1} as Na_2SeO_4) for 10 weeks in greenhouse.

Treatments		Shoot FW	Shoot DW	Root FW	Root DW	Root length	Root diameter
		'Homa'					
Control	–Se	539±19 ^{b}	119±7 ^{b}	299±36 ^{b}	52±6 ^{ab}	6.07±0.58 ^{b}	0.21±0.009 ^{b}
	+Se	592±28 ^{a}	132±6 ^{a}	346±18 ^{a}	60±4 ^{a}	7.50±0.72 ^{a}	0.30±0.003 ^{a}
Drought	–Se	155±10 ^{c}	60±5 ^{d}	89±12 ^{c}	37±9 ^{c}	2.65±0.57 ^{c}	0.13±0.006 ^{c}
	+Se	163±22 ^{c}	73±3 ^{c}	93±11 ^{c}	39±8 ^{bc}	2.57±0.21 ^{c}	0.21±0.005 ^{b}
		'Sara'					
Control	–Se	566±53 ^{b}	151±15 ^{a}	322±47 ^{b}	69±8 ^{ab}	7.49±0.42 ^{b}	0.18±0.005 ^{c}
	+Se	663±42 ^{a}	168±13 ^{a}	456±14 ^{a}	81±9 ^{a}	7.79±0.34 ^{a}	0.33±0.006 ^{a}
Drought	–Se	126±15 ^{c}	49±12 ^{b}	123±25 ^{c}	57±5 ^{b}	2.69±0.39 ^{c}	0.14±0.002 ^{d}
	+Se	153±26 ^{c}	60±8 ^{b}	136±24 ^{c}	63±5 ^{b}	2.10±0.28 ^{c}	0.28±0.001 ^{b}

Data of each column within each genotype indicated by the same letter are not statistically different (P≤0.05)

Table 2: Various growth parameters including yield of whole shoot, straw, spike and seed (g plant^{-1}) and weight of 1000 seeds (g) in two wheat genotypes grown under control (well-watered) and drought stress conditions without (–Se) or with two levels of foliar-applied Se (Se_1: 20 g ha^{-1} and Se_2: 60 g ha^{-1}) (as Na_2SeO_4) under field conditions.

Treatments		Whole shoot	Straw	Spike	Seed	1000 seeds
		'Homa'				
Control	–Se	19.7±1.6 ^{c}	8.9±1.0 ^{b}	1.07±0.07 ^{a}	7.62±0.72 ^{a}	48.08±3.97 ^{a}
	Se_1	21.5±1.7 ^{ab}	9.9±0.4 ^{ab}	1.15±0.13 ^{a}	8.28±0.68 ^{a}	50.38±4.68 ^{a}
	Se_2	23.7±1.9 ^{a}	12.8±1.2 ^{a}	1.09±0.16 ^{a}	8.08±0.75 ^{a}	44.71±3.82 ^{a}
Drought	–Se	11.8±1.1 ^{d}	5.0±1.0 ^{c}	0.68±0.11 ^{b}	4.63±0.39 ^{b}	35.97±2.29 ^{b}
	Se_1	12.9±0.7 ^{d}	5.4±0.4 ^{c}	0.74±0.04 ^{b}	5.14±0.25 ^{b}	36.37±2.27 ^{b}
	Se_2	13.0±1.2 ^{d}	5.5±1.1 ^{c}	0.75±0.21 ^{b}	4.92±2.10 ^{b}	37.68±3.31 ^{b}
		'Sara'				
Control	–Se	22.8±2.7 ^{a}	8.4±1.2 ^{ab}	1.44±0.16 ^{a}	10.68±0.61 ^{a}	37.57±1.94 ^{a}
	Se_1	25.7±2.4 ^{a}	10.4±2.3 ^{a}	1.53±0.13 ^{a}	11.20±0.97 ^{a}	38.93±2.42 ^{a}
	Se_2	23.2±2.8 ^{a}	11.7±3.9 ^{a}	1.14±0.18 ^{a}	9.70±0.94 ^{a}	38.06±2.21 ^{a}
Drought	–Se	10.7±1.8 ^{b}	3.7±1.1 ^{c}	0.70±0.11 ^{a}	4.89±0.71 ^{b}	27.41±3.18 ^{b}
	Se_1	13.2±1.5 ^{b}	5.4±0.7 ^{bc}	0.77±0.08 ^{a}	5.16±0.56 ^{b}	28.67±3.40 ^{b}
	Se_2	13.2±1.3 ^{b}	5.5±1.0 ^{bc}	0.77±0.13 ^{a}	4.76±0.96 ^{b}	27.30±1.38 ^{b}

Data of each column within each genotype indicated by the same letter are not statistically different ($P \leq 0.05$)

In general, leaf Chl a, b and Car concentrations were higher in drought-stressed plants being significant for Chl a and b in 'Homa'. In contrast, concentrations of anthocyanins were declined by drought treatment in both genotypes and that of flavonoids in 'Homa' (Table 3). Se treatment increased consistently concentrations of all leaf pigments. Compared with –Se treatment, the effect of Se application was much more pronouncedly observed for leaf anthocyanins under both well-watered and drought conditions being about 2.2 and 3.5 folds higher in 'Homa' and 'Sara', respectively. Se affected also significantly Chl a, b in 'Homa' and flavonoids in 'Sara' (Table 3).

Leaf Chl fluorescence parameters were affected by drought condition particularly in 'Homa' (Table 4). Maximum (F_v/F_m) and actual (F'_v/F'_m) efficiency of PSII and electron transport rate (*ETR*) were significantly lowered by drought stress in 'Homa'. Se treatment increased significantly F_v/F_m and *ETR* as well as non-photochemical quenching (*qN*) in 'Homa' under drought stress (Table 4).

Table 3: Concentration of leaf pigments (mg g^{-1} FW) in two wheat genotypes grown under control (100 % FC) and drought (20 % FC) conditions in the absence (–Se) or presence (+Se) of Se (15 µg Se L^{-1} as Na_2SeO_4) for 10 weeks in greenhouse.

Treatments		Chl *a*	Chl *b*	Car	Anthocyanins	Flavonoids
		'Homa'				
Control	–Se	1.66±0.32 ^{b}	0.81±0.05 ^{b}	0.50±0.08 ^{a}	7.98±1.46 ^{ab}	5.65±0.44 ^{a}
	+Se	2.29±0.30 ^{a}	0.94±0.05 ^{a}	0.52±0.07 ^{a}	9.78±2.75 ^{a}	5.87±0.34 ^{a}
Drought	–Se	2.31±0.11 ^{a}	0.98±0.07 ^{a}	0.57±0.03 ^{a}	2.30±1.30 ^{c}	3.78±0.08 ^{c}
	+Se	2.53±0.18 ^{a}	1.05±0.06 ^{a}	0.60±0.05 ^{a}	5.28±1.28 ^{bc}	4.88±0.24 ^{b}
		'Sara'				
Control	–Se	2.06±0.30 ^{b}	0.88±0.05 ^{a}	0.46±0.08 ^{a}	9.35±2.83 ^{bc}	3.10±0.14 ^{c}
	+Se	2.13±0.19 ^{ab}	0.93±0.02 ^{a}	0.49±0.04 ^{a}	18.0±2.53 ^{a}	4.51±0.24 ^{b}
Drought	–Se	2.25±0.21 ^{ab}	0.90±0.07 ^{a}	0.53±0.06 ^{a}	4.01±1.35 ^{c}	4.21±0.33 ^{b}
	+Se	2.55±0.17 ^{a}	0.98±0.08 ^{a}	0.57±0.05 ^{a}	13.89±3.74 ^{ab}	5.50±0.07 ^{a}

Data of each column within each genotype indicated by the same letter are not statistically different ($P \leq 0.05$)

Table 4: Chlorophyll fluorescence parameters: F_v/F_m (maximum quantum efficiency of PSII), F'_v/F'_m (excitation energy capture of PSII), *qP* (photochemical quenching), *qN* (non-photochemical quenching) and *ETR* (electron transport rate) in two wheat genotypes grown under control (100 % FC) and drought (20 % FC) conditions in the absence (–Se) or presence (+Se) of Se (15 µg Se L^{-1} as Na_2SeO_4) for 10 weeks in greenhouse.

Treatments		F_v/F_m	F'_v/F'_m	*qP*	*qN*	*ETR*
		'Homa'				
Control	–Se	0.86±0.00 [a]	0.75±0.00 [a]	0.99±0.02 [a]	0.07±0.04 [b]	124±3 [a]
	+Se	0.86±0.00 [a]	0.76±0.01 [a]	0.98±0.03 [a]	0.09±0.02 [b]	125±2 [a]
Drought	–Se	0.84±0.01 [b]	0.72±0.02 [b]	0.99±0.01 [a]	0.12±0.08 [b]	117±3 [b]
	+Se	0.86±0.01 [a]	0.72±0.01 [b]	0.98±0.01 [a]	0.25±0.05 [a]	120±3 [a]
		'Sara'				
Control	–Se	0.86±0.01 [a]	0.73±0.01 [a]	0.98±0.02 [a]	0.15±0.03 [a]	122±2 [a]
	+Se	0.87±0.01 [a]	0.74±0.01 [a]	0.95±0.03 [a]	0.20±0.02 [a]	118±5 [ab]
Drought	–Se	0.85±0.02 [a]	0.72±0.01 [a]	0.95±0.02 [a]	0.16±0.03 [a]	117±2 [ab]
	+Se	0.85±0.02 [a]	0.72±0.01 [a]	0.98±0.01 [a]	0.21±0.04 [a]	115±2 [b]

Data of each column within each genotype indicated by the same letter are not statistically different ($P \leq 0.05$)

Four weeks after starting treatments, a significant reduction of stomatal conductance was observed only in 'Homa'. Expectedly, transpiration rate was also lowered by drought stress but these changes were not statistically significant. In contrast, reduction of net photosynthesis rate by drought stress was significant in both genotypes (Table 5).

Se treatment in well-watered plants increased stomatal conductance, transpiration and photosynthesis rates in 'Homa' while decreased the latter parameter in 'Sara'. In drought-stressed plants Se application caused reduction of net assimilation rate in both genotypes (Table 5). Under long-term (6 and 8 weeks) drought stress, however, stomatal conductance and transpiration rates were significantly lowered in both genotypes. Se application increased all gas exchange parameters slightly or significantly in both genotypes. Eight weeks after starting treatments, leaf photosynthesis rate was increased by Se up to 23 % and 120 % in drought-stressed 'Homa' and 'Sara', respectively (Table 5). Gas exchange parameters under field conditions responded in the same way as observed in the pot experiment (Table 6).

Stomatal conductance was significantly lowered by drought stress in both young and flag leaves. A significant reduction of transpiration rate under drought stress, however, was observed only in the young and flag leaves of 'Sara'. Se-treated plants had higher stomatal opening and transpiration rate particularly in the flag leaves and two application levels of Se did not differ in this regard. Net assimilation rate was affected by both drought and Se treatments more pronouncedly than other two parameters. Both young and flag leaves had lower photosynthesis rate under drought irrespective to the level of Se treatments. In turn, Se application resulted in significantly higher photosynthesis under both watering treatments and in both genotypes. In contrast to stomatal conductance and transpiration rate, effect of higher Se concentration (60 g ha^{-1}) on the elevation of photosynthesis was greater than that of lower Se concentration (20 g ha^{-1}) (Table 6). Furthermore, the extent of Se effect on the increases in leaf photosynthesis of drought-stressed plants was higher under field conditions and reached up to 162 % and 179 % in the young leaves and 191 % and 202 % in the flag leaves of 'Homa' and 'Sara', respectively.

Table 5: Gas exchange parameters including net assimilation rate (A, μmol m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}) and stomatal conductance (g_s, mol m^{-2} s^{-1}) in two wheat genotypes grown under control (100 % FC) and drought (20 % FC) conditions in the absence (–Se) or presence (+Se) of Se (15 μg Se L^{-1} as Na_2SeO_4) at three measurement intervals in greenhouse.

Treatments		*A*	*E*	g_s	*A*	*E*	g_s
		'Homa'			'Sara'		
		4 weeks after starting treatments					
Control	–Se	13.71±0.91 [b]	2.08±0.70 [b]	0.50±0.09 [a]	15.09±0.02 [a]	1.42±0.09 [b]	0.23±0.16 [b]
	+Se	15.80±0.45 [a]	4.70±0.26 [a]	0.58±0.13 [a]	12.81±0.30 [b]	2.58±0.14 [a]	0.70±0.18 [a]
Drought	–Se	11.96±0.32 [c]	1.42±0.27 [bc]	0.26±0.05 [b]	11.06±0.33 [c]	1.43±0.63 [b]	0.14±0.05 [b]
	+Se	7.79±0.18 [bc]	0.81±0.22 [c]	0.10±0.04 [b]	9.08±0.56 [d]	1.16±0.40 [b]	0.21±0.08 [b]
		8 weeks after starting treatments					
Control	–Se	15.69±0.18 [b]	1.58±0.11 [a]	1.33±0.05 [a]	10.33±0.30 [c]	1.19±0.11 [b]	1.77±0.24 [a]
	+Se	18.04±0.45 [a]	1.60±0.15 [a]	1.41±0.17 [a]	12.52±0.24 [a]	1.57±0.06 [a]	1.91±0.12 [a]
Drought	–Se	8.61±0.49 [d]	0.93±0.05 [b]	0.90±0.21 [b]	9.91±0.48 [c]	0.86±0.02 [c]	0.38±0.09 [b]
	+Se	10.75±0.69 [c]	0.98±0.05 [ab]	1.24±0.19 [ab]	11.24±0.52 [b]	0.91±0.04 [c]	0.57±0.08 [b]
		10 weeks after starting treatments					
Control	–Se	16.70±0.54 [b]	1.52±0.34 [ab]	1.49±0.14 [ab]	8.85±0.13 [b]	1.43±0.36 [ab]	1.94±0.68 [a]
	+Se	19.02±0.36 [a]	1.72±0.48 [a]	2.06±0.18 [a]	13.01±0.45 [a]	1.99±0.33 [a]	2.44±0.56 [b]
Drought	–Se	5.86±0.13 [d]	0.92±0.04 [b]	0.67±0.14 [b]	2.14±0.20 [d]	0.78±0.11 [c]	0.10±0.03 [c]
	+Se	7.20±0.44 [c]	0.97±0.06 [b]	0.91±0.17 [b]	4.71±0.44 [c]	0.93±0.19 [bc]	0.11±0.06 [bc]

Data of each column within each measurement intervals indicated by the same letter are not statistically different ($P \leq 0.05$)

Table 6: Gas exchange parameters including net assimilation rate (A, μmol m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}) and stomatal conductance (g_s, mol m^{-2} s^{-1}) in the young and flag leaves in two wheat genotypes grown under control (well-watered) and drought stress conditions without (–Se) or with two levels of foliar-applied Se (Se_1: 20 g ha^{-1} and Se_2: 60 g ha^{-1}) (as Na_2SeO_4) under field conditions.

Treatments		*A*	*E*	g_s	*A*	*E*	g_s
		'Homa'			'Sara'		
		Young leaf					
Control	–Se	8.43±0.39 [c]	1.55±0.90 [ab]	0.35±0.04 [a]	8.48±0.36 [c]	1.21±0.17 [ab]	0.42±0.15 [ab]
	Se_1	11.95±0.38 [b]	1.86±0.37 [a]	0.55±0.17 [a]	13.14±0.72 [b]	1.51±0.62 [a]	0.61±0.10 [a]
	Se_2	15.27±0.82 [a]	1.51±0.66 [ab]	0.42±0.15 [a]	15.98±0.66 [a]	1.04±0.12 [abc]	0.43±0.13 [ab]
Drought	–Se	1.62±0.32 [f]	0.39±0.07 [b]	0.07±0.01 [b]	1.88±0.06 [e]	0.44±0.10 [c]	0.23±0.15 [b]
	Se_1	2.61±0.17 [e]	0.81±0.41 [ab]	0.09±0.04 [b]	2.97±0.38 [e]	0.53±0.09 [c]	0.30±0.19 [ab]
	Se_2	4.25±0.25 [d]	0.84±0.48 [ab]	0.14±0.02 [b]	5.24±0.80 [d]	0.68±0.09 [bc]	0.22±0.09 [b]
		Flag leaf					
Control	–Se	9.75±0.19 [c]	0.71±0.06 [b]	0.19±0.01 [bc]	9.95±0.17 [c]	0.65±0.09 [b]	0.24±0.05 [b]
	Se_1	12.0±0.12 [b]	1.08±0.24 [ab]	0.31±0.12 [ab]	15.6±0.21 [b]	0.94±0.08 [a]	0.49±0.16 [a]
	Se_2	14.9±0.54 [a]	1.15±0.25 [a]	0.34±0.08 [a]	17.3±0.11 [a]	0.91±0.09 [a]	0.48±0.14 [a]
Drought	–Se	1.51±0.17 [f]	0.77±0.20 [b]	0.06±0.03 [c]	1.75±0.15 [f]	0.26±0.00 [c]	0.06±0.01 [b]
	Se_1	3.55±0.22 [e]	1.13±0.39 [ab]	0.09±0.02 [c]	4.40±0.05 [e]	0.54±0.08 [b]	0.14±0.03 [b]
	Se_2	4.41±0.31 [d]	1.56±0.11 [a]	0.12±0.02 [c]	5.28±0.42 [d]	0.70±0.07 [b]	0.24±0.05 [b]

Data of each column within each type of leaf indicated by the same letter are not statistically different ($P \leq 0.05$)

Soluble protein concentration in the shoot was decreased slightly by drought treatment in 'Homa' while increased significantly in 'Sara' (Fig. 1A). In the roots, both genotypes had higher soluble proteins under drought conditions. Se treatment increased consistently soluble protein concentrations in the shoot and roots of both genotypes being significant in 'Homa' under drought and in 'Sara' under well-watered conditions (Fig. 1A).

Similar with soluble proteins, shoot concentration of free amino acids decreased upon drought treatment in 'Homa' while increased significantly in 'Sara' (Fig. 1B). In the roots of both genotypes higher free α-amino acids concentration was found in drought-stressed plants compared with well-watered ones. Effect of Se treatment depended on the plant organ, it resulted in slightly lower free amino acids concentration in the shoot while the opposite was observed in the roots that was significant in drought-stressed plants (Fig. 1B).

Expectedly, concentration of free proline was higher in the plants exposed to drought stress (Fig. 1C). This effect was more pronouncedly observed in 'Homa' compared with 'Sara' in both shoot and roots. Se effect on the shoot proline concentration of drought-stressed plants, however, differed between two genotypes. It decreased proline concentration of drought-stressed plants in 'Homa' whereas increased it in 'Sara'. In the roots, in contrast, both genotypes responded similarly to Se application as slightly or significantly lower proline concentration (Fig. 1C).

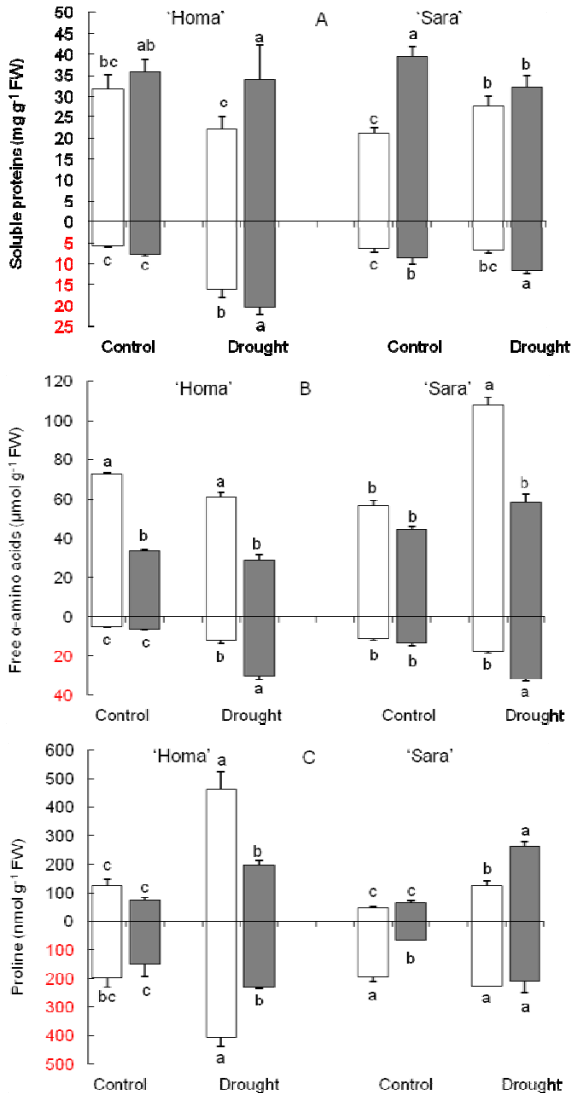


Figure 1: Concentration of soluble proteins (A) (mg g^{-1} FW), total free α-amino acids (B) (μmol g^{-1} FW) and proline (C) (nmol g^{-1} FW) in the leaves (above of the horizontal lines) and roots (below of the horizontal lines) of two wheat genotypes grown under control (100 % FC) and drought (20 % FC) conditions in the absence (–Se, open bars) or presence (+Se, closed bars) of Se (15 μg Se L^{-1} as Na_2SeO_4) for 10 weeks in greenhouse. Data of each column within each genotype indicated by the same letter are not statistically different ($P \leq 0.05$)

Concentration of soluble carbohydrates increased consistently by both drought stress and Se treatments in both genotypes in the shoot and roots (Fig. 2A). This led to the highest amount of soluble sugars in drought-stressed plants supplemented with Se. Root's concentration of starch showed the same pattern of changes in the soluble sugars concentration in response to the treatments. In the shoot, however, starch concentration was not affected by drought in both genotypes and increased by Se application only in 'Homa' under well-watered conditions.

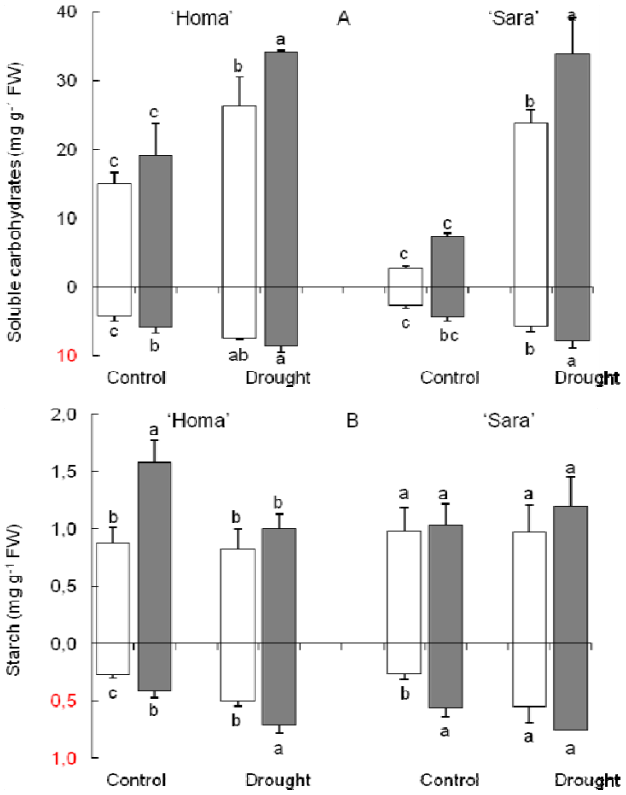


Figure 2: Concentration (mg g^{-1} FW) of soluble sugars (A) and starch (B) in the leaves (above of the horizontal lines) and roots (below of the horizontal lines) of two wheat genotypes grown under control (100 % FC) and drought (20 % FC) conditions in the absence (–Se, open bars) or presence (+Se, closed bars) of Se (15 µg Se L^{-1} as Na_2SeO_4) for 10 weeks in greenhouse. Data of each column within each genotype indicated by the same letter are not statistically different ($P \leq 0.05$).

Expectedly, leaf and root osmotic potentials decreased by drought stress in both genotypes (Fig. 3A). Effect of drought on reduction of roots osmotic potential was more pronounced in 'Homa' than 'Sara'. Se-treated plants had consistently higher leaf osmotic potential that was significant in 'Homa' under drought and in 'Sara' under well-watered conditions. Surprisingly, Se effect on the root osmotic potential was the opposite of that observed for the shoot. A significant effect of Se on declining root osmotic potential was observed in 'Homa' grown under drought conditions (Fig. 3B).

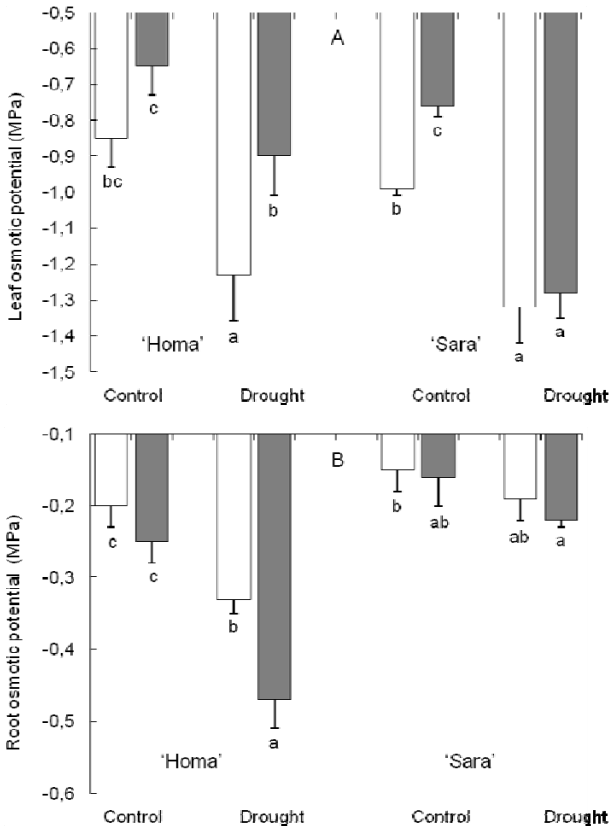


Figure 3: Leaf (A) and root (B) osmotic potentials (MPa) in two wheat genotypes grown under control (100 % FC) and drought (20 % FC) conditions in the absence (–Se, open bars) or presence (+Se, closed bars) of Se (15 µg Se L^{-1} as Na_2SeO_4) for 10 weeks in greenhouse. Data of each column within each genotype indicated by the same letter are not statistically different ($P \leq 0.05$)

Leaf RWC increased significantly by Se application in 'Homa' under well-watered conditions and in 'Sara' under drought stress. Instant *WUE* declined under drought conditions in both genotypes and in greenhouse and field experiments. Se treatment, however, increased this parameter slightly or significantly, under both watering treatments (Table 7).

Leaf concentration of K was lower in drought-stressed plants while that of Ca increased under these conditions in both genotypes. However, effect of drought on K concentration was not significant in 'Sara' (Table 8). Se application did not change K and Ca concentration in the leaves. In contrast to K and Ca, concentration of P in 'Homa' did not respond to drought while it was higher at higher Se application level (60 g ha^{-1}) under both watering regimes. In 'Sara', however, Se did not affect significantly P concentration of leaves while it was slightly lower in drought-stressed plants compared with well-watered ones (Table 8).

Table 7: Relative water content (RWC%) and instant water use efficiency (*WUE*, photosynthesis rate: transpiration rate) in two wheat genotypes grown under control or drought conditions in the absence (–Se) or presence (+Se) of added Se.

Treatments		RWC*	WUE*	WUE**	RWC*	WUE*	WUE**
		'Homa'			'Sara'		
Control	–Se	78.9±1.53 ^{b}	10.67±1.25 ^{ab}	6.49±1.52 ^{b}	84.7±1.35 ^{a}	6.24±0.98 ^{a}	7.07±0.76 ^{b}
	+Se	89.6±1.94 ^{a}	11.11±1.98 ^{a}	11.59±2.67 ^{a}	86.1±1.09 ^{a}	6.61±0.42 ^{a}	15.47±1.77 ^{a}
Drought	–Se	75.1±0.74 ^{c}	6.31±0.98 ^{c}	4.18±0.18 ^{b}	70.9±1.61 ^{c}	2.71±0.81 ^{b}	4.53±1.45 ^{b}
	+Se	77.0±1.92 ^{bc}	7.41±1.82 ^{bc}	6.02±1.21 ^{b}	75.0±1.94 ^{b}	5.11±0.70 ^{a}	7.86±1.78 ^{b}

Data of each column indicated by the same letter are not statistically different ($P \leq 0.05$)
* Measured or calculated for plants grown in greenhouse 10 weeks after starting treatments
** Calculated for young leaves of plants grown in field at Se level of 60 µg ha-1

Table 8: Concentration (mg g^{-1} DW) of K, Ca and P in the leaves of two wheat genotypes grown under control (well-watered) and drought conditions without (–Se) or with two levels of foliar-applied Se (Se_1: 20 g ha^{-1} and Se_2: 60 g ha^{-1}) (as Na_2SeO_4) under field conditions.

Treatments		K	Ca	P	K	Ca	P
		'Homa'			'Sara'		
Control	–Se	165±12 ^{a}	4.82±0.53 ^{b}	0.56±0.24 ^{b}	195±16 ^{a}	5.33±0.98 ^{d}	0.78±0.20 ^{a}
	Se_1	165±13 ^{a}	4.96±0.89 ^{b}	0.58±0.10 ^{b}	181±10 ^{ab}	7.39±0.86 ^{cd}	0.65±0.13 ^{ab}
	Se_2	178±19 ^{a}	4.91±0.52 ^{b}	1.08±0.33 ^{a}	183±12 ^{ab}	5.52±0.80 ^{d}	0.65±0.08 ^{ab}
Drought	–Se	128±18 ^{b}	8.37±1.31 ^{a}	0.35±0.03 ^{b}	171±11 ^{ab}	10.6±1.73 ^{bc}	0.41±0.08 ^{b}
	Se_1	128±15 ^{b}	9.48±1.92 ^{a}	0.37±0.08 ^{b}	163±17 ^{b}	12.8±1.25 ^{b}	0.43±0.11 ^{b}
	Se_2	132±2 ^{b}	7.82±1.76 ^{a}	1.16±0.11 ^{a}	167±4 ^{b}	17.2±2.58 ^{a}	0.49±0.04 ^{b}

Data of each column indicated by the same letter are not statistically different ($P \leq 0.05$)

4 DISCUSSION

4.1 Effect of drought and Se application on plants biomass

Plants biomass was significantly influenced by drought stress under both greenhouse and field conditions. The comparison of two genotypes showed that 'Sara' was more susceptible to drought stress compared with 'Homa' considering vegetative and reproductive stages. It was consistent with the instruction of providing institute on the ranking of drought tolerance in these genotypes. Beside the loss of cell turgor and reduction of cell expansion, the main mechanism reduce crop yield under drought conditions is lowered canopy absorption of photosynthetically active radiation following prolonged stomatal closing (Farooq et al., 2009). Reduction of seed yield and weight under drought, in turn, is related to both source and sink limitations. Apart from source limitation due to reduction of photosynthesis and limited sucrose export to the reproductive sinks, lower capacity of developing seeds to utilize the incoming assimilates and enhanced endogenous abscisic acid concentration are potential factors contributing to reduction of seed yield and weight under drought (Farooq et al., 2009).

Under well-watered conditions, Se application increased vegetative biomass of both genotypes in greenhouse. Effect of Se under field conditions, however, was significant only in 'Homa' and for vegetative but not reproductive growth parameters. Se at 20 g ha^{-1} could be regarded as optimum concentration because higher level (60 g ha^{-1}) did not improve significantly the vegetative growth and reduced slightly reproductive growth parameters. Root diameter was significantly higher in Se-treated plants under well-watered and drought conditions. It has been demonstrated that larger diameter roots would confer drought resistance because they have greater xylem vessel radii and lower axial resistance to water flux, with great penetration ability (Gowda et al., 2011).

Higher P (and partly K) concentration at higher Se application level (60 g ha^{-1}) resulted likely from Se-mediated changes in the root morphology (length and diameter) that improved uptake of nutrients particularly those with higher dependency to spatial availability.

4.2 Effect of drought and Se application on leaf pigments, photochemistry and gas exchange

Leaf concentrations of Chl (significantly) and Car (slightly) was higher in drought-stressed plants obviously because of a concentration effect following higher reduction of leaf weight and area but less destruction of Chl and Car. Reduction of leaf Chl concentration and damages to chloroplasts occur under drought stress when particularly associated with higher light intensity (Hajiboland, 2014). In our greenhouse experiment, drought stress in the absence of higher light intensity, was likely not effective for a high generation rate of reactive oxygen species, chloroplasts damages and Chl destruction.

Nevertheless, anthocyanins and flavonoids concentrtaions rather decreased under water deficiency conditions. It implies that effect of leaf desiccation on the anthocyanins and flavonoids synthesis was much more than that for Chl and Car.

The most prominent effect of Se on leaf pigments was observed for anthocynainns and flavonoids. Accumulation of anthocyanins is believed to protect the cellular structures from oxidative damage (Wahid and Ghazanfar, 2006). Plant tissues containing anthocyanins are usually tolerant to drought (Ichikawa et al., 2001). This protection is related to the superoxide radical scavenging activity of anthocyanins (Ichikawa et al., 2001) and their ability to stabilize the water potential (Chalker-Scott, 2002). The contribution of flavonoids to the antioxidant defense capacity of plants and its relevance in plant responses to drought have been widely accepted (Fini et al., 2011). Evidences showed that flavonoids constitute a secondary free radicals-scavenging system in plants exposed to severe and prolonged stress conditions (Fini et al., 2011).

In contrast to growth parameters, leaf photochemical parameters were affected adversely by drought in ‘Homa’ but not in ‘Sara’. The preservation of Chl fluorescence parameters in ‘Sara’ under drought conditions indicated that photochemical events conserved their normal activities in this genotype. Reduction of optimal photochemical efficiency of PSII in dark-adapted leaves (F_v/F_m) and excitation capture efficiency of light-adapted leaves (F'_v/F'_m) both indicated occurrence of photoinhibition and damage to PSII in ‘Homa’. Environmental stresses such as drought and salinity reduce the activity of the Calvin cycle directly or indirectly, and result in a decline of $NADP^+$ regeneration, thus, over-reduction of the electron transport chain and photoinhibition and dames to the photosystems (Hajiboland, 2014; Noctor et al., 2014). Amounts of F_v/F_m, F'_v/F'_m and *ETR* under drought conditions was completely returned by Se application to its control levels in ‘Homa’. In addition, higher non-photochemical quenching (*qN*) in drought-stressed plants due to Se application observed in this study implied elevation of capacity for dissipation of excess absorbed energy as heat and thus more protection of photosynthetic apparatus (Müller et al., 2001).

Stomatal conductance was decreased significantly 4 weeks after imposing drought in ‘Homa’ leading to lower transpiration rate in this genotype. This effect, however, was less pronouncedly observed in ‘Sara’ without reduction of transpiration rate. In the two following measurement intervals in greenhouse and field experiment, however, both genotypes showed similar reduction of stomatal conductance and transpirational water loss. Stomatal closure is a fast response of plants to water deficit allowing reduction of the transpiration rate and conservation of relative water content (Zhou et al., 2013). Reduction of stomatal conductance under drought decreased net assimilation rate that was significant at all measurement intervals in the greenhouse experiment and in both leaf types in the field experiment. In addition, reduction of CO_2 availability for photosynthetic dark reactions may result in generation of excess excitation energy under drought conditions and causes damages to photosynthetic apparatus (Hajiboland, 2014) that was also reflected in Chl fluorescence parameters in this work.

Se treatment influenced gas exchange parameters more prominently than photochemical events. In general, stomatal conductance, transpiration rate

and particularly net assimilation rates were higher in Se-treated plants compared with plants without Se under both watering regimes. A significant elevation of net assimilation rate may explain growth improvement by Se not only under drought but also under well-watered conditions. Higher ability for CO_2 fixation increases plants capability for dry matter production under well-watered and for osmolytes accumulation under drought conditions. However, significantly higher photosynthesis rate at 60 g ha^{-1} compared with application of lower Se (20 g ha^{-1}) in well-watered plants was not associated with higher vegetative or reproductive growth parameters. Similarly, an elevated photosynthetic rate up to 3 fold did not result in significantly higher biomass in drought-stressed plants. Lack of a close relationship between photosynthesis rate and plants growth under both watering regimes suggested that there were some limiting factors e.g. low nutrients availability, which prevent maximum response of dry matter production to added Se.

Mechanisms for Se-mediated increase in photosynthesis rate have not been studied in detail. Possible effect of Se on the H^+-pumping and K^+ currents in stomatal cells has not been characterized so far. In addition, non-stomatal mechanisms are also likely involved in the Se effect on photosynthesis. Through proteomic analysis, it was revealed that primary metabolism, photosynthesis and redox homeostasis are the most highly affected biological processes by Se treatments (Wang et al., 2012). Effect of Se on the activation of fructose 1,6 bisphosphatase in alfalfa (Owusu-Sekyere et al., 2013) and a concomitant activation of NO_3^- and CO_2 assimilation rate in wheat (Hajiboland and Sadeghzadeh, 2014) have been previously reported.

4.3 Effect of Se application and drought on the organic osmolytes

Increases in soluble proteins in both leaves and roots of Se-treated plants under both watering regimes that was associated with lower free α-amino acids in the leaves (but not in the roots) may imply depletion of amino acids pool following elevated protein synthesis by Se in the leaves. Lack of such depletion in the roots may be attributed to higher nitrate uptake and assimilation exceeding the requirement for protein synthesis in this organ. Our previous study showed that Se-treated plants have higher nitrate uptake and nitrate reductase activity being much more pronounced in the roots compared with the leaves (Hajiboland and Sadeghzadeh, 2014). Higher concentrations of soluble proteins and free amino acids under drought observed here may confer higher osmotic adjustment capacity and protect cell structures against desiccation (Krasensky and Jonak, 2012).

Proline concentration was expectedly higher in drought-stressed plants whereas Se effect depended on plants organ and genotype. In the roots of both genotypes, Se treatment did not cause accumulation of proline despite lower root osmotic potential. It has been stated that, lower proline accumulation is mainly a reflection of an increased salt resistance in plants, i.e. less injury (Lutts et al., 1999). Here lower proline content of Se-treated plants under drought suggests that they were less strained due to some ameliorating mechanisms. In the leaves genotypic difference was observed in proline accumulation in response to Se. In another study, drought-stressed wheat had higher leaf proline concentration when exposed to Se (Yao et al., 2009).

Desiccation-induced accumulation of soluble sugars observed in this work is a well-known response that is either the result of increased partitioning of photoassimilates to the synthesis of free sugars and/or enhanced starch degradation (Lee et al., 2008). Free soluble carbohydrates are effective compounds in osmotic homeostasis, protection of membranes and cell structures against dehydration and have free radicals scavenging activity (Niedzwiedz-Siegien et al., 2004). Se-mediated accumulation of soluble sugars and starch has previously been reported in lettuce (Pennanen et al., 2002) and potato (Turakainen et al., 2004). However, effect of Se application on this parameter under different water supply level and its role in drought tolerance has not been investigated so far. In this work, Se treatment increased concentration of soluble sugars in both leaves and roots of well-watered and drought-stressed plants. It may be primarily attributed to the higher photosynthesis and CO_2 assimilation. This suggestion is confirmed by concomitantly higher starch concentration of Se-treated plants that in turn, excludes the contribution of starch

degradation to the increased concentration of soluble sugars upon Se application.

4.4 Effect of Se application and drought on water relation parameters

Se treatment increased leaf osmotic potentials while decreased it in the roots. Such differential effect of Se on leaf and root osmotic potentials may be resulted from higher root-shoot water transport that could be related in turn, to higher root hydraulic conductivity in Se-treated plants. Root-to-leaf conductance declines during drought and have the greater influence under transpirational conditions compared with soil-to-root component of water pathway (Sperry, 2000). Much of the decline in root-to-shoot hydraulic conductance could be explained by xylem cavitation under drought (Sperry, 2000). Regarding changes in the root morphology and increase in the ratio of thick roots in the wheat plants of this study under Se treatment it could be speculated that hydraulic conductivity of whole root system was higher in Se-treated plants likely because of higher diameter of xylem conduits in the thick roots as was observed in the tick roots of rice (Gowda et al., 2011). To our best knowledge, there is no published work on the changes in water relation parameters as affected by Se in drought-stressed plants. Thus, comparison of our results with other works was not possible. Regardless of mechanism, elevation of leaf osmotic potential upon Se treatment may contribute significantly to the maintenance of biochemical reactions under drought. Lower root osmotic potential, in turn, may allow plants to take up water more efficiently from dry substrate. This parameter together with greater root diameter plays likely important roles for improvement of water uptake capacity in Se-treated plants.

5 REFERENCES

Ashraf, M. 2010 Inducing drought tolerance in plants: Recent advances. Biotechnol. Adv. 28: 169-183, DOI: 10.1016/j.biotechadv.2009.11.005

Bates, L.S., Waldren, R.P., Teare, I.D. 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39: 205-207, DOI: 10.1007/BF00018060

Chalker-Scott. L. 2002. Do anthocyanins function as osmoregulators in leaf tissues? Adv. Bot. Res. 37: 103-106, DOI: 10.1016/S0065-2296(02)37046-0

Chaves, M.M., Flexas, J., Pinheiro, C. 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann. Bot. 103: 551-560.DOI: 10.1093/aob/mcn125

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S.M.A. 2009. Plant drought stress: effects, mechanisms and management. Agron. Sustain. Dev. 29: 185-212, DOI: 10.1051/agro:2008021

Feng, R., Wei, C., Tu, S. 2013. The roles of selenium in protecting plants against abiotic stresses. Environ. Exp. Bot. 87: 58-68, DOI: 10.1016/j.envexpbot.2012.09.002

Fini, A., Brunetti, C., Di Ferdinando, M., Ferrini, F., Tattini, M. 2011. Stress-induced flavonoid biosynthesis and the antioxidant machinery of plants. Plant Signal. Behav. 6: 709-711, DOI: 10.4161/psb.6.5.15069

Giusti, M.M., Wrolstad, R.E. 2001. Characterization and measurement of anthocyanins by UV–Visible spectroscopy. In: Current Protocols in Food Analytical Chemistry. Wrolstad, R.E., Acree, T.E., An, H., Decker, E.A., Pennere, M.H., Reid, D.S., Schwartz, S.J., Shoemaker, C.F., Sporns, P. (eds.). New York, John Wiley: F1.2.1–F1.2.13, DOI: 10.1002/0471142913.faf0102s00

Gowda, V.R.P., Henry, A., Yamauchi, A., Shashidhar, H.E., Serraj, R. 2011. Root biology and genetic improvement for drought avoidance in rice. Field Crops Res. 122: 1–13, DOI: 10.1016/j.fcr.2011.03.001

Grayer, R.J. 1989. Flavonoids. In: Methods in Plant Biochemistry, Vol. 1, Plant Phenolics. Dey, P.M., Harborne, J.B. (eds.) London, academic Press: 283-323.

Habibi, G. 2013. Effect of drought stress and selenium spraying on photosynthesis and antioxidant activity of spring barley. Acta Agric. Slov. 101: 31-39, DOI: 10.2478/acas-2013-0004

Hajiboland, R. 2012. Effect of micronutrient deficiencies on plant stress responses. In: Abiotic Stress Responses in Plants. Ahmad, P., Prasad, M.N.V. (eds.). New York, Springer: 283-329, DOI: 10.1007/978-1-4614-0634-1_16

Hajiboland, R. 2014. Reactive oxygen species and photosynthesis. In: Oxidative Damage to Plants,

Antioxidant Networks and Signaling. Ahmad, P. (ed.). New York, Springer: 1-63, 10.1016/B978-0-12-799963-0.00001-0

Hajiboland, R., Sadeghzadeh, N. 2014. Effect of selenium supplementation on CO_2 and NO_3^- assimilation under low and adequate N supply in wheat (*Triticum aestivum* L.) plants. Photosynthetica, DOI: 10.1007/s11099-014-0058-1.

Hajiboland, R., Yang, X.E., Römheld, V. 2003. Effects of bicarbonate and high pH on growth of Zn-efficient and Zn-inefficient genotypes of rice, wheat and rye. Plant Soil 250: 349-357, DOI: 10.1023/A:1022862125282

Hasanuzzaman, M., Fujita, M. 2011. Selenium pretreatment upregulates the antioxidant defense and methylglyoxal detoxification system and confers enhanced tolerance to drought stress in rapeseed seedlings. Biol. Trace Element Res. 143: 1758-1776, DOI: 10.1007/s12011-011-8998-9

Ichikawa, H., Ichiyanagi, T., Xu, B., Yoshii, Y., Nakajima, M., Konishi, T. 2001. Antioxidant activity of anthocyanin extract from purple black rice. J. Med. Food 4: 211-218, DOI: 10.1089/10966200152744481

Jaiswal, P.C. 2004. Soil, plant and water analysis. New Delhi, Kalyani Publishers.

Krall, J.P., Edwards, G.E. 1992. Relationship between photosystem II activity and CO_2 fixation in leaves. Physiol. Plant. 86: 180-187, DOI: 10.1111/j.1399-3054.1992.tb01328.x

Krasensky, J., Jonak, C. 2012. Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. J. Exp. Bot. 63: 1593-1608, DOI: 10.1093/jxb/err460

Lee, B.R., Jin, Y.L., Jung, W.J., Avice, J.C., Morvan-Bertrand, A., Ourrt, A., Park, C.W., Kim, T.H. 2008. Water deficit accumulates sugars by starch degradation-not by de novo synthesis-in white clover leaves (*Trifolium repens*). Physiol. Plant. 134: 403-411, DOI: 10.1111/j.1399-3054.2008.01156.x

Liang, Y.C., Sun, W., Zhu, Y-G., Christie, P. 2007. Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: a review. Environ. Pollut 147: 422-428, DOI: 10.1016/j.envpol.2006.06.008

Lichtenthaler, H.K., Wellburn, A.R. 1985. Determination of total carotenoids and chlorophylls a and b of leaf in different solvents. Biol. Soc. Trans. 11: 591-592, DOI: 10.1042/bst0110591

Lutts, S., Majerus, V., Kinet, J.M. 1999. NaCl effects on proline metabolism in rice (*Oryza sativa*) seedlings. Physiol. Plant. 105: 450-458, DOI: 10.1034/j.1399-3054.1999.105309.x

Lyons, G., Stangoulis, J., Graham, R. 2003. High-selenium wheat: biofortification for better health. Nutr. Res. Rev. 16: 45-60, DOI: 10.1079/NRR200255

Mitra, J. 2001. Genetics and genetic improvement of drought resistance in crop plants. Curr. Sci. 80: 758-763

Müller, P., Li, X.P., Niyogi, K.K. 2001. Non-photochemical quenching. A response to excess light energy. Plant Physiol. 125: 1558-1566, DOI: 10.1104/pp.125.4.1558

Niedzwiedz-Siegien, I., Bogatek-Leszczynska, R., Come, D., Corbineau, F. 2004. Effects of drying rate on dehydration sensitivity of excised wheat seedling shoots as related to sucrose metabolism and antioxidant enzyme activities. Plant Sci. 167: 879-888, DOI: 10.1016/j.plantsci.2004.05.042

Noctor, G., Mhamdi, A., Foyer, C.H.A. 2014. The roles of reactive oxygen metabolism in drought: not so cut and dried. Plant Physiol. 164: 1636-1648, DOI: 10.1104/pp.113.233478

Owusu-Sekyere, A., Kontturi, J., Hajiboland, R., Rahmat, S., Aliasgharzad, N., Hartikainen, H., Seppänen, M.M. 2013. Influence of selenium (Se) on carbohydrate metabolism, nodulation and growth in alfalfa (*Medicago sativa* L.). Plant Soil 373: 541-552, DOI: 10.1007/s11104-013-1815-9

Pennanen, A., Xue, T., Hartikainen, H. 2002. Protective role of selenium in plant subjected to severe UV irradiation stress. J. Appl. Bot. 76: 66-76

Sperry, J.S. 2000. Hydraulic constraints on plant gas exchange. Agric. Forest Meteorol. 104: 13-23, DOI: 10.1016/S0168-1923(00)00144-1

Tardieu, F., Parent, B., Caldeira, C.F., Welcker, C. 2014. Genetic and physiological controls of growth under water deficit. Plant Physiol. DOI:10.1104/pp.113.233353.

Tennant, D. 1975. A test of modified line intersect method of estimating root length. J. Ecol. 63: 995-1001, DOI: 10.2307/2258617

Turakainen, M., Hartikainen, H., Seppänen, M.M. 2004. Effects of selenium treatments on patato (*Solanum tuberosum* L.) growth and concentrations of soluble sugars and starch. J. Agric. Food Chem. 52: 5378-5382, DOI: 10.1021/jf040077x

Valliyodan, B., Nguyen, H.T. 2006. Understanding regulatory networks and engineering for enhanced

drought tolerance in plants. Curr. Opin. Plant Biol. 9: 1-7, DOI: 10.1016/j.pbi.2006.01.019

Verbruggen, N., Hermans, C. 2008. Proline accumulation in plants: a review. Amino Acids 35: 753-759, DOI: 10.1007/s00726-008-0061-6

Wahid, A., Ghazanfar, A. 2006. Possible involvement of some secondary metabolites in salt tolerance of sugarcane. J. Plant Physiol. 163: 723-730, DOI: 10.1016/j.jplph.2005.07.007

Wang, C.Q. 2011. Water-stress mitigation by selenium in *Trifolium repens* L. J. Plant Nutr. Soil Sci. 174: 276-282, DOI: 10.1002/jpln.200900011

Wang, Y.D., Wang, X., Wong, Y.S. 2012. Proteomics analysis reveals multiple regulatory mechanisms in response to selenium in rice. J. Proteomics 75: 1849-1866, DOI: 10.1016/j.jprot.2011.12.030

Yao, X.Q., Chu, J.Z., Wang, G.Y. 2009. Effects of drought stress and selenium supply on growth and physiological characteristics of wheat seedlings. Acta Physiol. Plant. 31: 1031-1036., DOI: 10.1007/s11738-009-0322-3

Yemm, E.W., Cocking, E.C. 1955. The determination of amino acids with ninhydrin. Analyst 80: 209-213, DOI: 10.1039/an9558000209

Yemm, E.W., Willism, A.J. 1954. The estimation of carbohydrates extracts by anthrone. Biochem. J. 57: 508-514, DOI: 10.1042/bj0570508

Zhou, S., Duursma, R.A., Medlyn, B.E., Kelly, J.W.G., Prentice, I.C. 2013 How should we model plant responses to drought? An analysis of stomatal and non-stomatal responses to water stress. Agric. Forest Meteorol. 182/183: 204-114, DOI: 10.1016/j.agrformet.2013.05.009

COBISS Code 1.01

DOI: 10.14720/aas.2015.105.2.02
Agrovoc descriptors: allelopathy, amaranthaceae, weeds, biological competition, crops, tolerance, resistance to injurious factors, germination, growth

Agris category code: f62, f40, h60

Allelopathic effects of redroot pigweed (*Amaranthus retroflexus* L.) on germination & growth of cucumber, alfalfa, common bean and bread wheat

Hamideh BAKHSHAYESHAN-AGDAM[1], Seyed Yahya SALEHI-LISAR[1]*, Rouhollah MOTAFAKKERAZAD[1], Amirhosein TALEBPOUR[2] and Nader FARSAD[1]

Received March 09, 2015; accepted July 27, 2015.
Delo je prispelo 09. marca 2015, sprejeto 27. julija 2015.

ABSTRACT

Allelopathy is one of the important interactions among plants. Weeds can reduce crops productions in farms by their allelopathic effects. Redroot pigweed (*Amaranthus retroflexus* L.) is the most common weed in Iran with well-known allelopathic potential. In the presented experiment, the allelopathic effects of redroot pigweed on germination and growth of four important crop species including cucumber (*Cucumis sativus* L.), alfalfa (*Medicago sativa* L.), common bean (*Phaseulus vulgaris* L.) and bread wheat (*Triticum aestivum* L.) was studied. The effect of different concentrations of redroot pigweed leachate on seed germination and seedlings growth parameters of tested plants was significant, but not same in all studied species. Bread wheat and cucumber were more resistance in seed germination stage in comparison to common bean and alfalfa. Except alfalfa, all plant species showed certain rate of resistance in the most measured parameters. According to the obtained results, bread wheat and common bean were the most resistant species, cucumber was resistant at low concentration but sensitive at high concentration, and alfalfa was the most sensitive species to the redroot pigweed leachate treatments. Therefore, the cultivation of resistant plant species (such as bread wheat and common bean plants) in the regions with redroot pigweed's invasion is appropriate way in management of the farms.

Key words: allelopathy, redroot pigweed, resistance, crop species, leachate concentration

IZVLEČEK

ALELOPATIČNI UČINEK NAVADNEGA ŠČIRA (*Amaranthus retroflexus* L.) NA KALITEV IN RAST KUMAR, LUCERNE, NAVADNEGA FIŽOLA IN KRUŠNE PŠENICE

Alelopatija je ena izmed najpomembnejših interakcij med rastlinami. Pleveli lahko zmanjšajo pridelek zaradi njihovih alelopatičnih učinkov. Navadni ščir (*Amaranthus retroflexus* L.) je v Iranu najpogostejši plevel z dobro znanim alelopatičnim učinkom. V tej raziskavi smo preučevali alelopatski učinek navadnega ščira na kalitev in rast štirih pomembnih kulturnih rastlin in sicer kumar (*Cucumis sativus* L.), lucerne (*Medicago sativa* L.), navadnega fižola (*Phaseulus vulgaris* L.) in krušne pšenice (*Triticum aestivum* L.). Učinek različnih koncentracij izvlečka navadnega ščira na kalitev in rastne parameter preiskuševanih rastlin je bil značilen toda ne enak pri vseh rastlinah. Krušna pšenica in kumare so bile bolj odporne na stopnji kalitve v primerjavi s fižolom in lucerno. Z izjemo lucerne so vse preiskušene vrste pokazale določeno odpornost pri vseh merjenih parametrih. Glede na rezultate te raziskave sta se krušna pšenica in navadni fižol izkaza kot najbolj odporna, kumare so bile pri manjših koncentracijah ekstrakta navadnega ščira odporne, a občutljive pri velikih koncentracijah. Lucerna je bila najbolj občutljiva na izločke navadnega ščira pri vseh obravnavanjih. Na osnovi dobljenih rezultatov priporočamo kmetovalcem na območjih z večjim pojavljanjem navadnega ščira gojenje nanj odpornih rastlin kot sta krušna pšenica in navadni fižol.

Ključne besede: alelopatija, navadni ščir, odpornost, kmetijske rastlines, koncentracije izvlečkov

[1] Department of Plant Sciences, Faculty of Natural Sciences, University of Tabriz, Tabriz-5166616471, Iran; *Corresponding author: y_salehi@tabrizu.ac.ir

[2] Agricultural and Natural Resources Research Center of East Azerbaijan, Tabriz- 5716964455, Iran

1 INTRODUCTION

Farmers have realized long-time ago that in addition to cultivated crop species, specific plants are growing in agricultural land called weeds (Dhole et al., 2011; Salehian and Eshaghi, 2012; Modhej et al., 2013). In addition to reduction of resources available to crop species (competition), allelochemical compounds produced by weed may also affect plants growth (allelopathy) (Shahrokhi et al., 2011; Modhej et al., 2013; Konstantinović et al., 2014). Allelopathy is one of the most important interactions between plants (Amini et al., 2012; Amini, 2013; Konstantinović et al., 2014), which occurs via production of certain compounds called allelochemicals, mainly a subset of secondary metabolites (Khan et al., 2010; Amini et al., 2012; Soltys et al., 2013). Plants can release these compounds into the environment by different ways such as leaching the allelopathic materials from the shoot by rainfall, releasing volatile phytotoxic compounds from plant's green parts, releasing phytotoxic compounds from decomposed plant material and eventually, releasing phytotoxic compounds by the root exudates (Weir et al., 2004; Terji, 2008; Amini et al., 2012; Soltys et al., 2013). Almost 250 weed species that have invaded farms have been identified which have the potential to produce allelopathic compounds (Shahrokhi et al., 2011). Although many species from the genus *Amaranthus* are weeds, the redroot pigweed *(Amaranthus retroflexus* L.*)* is the most famous with the well-known allelopathic effects (Costea et al., 2004; Shahrokhi et al., 2011; Shahrokhi et al., 2012; Konstantinović et al., 2014). Moreover redroot pigweed is a common weed in Iran and can be seen frequently on agricultural lands (Shahrokhi et al., 2011; Shahrokhi et al., 2012). This plant is one of the main components of desert and semi-desert's flora (Lamonico, 2010; Duretto and Morris, 2011) and expansion from Iran in the desert belt of the world could be the reason of its high distribution in this country. Redroot pigweed is summer annual C_4 species with high biological potential and can produce a lot of seeds (Lamonico, 2010; Duretto and Morris, 2011; Amini et al., 2012; Shahrokhi et al., 2012). It is one of the few resistant plants to several herbicides, including atrazine, simazine, imazethapyr, thifensulfuron, and linuron (Costea et al., 2004; Sarabi et al., 2011).

Many researchers have reported the allelopathic effects of redroot pigweed on different crops (Shahrokhi et al., 2011; Tejeda-Sartorius et al., 2011; Amini et al., 2012; Dogaru et al., 2012; Mlakar et al., 2012; Namdari et al., 2012; Shahrokhi et al., 2012; Konstantinović et al., 2014). Nodaway, several allelic compounds such as aldehydes, alkaloids, apocarotenoids, flavonoids, steroids, xyloids, chlorogenic acid and saponins have been identified from amaranth residues (Shahrokhi et al., 2011; Shahrokhi et al., 2012).

The recent study was carried out in order to evaluate the allelopathic effects of different leachate concentrations of redroot pigweed on germination and growth of some important and common crop species in Iran in order to determine their sensitivity and resistance to chemicals produced by redroot pigweed.

2 MATERIALS AND METHODS

2.1 Experimental design and treatments

The experiment was conducted as factorial based on completely randomized design (CRD) with 3 replications. Experimental factors were crop species at four levels including cucumber (*Cucumis sativus* 'Basmenj'), alfalfa (*Medicago sativa* 'Hamedan'), common bean (*Phaseulus vulgaris* 'Dorsa') and bread wheat (*Triticum aestivum* 'Pishgam') and different concentrations of redroot pigweed leachate (5 % and 10 %). Double distilled water was considered as control.

2.2 Sampling and plant extract preparation

Redroot pigweed fresh material including root, stem, leaf and flower was collected from crop fields of Khosroshahr (East Azerbaijan, Iran) and powdered after air drying under lab conditions. For leachate preparation, 10 grams of powdered material were suspended in100 ml double distilled

water and mixed for 24 hours by a horizontal rotary shaker for producing uniform suspension (Shahrokhi et al., 2011; Mlakar et al., 2012; Shahrokhi et al., 2012). Suspension was filtered using two layers of sterile cheese cloth and this filtrate was considered as leachate. Furthermore, leachate 5 % was prepared by dilution of leachate 10 % using double distilled water.

2.3 Plant culture and bioassay tests

For evaluation of the allelopathic effect of weed extracts on germination and growth of cucumber, alfalfa, common bean and bread wheat seeds were disinfected using 1 % (v/v) sodium-hypochlorite solution for 5 minutes and washed sufficiently using sterile distilled water. Ten seeds of each species were placed in Petri dishes containing sterile filter paper and 5 ml of leachate with appropriate concentration were added. Control seeds were moistened with 5 ml of sterile double distilled water. Petri dishes were sealed with parafilm for prevention of pollution and water evaporation and transferred to darkness. After 2 days all germinated seeds were transferred to climate chambers with controlled conditions (25-30 °C, 16/8 (light/dark) photoperiod and relative humidity of 60 %). The percentage of germinated seeds was recorded daily and growth parameters like seedling length, shoot length, seminal root length, fresh and dry weight of seedlings were determined after 7 days. Relative growth rate (Equation 1) (Tomlinson et al., 2012) and seedling survival rate (Equation 2) (Kusmana, 2010) were calculated using following formulae:

$$\text{Equation 1: } RGR = \frac{\Delta y}{y \Delta t} \times 100$$

Where RGR is relative growth rate, Δy is growth amount, Δt is growth time (day) and y is the fresh/dry weight of primary tissue or organ.

$$\text{Equation 2: } SSR = \frac{A}{B} \times 100$$

Where SSR is seedling survival rate, A is number of germinates and B is the number of germinated seeds.

2.4 Data analysis

The data were analyzed using GLM procedure by SPSS software (Ver.16) and Tukey's multiple range tests was used for mean comparisons at 1 % probability level.

3 RESULTS

According to statistical evaluation (analysis of variance), the effect of different concentrations of redroot pigweed leachate on seed germination percentage; seedling, shoot and seminal root length; fresh and dry weight of seedlings and its interaction with crop species was significant ($p < 0.01$). However, the effect of different leachate concentrations on germination and growth of species was not the same (Table 1).

Table 1: Mean squares of redroot pigweed leachate's concentrations effect on germination and growth related characteristics of crop species

Source of variation	Df	Shoot Length	Seminal root Length	Seedling Length	Fresh Weight	Dry Weight	Germin. Percentage
Treatments	2	3148.0 **	16473.4 **	31332.8 **	88395.3 **	6706.9 **	5077.8 **
Species	3	2431.3 **	2949.7 **	10382.9 **	194355.7 **	74284.8 **	3425.9 **
Treatments*Species	6	852.1 **	1585.8 **	3835.2 **	19268.8 **	4578.5 **	848.2 **
Error	24	17.67	46.53	122.22	3046.29	844.98	75.0
Coefficient of variation (%)		11.67	18.94	30.70	153.31	80.74	24.05

**: significant at $p < 0.01$ by Tukey's multiple range tests, n = 3

3.1 Seed germination

Except for bread wheat plants, treatment with 5 % redroot pigweed leachate of led to decrease in seed germination. The highest reduction was recorded in alfalfa. Treatment of plants with 10 % redroot pigweed leachate led to a decrease in seed germination in all species. Bread wheat and alfalfa plants showed the lowest and the highest decrease in seed germination respectively. According to the results of germination percentage, bread wheat and cucumber were identified as the most resistant, and alfalfa and common bean were classified as the most sensitive plants among studied species (Figure 1). Daily recording of germination percentage indicated a delay phase in germination of treated plants in all species (Figure 2). The results of these records were also similar to the germination percentage. On the other hand, bread wheat plants showed the lowest delay time, whereas the highest delay time was observed in alfalfa.

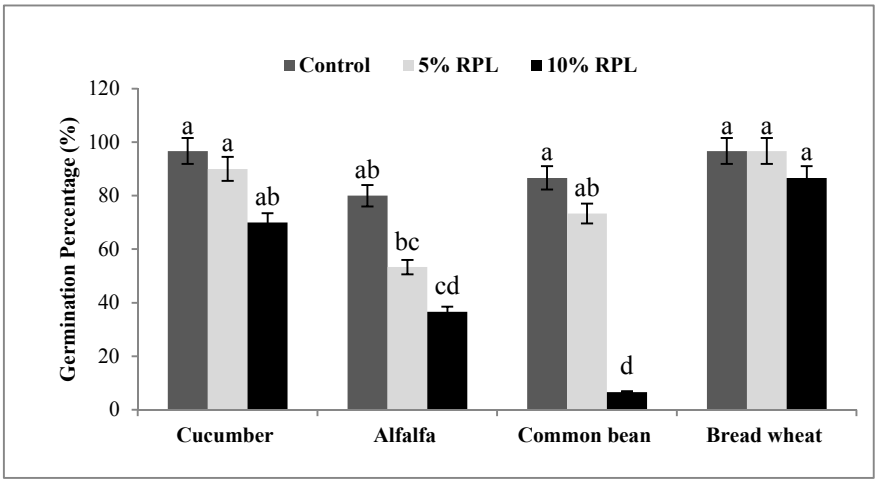
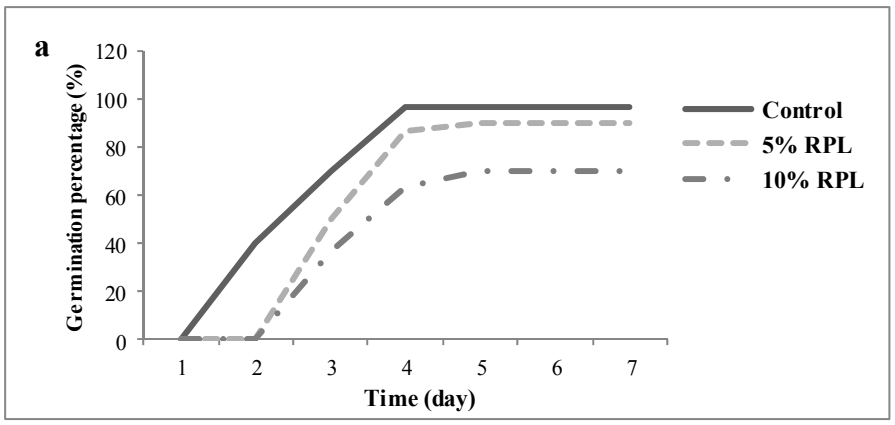


Figure 1: The effect of different concentrations (5 % and 10 %) of the redroot pigweed's leachate (RPL) on seed germination of crop species (n = 3, $p < 0.01$).



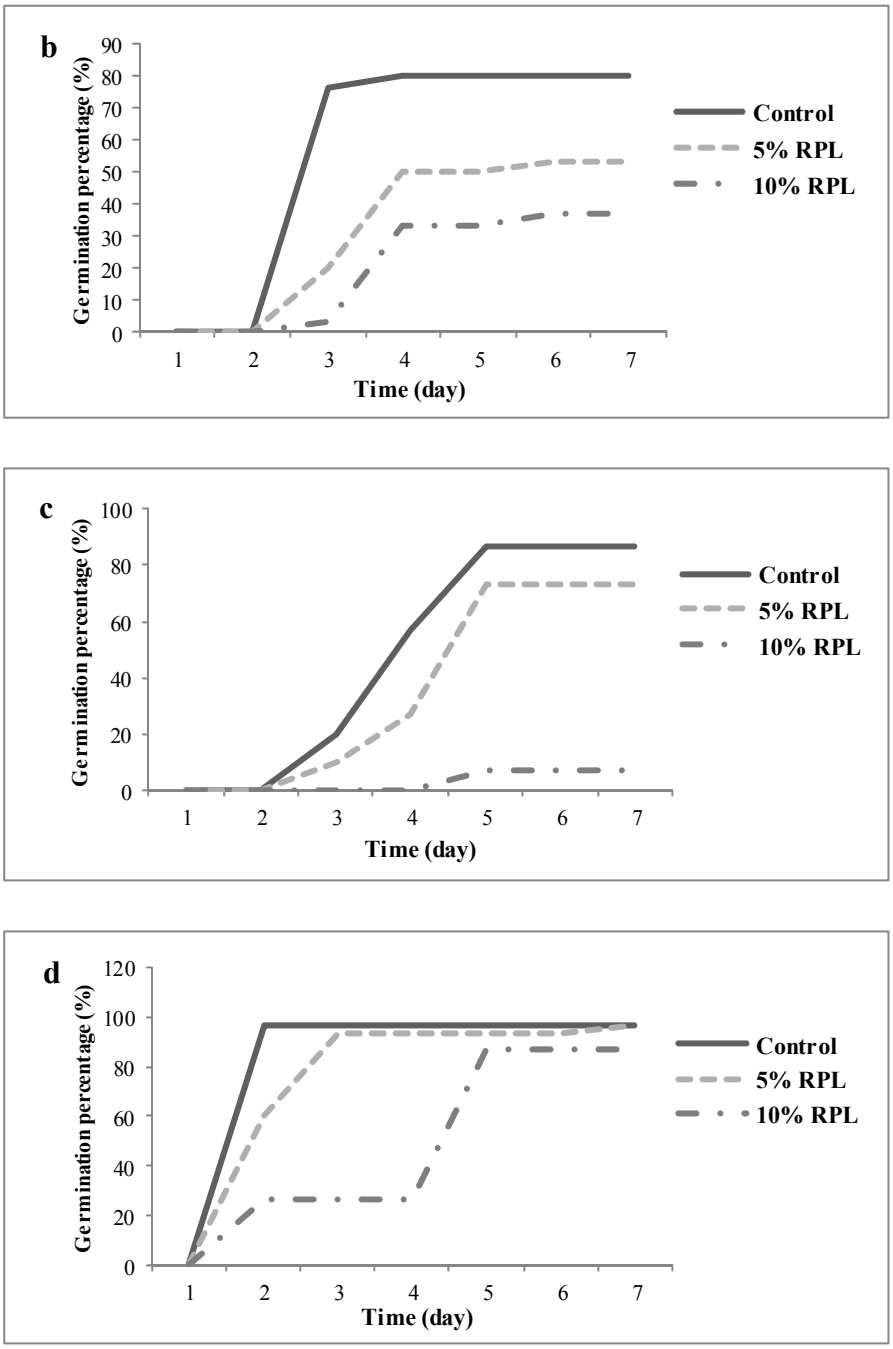


Figure 2: Effects of redroot pigweed leachate (RPL, 5 and 10 %) on seed germination of (a) cucumber, (b) alfalfa, (c) common bean, (d) bread wheat during 7 days after treatment (n = 3, $p < 0.01$).

3.2 Seedling survival rate (SSR)

Only the alfalfa seedling survival rate (SSR) significantly decreased in plants treated with 5 % redroot pigweed leachate and other species were not affected significantly ($p < 0.01$). Treatment of 10 % redroot pigweed leachate led to considerable decrease in alfalfa and cucumber seedling survival rate, while this was not notably affective on bread wheat and common bean plants. Analysing this parameter, bread wheat and common bean were the most resistant, cucumber was relatively sensitive and alfalfa was the most sensitive plants in response to the redroot pigweed leachate (Figure 3).

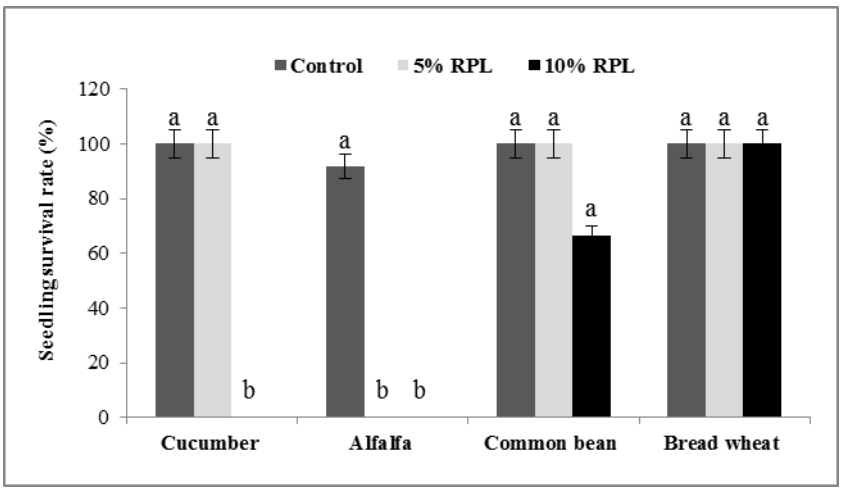


Figure 3: The effects of different concentrations (5 % and 10 %) of the redroot pigweed's leachate (RPL) on seedling survival rate of crop species (n = 3, $p < 0.01$).

3.3 Shoot, seminal root and seedling length

Shoot length of cucumber was not notably affected by treatment with 5 % of redroot pigweed leachate, but in bread wheat and alfalfa this parameter decreased significantly ($p < 0.01$). In common bean plants shoot length decreased 19.24 % in comparison with control plants, but was not significant (Table 2). Significant decrease in shoot length of bread wheat; cucumber and alfalfa were observed in plants treated by leachate 10 %. In common bean plant, this parameter decreased by 27.62 %, but the trend was not significant ($p < 0.01$) (Table 2). According to the obtained results, common bean and bread wheat were the most resistant plants; cucumber was moderately sensitive and alfalfa was the most sensitive species to the redroot pigweed leachate, respectively.

In all studied species seminal root length was significantly shorter in plants treated with 5 % and 10 % leachate of redroot pigweed plant, however, the difference among plants treated with 5 % and 10 % leachate was only significantly different in cucumber plant ($p < 0.01$) (Table 2). Therefore, according to this parameter, common beans and bread wheat were the most resistant plants; cucumber and alfalfa were the most sensitive species to redroot pigweed plant.

The results of seedling length were similar to the results of shoot and seminal root length. Common bean and bread wheat were classified as the most resistant species, cucumber was moderately susceptible and alfalfa was the most sensitive species to redroot pigweed leachate (Table 2).

Redroot pigweed leachate increased shoot/seminal root length ratio of cucumber, bread wheat and common bean plants and the ratio was higher in plants treated with 10 % leachate (Table 2). The shoot/seminal root length ratio in cucumber, common bean and bread wheat plants treated with 5 % leachate were 4.21, 2.98 and 3.71 times bigger than in control plants, respectively. In bread wheat and common bean plants treated with 10 % leachate shoot/seminal root length ratio was 6.01 and 8.154 times higher, respectively, when compared to controls. However, the effect of redroot pigweed leachate on shoot/seminal root length ratio and was not significant ($p < 0.01$). Considering this parameter, among studied species bread wheat and common bean can be classified as the most resistant, cucumber as moderately susceptible and alfalfa as the most sensitive to redroot pigweed leachate.

Table 2: The effect of different concentrations (5 and 10 %) of the redroot pigweed's leachate on shoot, seminal root and seedling length (mm) and shoot/seminal root length ratio of crop species (n = 3, $p < 0.01$).

Plant Species		Parameters			
		Shoot Length	Seminal root Length	Seedling Length	Shoot/ Seminal root
Cucumber	Control	61.07±2.99 [a]	123.79±11.18 [a]	181.09±19.04 [a]	0.494±0.02 [b]
	5 %	63.67±2.46 [a]	30.99±4.41 [b]	94.68±5.23 [b]	2.081±0.31 [a]
	10 %	0.0±0.0 [b]	0.0±0.0 [c]	0.0±0.0 [c]	0.0±0.0 [c]
Alfalfa	Control	11.46±1.10 [a]	30.28±4.78 [a]	41.75±5.35 [a]	0.383±0.06 [a]
	5 %	0.0±0.0 [b]	0.0±0.0 [b]	0.0±0.0 [b]	0.0±0.0 [b]
	10 %	0.0±0.0 [b]	0.0±0.0 [b]	0.0±0.0 [b]	0.0±0.0 [b]
Common bean	Control	19.33±0.72 [a]	41.24±5.93 [a]	60.57±6.46 [a]	0.474±0.06 [a]
	5 %	15.61±1.64 [a]	11.95±4.04 [b]	27.56±5.21 [b]	1.416±0.51 [a]
	10 %	13.99±0.83 [a]	6.12±3.71 [b]	20.12±2.88 [b]	2.848±1.86 [a]
Bread wheat	Control	54.13±10.99 [a]	87.65±17.76 [a]	141.79±28.69 [a]	0.617±0.01 [b]
	5 %	22.66±1.70 [b]	10.15±2.20 [b]	32.81±3.70 [b]	2.285±0.40 [ab]
	10 %	9.12±1.81 [b]	1.89±0.32 [b]	11.02±1.48 [b]	5.031±1.98 [a]

3.4 Fresh and dry weight of seedlings

Both 5 and 10 % leachate leads to significant decrease in seedling fresh weight of bread wheat, cucumber and alfalfa plants ($p < 0.01$). In common bean seedling fresh weigh decreased up to 34 %, but the trend was not significant ($p < 0.01$). Alfalfa growth was completely inhibited by both treatments, whereas cucumber growth was only inhibited with 10 % redroot pigweed leachate (Table 3). Therefore, according to this parameter, common beans and bread wheat were the resistant plants; cucumber and alfalfa were the sensitive species to redroot pigweed allelopathic effects.

The effect of redroot pigweed leachate on seedling dry weight of cucumber and alfalfa plants was similar to its effect on fresh weight. 5 % leachate stimulated common bean dry weight, but significant decrease occurred in plants treated with 10 % leachate in comparison with control plants ($p < 0.01$). However, the effect of leachate on bread wheat plants dry weight was in contrast and not significant. On the other hand, leachate of 5 % reduced and leachate of 10 % stimulated dry material accumulation in bread wheat plants (Table 3). Considering this parameter, bread wheat is the most resistance and alfalfa is the most sensitive species to redroot pigweed leachate.

3.5 Related growth rate (RGR)

Relative growth rate (RGR) varied between studied species and redroot pigweed leachate treatments (Table 3). In cucumber, RGR was considerably decreased, while in bread wheat plants it was slightly stimulated. Common bean was differently affected by redroot pigweed leachate. In this plant RGR was significantly increased ($p < 0.01$) by 5 % leachate treatment, whereas 10 % leachate led to considerable reduction. Considering the results of this parameter, bread wheat and common bean are the most resistance, cucumber is moderately sensitive and alfalfa is the most sensitive species to redroot pigweed leachate.

Table 3: The effect of different concentrations (5 and 10 %) of the redroot pigweed's leachate on fresh and dry weight of seedlings (mg) and relative growth rate (RGR, $1.day^{-1}$) of crop species in 7 day after treatment (n = 3, $p < 0.01$).

Plant Species	Treatments	Parameters		
		Fresh Weight	Dry Weight	RGR
Cucumber	Control	320.12±31.86 [a]	31.72±5.44 [a]	10.05±2.11 [a]
	5 %	239.27±36.76 [b]	24.94±3.08 [a]	7.89±0.98 [a]
	10 %	0.0±0.0 [c]	0.0±0.0 [b]	0.0±0.0 [b]
Alfalfa	Control	25.08±2.69 [a]	0.64±0.05 [a]	4.52±0.36 [a]
	5 %	0.0±0.0 [b]	0.0±0.0 [b]	0.0±0.0 [b]
	10 %	0.0±0.0 [b]	0.0±0.0 [b]	0.0±0.0 [b]
Common bean	Control	438±32.94 [a]	233.15±16.93 [ab]	13.22±0.96 [ab]
	5 %	425.44±6.11 [a]	251.16±25.97 [a]	14.27±1.47 [a]
	10 %	288.75±86.62 [a]	155.01±46.41 [b]	8.79±2.64 [b]
Bread wheat	Control	142.33±37.37 [a]	27.44±1.32 [a]	9.55±0.46 [a]
	5 %	74.82±10.60 [b]	26.70±5.77 [a]	9.29±2.01 [a]
	10 %	67.64±2.44 [b]	31.22±1.76 [a]	10.87±0.62 [a]

4 DISCUSSIONS

In the present study, different concentrations of redroot pigweed's leachate caused 6.90-100 % reduction in seed germination of studied species. Allelopathic inhibitory potential of redroot pigweed was frequently reported in the literatures (Shahrokhi et al., 2011; Tejeda-Sartorius et al., 2011; Amini et al., 2012; Dogaru et al., 2012; Mlakar et al., 2012; Namdari et al., 2012; Shahrokhi et al., 2012; Konstantinović et al., 2014). Control of redroot pigweed is not necessary in natural environments because its populations have disturbed distribution and displaced by other plants after several years over natural succession (Costea et al., 2004). Most control methods documented for this weed are specific to agricultural systems (Costea et al., 2004; Rosskopf et al., 2005; Zhang and Mu, 2008; Dogaru et al., 2012). The use of herbicides as a thin layer on the surface of soil is one of these methods (Sarabi et al., 2011; Sodaei zadeh and Hosseini, 2012). The development of resistant varieties from redroot pigweed by using herbicides showed that these methods were not effective in long time periods (Costea et al., 2004; Rosskopf et al., 2005; Sarabi et al., 2011). Also, biological control of redroot pigweed using insects was ineffective (Costea et al., 2004; Rosskopf et al., 2005); however, there are reports on different pigweed species control by fungi *Phomopsis amaranthicola* Rosskopf, Charud, Shabana & Benny. Sp. nov. in field conditions (Rosskopf et al., 2005). Nowadays, only cultivation of crop species which are resistant to redroot pigweed allelochemicals in fields aggressed with this plant appears to be promising. The results of this study clearly showed that seed germination and seedling growth of studied species were differently affected by redroot pigweed allelochemicals. According to recorded parameters, common bean was sensitive in seed germination but resistant in seedling growth stages. The exactly contrast responses were observed in cucumber. However, bread wheat plant was relatively resistance species in both germination and subsequent seedling growth phases. Although

alfalfa slightly germinated in the presence of redroot pigweed allelochemical but its seedling growth and development was completely inhibited. Therefore, this species was the most sensitive species. The result of germination rate clearly showed that redroot pigweed can reduce crop plants seed germination, but the effective time is different in species. While bread wheat seeds germination started in first day, in other species germination showed a delay phase up to 3 days. Furthermore, seeds germination rate in treated bread wheat plants was lower in comparison to control, but the germination rate increased over time and finally reached to control. Weaker compensation ability was observed in other species. Therefore, the allopathic effect of redroot pigweed on the seeds germination of crops is higher in short time and compensation capability was dependent on plant species and leachate concentration. The effect of redroot pigweed leachate on seminal root length was higher than that in shoot lenght. Measurements of other growth parameters and studying of mechanisms involved in resistance of these organs can be useful in fine evaluation of this finding. Also conducting of this experiment using soil, perlite or hydroponic culture of plants could lead to reliable results for evaluation of allopathic effects of redroot pigweed on crops.

5 CONCLUSIONS

According to the results of presented study, bread wheat and common bean were the most resistant species, cucumbers was resistant species at low redroot pigweed leachate concentration but sensitive at high concentration, and alfalfa was the most sensitive species to the redroot pigweed leachate. Therefore, among the four studied crop species the cultivation of bread wheat and common bean plants in the regions with redroot pigweed's invasion is affordable, and avoidance of alfalfa cultivation in these regions is essential because this species is quite sensitive to compounds produce by redroot pigweed.

6 REFERENCES

Abdul Raoof K.M., Siddiqui M.B. 2012. Allelopathic impact of rhizosphere soil of *Tinospora cordifolia* on growth and establishment of some weed plants. African Journal of Agricultural Research, 7: 3952-3956, doi: 10.5897/AJAR11.2163

Amini R.A. 2013. Allelopathic potential of little seed canary grass (*Phalaris minor* Retz.) on seedling growth of barley (*Hordeum vulgare* L.). Journal of Biodiversity and Environmental Sciences, 3: 85-91

Amini R.A., Movahedpour F., Ghassemi-Golezani K., Dabbagh Mohammadi-Nasab A., Zafarani-Moattar P. 2012. Allelopathic assessment of common amaranth by ECAM. International Research Journal of Applied and Basic Sciences, 3: 2268-2272

Costea M., Weaver S., Tardif F. 2004. The biology of Canadian weeds. 130. *Amaranthus retroflexus* L., *A. powellii* S. Watson and *A. hybridus* L. (Update). Canadian Journal of Plant Science, 84: 631-668, doi: 10.4141/P02-183

Dhole J.A., Bodke S.S., Dhole N.A. 2011. Allelopathic effect of aqueous extract of five selected weed species on seed mycoflora, seed germination and seedling growth of *Sorghum vulgare* Pers. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2: 142-148

Dogaru G.V., Budoi S.G., Sandoiu D.D.I. 2012. Determination of the *Amaranthus retroflexus* Damage Threshold in Maize Crop. Advances in Agriculture and Botanic, 4: 1-5

Duretto M.F., Morris D.I. 2011. Amaranthaceae, Version 2011:1. In: *Flora of Tasmania Online.* Duretto M.F. (Ed.). Tasmanian Museum and Art Gallery, Australia: 1-29

Khalaj M.A., Amiri M., Azimi M.H. 2013. Allelopathy: physiological and sustainable agriculture important aspects. International journal of Agronomy and Plant Production, 4: 950-962

Khan A.L., Hussain J., Hamayun M., Kang S.M., Kim H.Y., Watanabe K.N., Lee I.N. 2010. Allelochemical, eudesmane-type sesquiterpenoids from *Inula falconeri*. Molecules Journal, 15: 1554-1561, doi: 10.3390/molecules15031554

Konstantinović B., Blagojević M., Konstantinović B., Samardžić N. 2014. Allelopathic effect of weed

species *Amaranthus retroflexus* L. on maize seed germination. Romanian Agricultural Research, 31: 1-7

Kusmana C. 2010. The growth of *Rhizophora mucronata* and *Avicennia marina* seedlings planted using Guludan technique in coastal area of Jakarta. Conference of the Earth and Space Sciences, Indonesia: 1-7

Iamonico D. 2010. Biology, life-strategy and invasiveness of *Amaranthus retroflexus* L. (Amaranthaceae) in central Italy: preliminary remarks. Botanica Serbica, 34: 137-145

Mlakar S.G., Jakop M., Bavec M., Bavec F. 2012. Allelopathic effects of *Amaranthus retroflexus* and *Amaranthus cruentusextracts* on germination of garden cress. African Journal of Agricultural Research, 7: 1492-1497

Modhej A., Rafatjoo A., Behdarvandi B. 2013. Allelopathic inhibitory potential of some crop species (wheat, barley, canola, and sunflower) and wild mustard (*Sinapis arvensis*). International Journal of Biosciences, 3: 212-220, doi: 10.12692/ijb/3.10.212-220

Namdari T., Amini R.A., Sanayei S., Alavi-Kia S., Dabbagh Mohammadi-Nasab A. 2012. Allelopathic effects of redroot pigweed (*Amaranthus retroflexus* L.) root exudates on common bean seedling growth. International Research Journal of Applied and Basic Sciences, 3: 1230-1234

Rosskopf E.N., Yandoc C.B., Charudattan R. 2005. Genus-specific host range of *Phomopsis amaranthicola* (Sphaeropsidales), a bioherbicide agent for Amaranthus spp. Biocontrol Science and Technology, 16: 27-35, doi: 10.1080/09583150500187975

Salehian H., Eshaghi O. 2012. Growth analysis some weed species. International Journal of Agriculture and Crop Sciences, 4: 730-734

Sarabi V., Rashed Mohassel M.H., Valizadeh M. 2011. Response of redroot pigweed (*Amaranthus retroflexus* L.) to tank mixtures of 2,4-D plus MCPA with foramsulfuron. Australian Journal of Crop Science, 5: 605-610

Shahrokhi S., Darvishzadeh M., Mehrpooyan M., Farboodi M. 2012. Comparison of allelopathic effects of *Amaranthus retroflexus* L. different organs extracts on germination and initial growth of Alvand and Zarrin wheat cultivars. International journal of Agronomy and Plant Production, 3: 489-494

Shahrokhi Sh., Hejazi S.N., Khodabandeh H., Farboodi M., Faramarzi A. 2011. Allelopathic effect of aqueous extracts of pigweed, *Amaranthus retroflexus* L. organs on germination and growth of five barley cultivars. International Conference on Chemical, Biological and Environmental Engineering, 20: 80-84

Sodaeizadeh H., Hosseini Z. 2012. Allelopathy an environmentally friendly method for weed control. International Conference on Applied Life Sciences, Turkey, 387-392

Soltys D., Krasuska U., Bogatek R., Gniazdowska A. 2013. Allelochemicals as bioherbicides — present and perspectives. V: *Herbicides – Curent Research and Case Studies in Use*. Price A.J., Kelton J.A. (Eds.). InTech Publisher, Rijeka, Croatica: 517-542

Tejeda-Sartorius O., Vaquera-Huerta H., Cadena-Iñiguez J. 2011. Effect of amaranth residues (*Amaranthus hypochondriacus* L.) on weed control and yield or radish, onion and carrot. Spanish Journal of Agricultural Research, 9: 284-295, doi: 10.5424/sjar/20110901-040-10

Terji I. 2008. Allelopathic effects of juglone and decomposed walnut leaf juice on muskmelon and cucumber seed germination and seedling growth. African Journal of Biotechnology, 7: 1870-1874

Tomlinson K.W., Sterck F.J., Bongers F., da Silva D.A., Barbosa E.R.M., Ward D., Bakker F.T., van Kaauwen M., Prins H.H.T., de Bie S., van Langevelde F. 2012. Biomass partitioning and root morphology of savanna trees across a water gradient. Journal of Ecology, 100: 1113-1121, doi: 10.1111/j.1365-2745.2012.01975.x

Weir T.L., Park S.W., Vivanco J.M. 2004. Biochemical and physiological mechanisms mediated by allelochemicals. Current Opinion in Plant Biology, 7: 472–479, doi: 10.1016/j.pbi.2004.05.007

Zhang Y., Mu X. 2008. Allelopathic effects of *Amaranthus retroflexus* L. and its risk assessment. Acta Botanica Boreali Occidentalia Sinica, 4: 771-776

COBISS Code 1.01

DOI: 10.14720/aas.2015.105.2.03
Agrovoc descriptors: sesame, seed germination, germination, seedlings, growth, seed treatment ultrasound, enzyme activity, water uptake

Agris category code: f02, f60

Sonication of seeds increase germination performance of sesame under low temperature stress

Fariborz SHEKARI[1], Seyyed-Hamid MUSTAFAVI[1], Amin ABBASI[1]

Received April 14, 2015; accepted August 13, 2015.
Delo je prispelo 14. aprila 2015, sprejeto 13. avgusta 2015.

ABSTRACT

A laboratory experiment was conducted to determine the effect of ultrasound (US) exposure time on germination behavior of sesame seeds. All tests were carried out at 20 kHz in a water bath ultrasonic device varying two factors, treatment duration (10, 20 and 30 min) and germination temperature (15, 20 and 25 ºC). Parallel tests were run in which seeds were soaked in water without sonication in order to eliminate the effect of water from US test results. US treatments enhanced seeds water uptake. At mild exposure time it improved sesame seed germination performance and seedling growth at suboptimal temperatures as indicated by higher germination percentage and germination rate. US applying for 20 min had relatively high superoxide dismutase activity; however, had not significant differences with control and US duration for 10 min. The catalase activity was strongly increased by applying the US for a 10 and 20 min. Among the treatments, application of US vibration for 10 and 20 min reduced both of malondialdehyde and H_2O_2 contents, however high US duration (30 min) increased both of the traits. In general, ultrasonic priming technique can be useful for early planting the sesame seeds, and lead to higher yields.

Key words: enzyme activity, germination performance, seedling growth, ultrasound, water uptake

IZVLEČEK

SONIFIKACIJA SEMEN SEZAMA Z ULTRAZVOKOM POVEČA NJIHOVO KALITEV V RAZMERAH HLADNEGA STRESA

V laboratorijskem poskusu smo določali učinke ultrazvoka (US) na kalitev semen sezama. Vsi poskusi so bili izvedenei v ultrazvočni vodni kopeli s frekvenco ultrazvoka 20 kHz , pri čemer smo spreminjali dva dejavnika in sicer trajanje obdelave z ultrazvokom (10, 20 in 30 min) in temperaturo kalitve (15, 20 in 25 ºC). Vzporedno so potekali poskusi, v katerih so bila semena samo namočena v vodi brez ultrazvočne sonifikacije, da bi odpravili učinke vode pri ultrazvočno obdelanih semenih. Obdelava semen z ultrazvokom je v njih povečala privzem vode. Pri srednjih obravnavanjih z ultrazvokom se je izboljšala kalitev in rast kalic pri suboptimalnih temperaturah, kar se je odrazilo kot večji odstotek kalitve in njen hitrejši potek. US obdelava za 20 min je rahlo povečala aktivnost superoksid dizmutase, vendar v primerjavi s kontrolo in obdelavi z US 10 min ni bilo značilnih razlik. Aktivnost katalaze se je pri obdelavah z 10 in 20 min močno povečala. Obdelava z ultrazvokom za 10 in 20 min je zmanjšala vsebnost malondialdehida in H_2O_2, obdelava za 30 min pa je vsebnost obeh parametrov povečala. Na splošno lahko na osnovi te raziskave povzamemo, da je ultrasonična predobdelava semen sezama koristna tehnika za njegovo zgodnjo setev, kar vodi v večje pridelke.

Ključne besede: encimska aktivnost, kalitev, rast kalic, ultrazvok, privzem vode

[1] Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Maragheh, Maragheh, Iran

1 INTRODUCTION

Sesame (*Sesamum indicum* L.) is one of the most important oilseed crops used by humans (Weiss, 2000). It has a high resistance level in drought stress condition, and is therefore appropriate crop for cultivation in dry-land conditions. But in these regions, water availability in the soil can be insufficient to support seed germination and early seedling growth, which are the most sensitive stages for water shortage. In order to conquer for this problem, sesame seeds should be sown earlier in the season, when a lot of water is available, so, canopy closing and growth degree day (GDD) requirement for completing the growing stage is gain earlier and reproductive stage and following seed development and maturity complete early. One of the most important obstacles of this approach is low temperature in the early stages of season that negatively affects seed germination and seedling establishment.

Seed germination and seedling establishment are the most critical stages for survival of plants under unfavorable conditions. One of the most important factors that affect germination and subsequently later stages is low temperature. Seeds can germinate over a wide range of temperatures, but maximum percentage germination is typically reduced at the extremes of the range (Probert, 2000). Germination is divided into three phases: imbibition, activation and post germination growth. The major negative effects of low temperature during germination seem to be associated to the imbibition phase (Hoekstra *et al*., 1992). Arin and Kiyak (2003) reported that difficulty in water uptake under low temperature can influence the later stage of growth and development. For example, emergence percentage decreased under the low temperatures.

Low temperature during imbibition phase of germination leads to the increase of electrolyte leakage from the seeds, which has been attributed to the disturbance of plasma membrane integrity (Hoekstra *et al*., 1992). Low temperature promotes gel phase formation and increase rigidity, thereby increasing the likelihood of imbibitional injury (Crowe *et al*., 1989). Bochicchio *et al*., (1991) reported that imbibition of seeds is necessary to reorganize fully the structure of cell membrane lipids, but, if during the imbibition process, the temperature is such that membrane lipids are in a gel phase, formation of a continuous bilayer might not be possible, or the bilayer formed might be functionally imperfect. Sharma *et al*., (2011) reported that oxidative stress may be a significant factor in relation to low temperature damage in plants. Reactive Oxygen Species (ROS) play a key role in various events of seed life. In seeds, ROS production has been considered for a long time as being very detrimental, since the works dealing with ROS were mainly focused on seed ageing or seed desiccation, two stressful situations which often lead to oxidative stress. At the opposite, it now appears more and more clearly that ROS would play a key signaling role in the achievement of major events of seed life, such as germination or dormancy release. Many reports have shown that the transition from a quiescent seed to a metabolically active organism (phase II of germination) is associated with ROS generation, suggesting that it is a widespread phenomenon (Bailly, 2004). ROS are involved in endosperm weakening during germination. Cellular antioxidant mechanisms seem to tightly control ROS concentrations, rather than to eliminate them completely, suggesting that some ROS might play normal physiological roles and act as signaling molecules. The reactivation of metabolism following seed imbibition may provide an important source of active oxygen species (AOS). For example, H_2O_2 is produced at the early imbibition period of soybean (Puntarulo *et al*., 1991).

There are several techniques that have positive effect on low temperature tolerance at germination stage. Ultrasound is a novel physical method that involves the application of sound frequencies in the inaudible range (20–100 kHz) to interact with the materials. It was proven that application of ultrasound treatment could change the state of the substances and even accelerate the reactions (Aladjadjiyan, 2007). This technique is unique among existing seed pretreatment methods in that it is simple, cheap, environmentally friendly and multifunctional (Goussous *et al*., 2010). Ultrasound treatment to stimulate germination has been investigated in many seed types including maize, barley, rice and sunflower (Aladjadjiyan, 2002; Florez *et al*., 2007; Yaldagard *et al*., 2008a,

b). Goussous *et al*., (2010) and Yaldagard *et al*., (2008a) showed that US treatments increase seed water uptake, the important stage that was inhibited by low temperatures; so, we use this technique to investigate the beneficial effect of US on seed germination performance in low temperatures. Besides, there has not been any investigation on the effects of US treatment on ROS and antioxidant enzyme content following the ultrasonication. So this research was aimed to determine whether ultrasonic treatment is usable as a seed priming method for sesame.

2 MATERIALS AND METHODS

2.1 Seed materials

Native seed lots of sesame (*Sesamum indicum* 'Oltan') were obtained from Tabriz University, Iran which collected the seed in the same year that the study was undertaken.

2.2 Seed treatment

The ultrasonication experiments were carried out at 20 kHz on the ultrasonic generator (UW2200, Berlin). All tests were performed on samples (50 seeds for each treatment) dispersed in 100 ml of distilled water with direct sonication for 0, 10, 20 and 30 min. To rule out the effect of water in these tests, a control replicate of each test was soaked in a similar volume of water but without sonication. Water circulating in ultrasonic equipment as well as in the water bath was kept at 25 ºC.

2.3 Germination tests

Three replicates of 50 seeds were placed in covered 9-cm Petri dishes containing a single filter paper with 5 mL test solutions. The Petri dishes with seeds were put in sealed plastic bags to avoid moisture loss. Seeds were germinated at constant temperatures (T) of 15, 20 and 25 ºC the dark in an incubator. Germination was scored when the seminal root was 2 mm long.

2.4 Germination performance measurements

At the end of experiment, Final germination percentage (FGP), germination rates (GR), germination uniformity (GU), seminal root and shoot length were recorded to evaluate germination performance. Daily germination percentage was recorded and subjected to statistical analysis. GR (T_{50}) was defined as days needed to reach 50 % of FGP. GU (T_{10-90}) was defined as days needed for 10 % of FGP to 90% of FGP.

2.5 Seed water uptake

The water uptake of seeds necessary for germination was determined under 15 ºC. For this purpose, three replications of 50 seeds were placed in petri dishes as described for germination experiments, removed at 15 min after initiation of imbibition, drained and blotted with absorbent paper, weighted and placed again into the petri dishes. After 30 min, 1 h and 2 h, the seeds were reweighted as described above. The water uptake was expressed as the seed moisture content at different times.

2.6 Enzyme extractions and assays

Measurements of enzyme activities, lipid peroxidation and H_2O_2 contents were carried out with imbibed but non-germinated seeds and with whole seedlings at 3 days after germination. In order to better discriminate the behavior of the seeds and seedlings, the temperature of 15 °C only has been chosen.

Two grams of peeled seeds or whole seedlings were used for enzymes extraction. The samples were homogenized in 20 ml of 0.1 M potassium phosphate buffer (pH 7.8) centrifuged for 15 min at 16,000×g, at 0 ºC. Extraction was at 4 ºC. The supernatant sample was then stored at -20 ºC prior to assaying. Superoxide dismutase (SOD, EC 1.15.11), catalase (CAT, EC 1.11.1.6) were extracted and assayed according to Bailly *et al*., (1996), and expressed as units per gram FW.

2.7 Evaluation of hydrogen peroxide and malondialdehyde contents

H_2O_2 concentration was measured by the methods of Moloi and Westhuizen (2006). Results are expressed in nmole per gram dry weight. Lipid peroxidation was evaluated by measuring malondialdehyde (MDA) content from 0.2 g (fresh weight) of seeds or seedlings, according to Predieri

et al. (1995). The samples were homogenized in 5 ml sodium phosphate buffer followed by centrifugation for 15 min at 8,000×g. A 0.5 ml aliquot of the supernatant was combined with an equal volume of thiobarbituric acid (TBA) reagent and boiled for 20 min. Absorbance was determined at 532 and 600 nm. MDA concentration was expressed in micromoles per gram dry weight.

2.8 Data analysis

Analysis of variance appropriate to the experimental design was conducted, using SPSS software. Means of each trait were compared according to Duncan multiple range test at $P \leq 0.05$. Excel software was used to draw figures.

3 RESULTS AND DISCUSSION

3.1 Effect of different exposure times on seed water uptake

Before any treatment, seed moisture was 13 %. With increasing the US exposure time, seed moisture increased. At the early times, US treated seeds, particularly US treatment for 30 min, had rapid water uptake compared with control, then water uptake slope (rate of water uptake) was enhanced in control, while reduction was observed in US for 20 and 30 min (Fig. 1). At the end, seed moisture content of control and mild treatment duration (10 and 20 min) were approximately similar. However, at high US duration, seed moisture content was lower than control. One of the possible explanations could be that sonication (for short periods) may create or enlarge fissures in the protective coating surrounding the seed and pericarp and after steeping in the water a significant rise in seedling moisture resulted. US treatment for high duration (30 min) might have led to much cell wall disruption that allowed greater accessibility of cell membranes to the effects of US, thus increasing their permeability and finally leading to post treatment water leakage. Similar results were observed by Goussous *et al*., (2010).

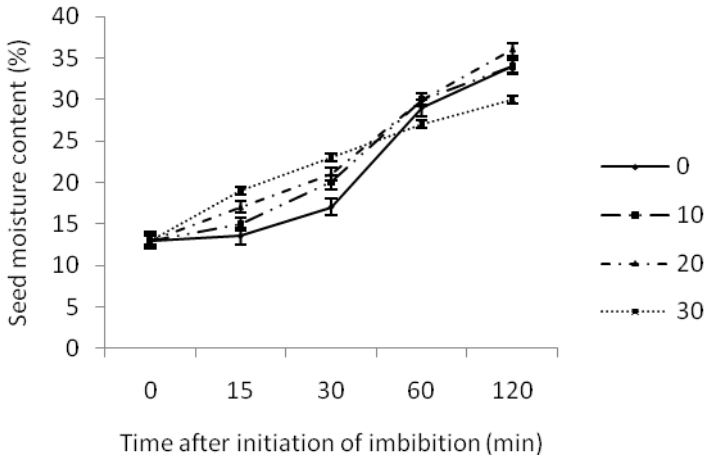


Figure 1: Seed moisture content of sesame seeds soaked in water in ultrasonic device (US) for 0 - 120 min

3.2 Effect of different us exposure times and temperatures on germination performance

Germination percentage (GP) of sesame seeds increased with increasing the treatment duration, reaching the limit (80 %) at 20 min. this GP value was approximately 12 % above that of the control seeds. However, at long duration (30 min), GP significantly decreased (approximately 18 %). Similar result was reported by Goussous *et al*., (2010) that showed that application of mild US duration improved germination percentage of wheat seeds. The effects of ultrasonic treatment on seed germination depend on frequency of

ultrasonic wave and exposure time as well as on plant species (Aladjadjiyan, 2012). It is noticeable from Table 1 that significant differences also existed in GP among incubation temperatures. Seeds of sesame germinated better at 25 ºC. It is also observed that, at suboptimal temperature (15 ºC), 20 min US treatment, enhanced GP by 22 %, but at optimal temperatures improvement of GP by 20 min US was only 2 % above control. Therefore, US treatment had more pronounced effects on germination at low temperatures than at optimum ones. US duration treatment significantly affected germination rate (GR) and uniformity (GU) (Table 1). With increasing the US duration, GR increased. The highest GR (0.27) was obtained at 30 min, reaching 28 % above control. GU decreased by US treatments, however, difference between control and 10 min US was not significant. In general, US priming technique improved sesame seed germination performance at 15 ºC as indicated by higher GP and GR (Table 1), indicating an improved of chilling tolerance.

Germination performance improvement by US application had been reported by Goussous *et al*. (2010) on wheat, Yaldagard *et al*. (2008) on barley and Aladjadjiyan (2002) on carrot. It is demonstrated that US exerts its major effects by inducing mechanical effects (acoustic cavitation) and disruption of plants cell walls, thereby increasing water uptake, important phase of germination that are negatively affected by low temperatures. The extra absorbed water reacts freely and readily with the cell embryo, so, metabolic processes such as gibberellic acid release and activation of enzymes expedited (Yaldagard *et al*. 2008a), and then will significantly reduce the mean germination time and will increase the rate and yield of germination. At long period of US (30 min) decreased germination performance. It is possible that US treatment for 30 min was too extreme to be tolerated by the small and fragile seeds of sesame leading to lysis of cells.

In this study non-sonicated seeds only exposed to water were used as control. Hydration of seeds by soaking in water for various periods without allowing seminal root emergence is one method of priming, which generally improves germination performance (Ashraf and Foolad 2005). Therefore, sonication priming can be used instead of usual priming ways (hydropriming).

Table 1: Germination percentage, rate and uniformity of sesame seeds exposed to different duration of ultrasound (US) treatment and incubated at three temperatures; 15, 20 or 25 ºC

US duration	Temperature (ºC)	Germination		
		Percentage (%)	Rate (1/day)	Uniformity (day)
0	15	62^{f}	0.19^{j}	4.73^{a}
	20	70^{def}	0.21^{h}	4.07^{bc}
	25	82^{ab}	0.23^{f}	3.69^{cd}
10	15	72^{cdf}	0.20^{i}	4.32^{b}
	20	74^{bcd}	0.23^{f}	3.8^{cd}
	25	84^{a}	0.24^{e}	3.77^{cd}
20	15	76^{abcd}	0.25^{d}	3.67^{cd}
	20	80^{abc}	0.26^{c}	3.5^{d}
	25	84^{a}	0.22^{g}	3.37^{d}
30	15	51^{g}	0.31^{a}	3.74^{cd}
	20	62^{f}	0.29^{b}	3.72^{cd}
	25	64^{ef}	0.22^{g}	3.52^{d}

Different letters in each column indicate significant difference at $P \leq 0.05$.

3.3 Seed germination trends

Control seeds were germinated well when incubation temperature was 25 ºC, but US treatment seeds were not significantly affected by temperatures at early stages of germination. Higher temperature caused rapid germination in US and control seeds (Fig. 2). The optimum temperature for sesame seed germination is 25 ºC (Bennet, 2011). The temperature below the optimum, decreased germination rate (Bradford, 2002). Langham (2007) reported that with soil temperatures around 25°C, the seed imbibes enough moisture and encourage a rapid germination. At all of the temperatures, high US duration (30 min) had lowest GP, but cumulative GP slop (i.e. GR) was high. At low temperature (15 ºC), GP curve for control seeds was lower than 10 and 20 min US, however, with increasing the temperature, GP curve for control seeds was approached to 10 and 20 min US duration curves. At 25 ºC, control and mild US duration treatments (10 and 20 min) had approximately similar curves (Fig. 2). Therefore, US priming technique for appropriate duration were more beneficial at suboptimal temperature than optimal ones. And it can improve germination performance of sesame seeds under chilling stress.

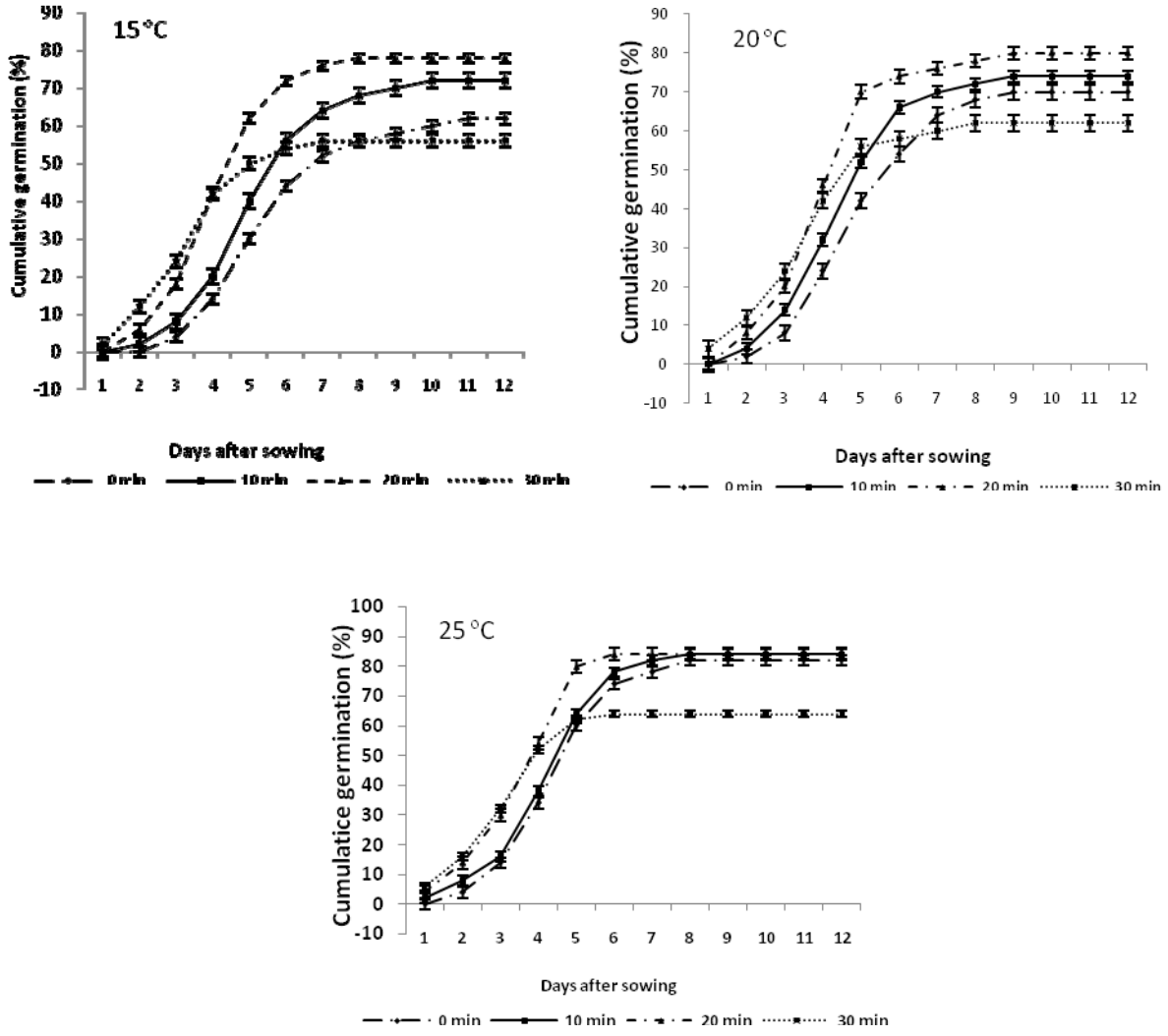


Figure 2: Cumulative seed germination of US treatment and control seeds under different temperatures (15, 20 and 25 ºC)

3.4 Effect of us and temperature treatments on seedling growth

Seedling length and dry weight were significantly affected by priming technique. The size of the seedlings was larger in treatments where seeds were primed by 20 min or 10 min, and smaller in control and 30 min US treatment. At all temperatures, seedling size of mild US treated seeds (20 min), was higher than control, but high US duration at low incubation temperatures produce more slender seedlings (compared to control) (Fig. 3). At higher temperatures, 30 min US treatment of seeds for 30 min decreased seedling's growth. Their size is lower than in controls. According to literature low temperature promotes gel phase formation and increase rigidity of membrane, so, it seems that, in this situation, membrane may be able to tolerate mechanical changes that caused by US duration for 30 min. At higher temperatures, however, membrane rigidity is eliminated (Nijsse *et al*., 2004), which presumably decrease tolerance to high duration US treatment. In a research conducted by Fateh *et al*. (2012) on fennel, they showed that US treatment had adverse effect on seedling growth. Aladjadjiyan (2012) reported that seedling length of lentil had approximately linear relationship with US exposure time, and with increasing the US time, these traits increased.

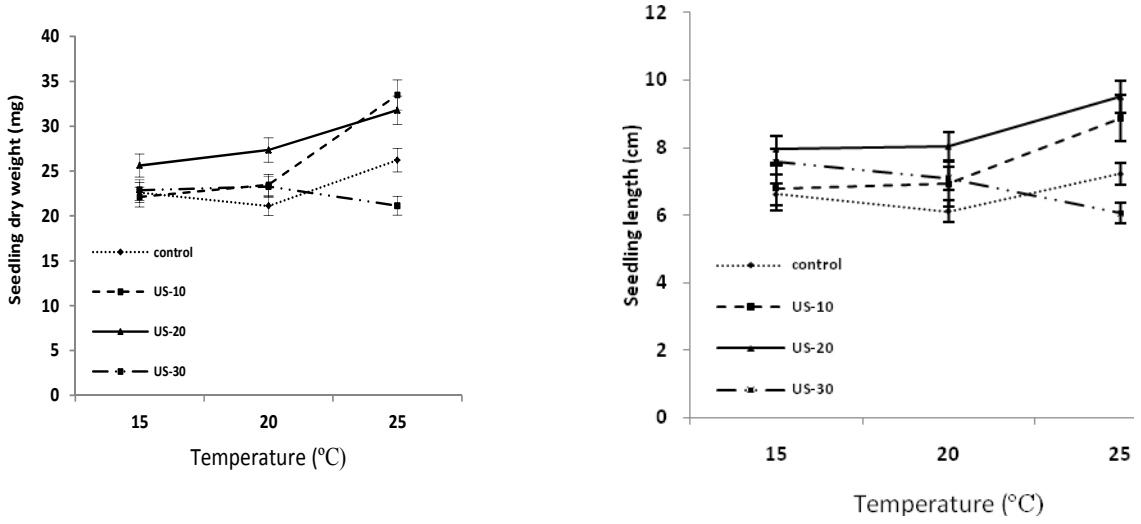


Figure 3: Effect of US treatments on seedling dry weight and length at different temperatures

3.5 Enzyme activities

SOD activity did not significantly change during germination phase. So this enzyme activity was similar from imbibition phase to seedling growth. Whereas, about CAT activity; after imbibition, CAT activity had increased by 29 % and the activity of this enzyme was higher at seedling stage than imbibition time (Fig. 4). It can be concluded that SOD activity is not correlated with seed germination, and it may be mainly involved in preserving the viability of seeds and protecting them from reactive oxygen species formed during storage. CAT activity changes during germination are demonstrating that this enzyme in addition to had role in storage time; it had a detoxifying role at germination stage too. These results are in agreement with certain finding of Yeh *et al*. (2005), which showed that activity of antioxidant enzymes such as CAT and SOD is closely related with germination and storage longevity.

Among the treatments, 20 min US treatment induced relatively high SOD activity. There was, however, not significant difference between this treatment and shorter US treatment (10 min) and the control. High duration of US (30 min) decreased SOD activity. At all of the treatments, SOD activity changes were not significant between imbibed seeds and seedlings (Fig. 4). The CAT activity was strongly increased by applying the US for a moderate time (10 and 20 min), thus

enhancing the antioxidant defence of the cells. Our data also showed that US treatment for a long time (30 min) reduced CAT activity in the both of imbibed seeds and seedlings. Chen *et al*. (2013) reported that seeds exposed to ultrasonic vibration showed high CAT and SOD activities, so, improved resistance to cadmium and lead in wheat seedling. Stimulation of CAT activity by seed priming was also reported by Bailly *et al*. (2002).

The catalase intervenes in the respiration of plants, which caused degradation of the endosperm and cotyledons reserve substances and synthesis of some necessary substances for nutrition and embryo growth. Therefore, it seems that stimulation of germination process and seedling growth by ultrasonic vibration may be attributed to CAT activity. Bailly *et al*. (2002) showed that improvement of germination performance by priming was clearly associated with higher CAT activity.

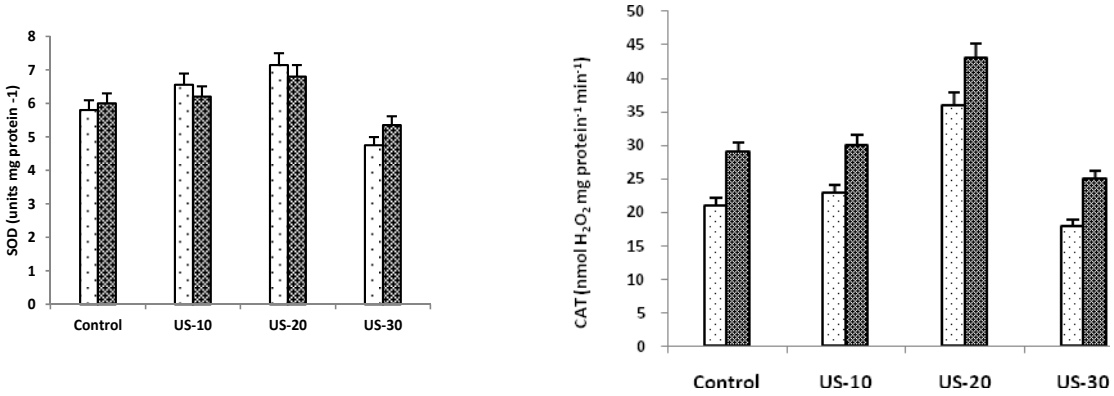


Figure 4: Effects of US treatments on SOD and CAT activities in imbibed seeds and seedlings

3.6 Lipid peroxidation and H_2O_2 content

Lipid peroxidation (degradation) was evaluated by determination of seed malondialdehyde content. MDA is one of the final products of the peroxidation of unsaturated fatty acids in phospholipids and is responsible for cell membrane damage (Sharma *et al*., 2011). MDA contents of seeds and seedlings are summarized in Fig. 5. In all of the treatments, MDA contents were higher in imbibed seeds than in seedlings. Despite an active metabolism associated with seedling growth, there was no increase in lipid peroxidation. Our data showed that hydrogen peroxide content was not significantly changed during seedling development following the imbibition, despite of producing by mobilization of stored reserves by β-oxidation in glyoxysomes, a type of peroxisome (Bewley and Black, 1994). Indeed, peroxisomes are also the site of localization of catalase, which eliminates H_2O_2. Therefore, production of H_2O_2 by converting the lipid reserves into sugars during the first stages of seedling development can be reduced by catalase activity that increased in this period.

Among the treatments, application of US vibration for 10 and 20 min reduced both of MDA and H_2O_2 contents, however high US duration (30 min) increased both of the traits. It is notable that MDA and H_2O_2 enhancement in imbibed seeds is higher than seedlings (Fig. 5). Imbibed control and US treated seeds for 10 min, and further growth of seedlings generated by these seeds, did not lead to significant changes in H_2O_2 content. It seems that increase in MDA and H_2O_2 content by US priming may be attributed to increase in antioxidant enzymes activity such as catalase that could be able to eliminate these detoxifying agents.

The results obtained underline the likely involvement of CAT in germination and early growth of seedlings of sesame thus suggesting that control of H_2O_2 homeostasis is an important event in expression of seed vigor. However, H_2O_2, together with other ROS, may have many cellular

targets, such as proteins and DNA (Sharma *et al*., 2011). Alternatively, CAT also plays a key role in H_2O_2 removal during fatty acid β-oxidation in glyoxysomes (Olsen and Harada, 1995). Therefore, high CAT activity could be associated with better mobilization of lipid reserves and faster seedling development.

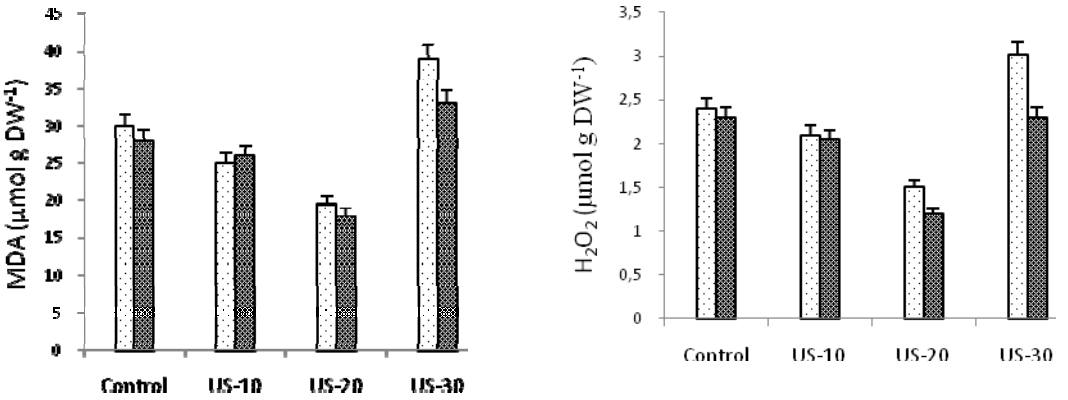


Figure 5: Effects of US treatments on MDA and H_2O_2 contents in imbibed seeds ⬚ and seedlings ▩

4 CONCLUSION

Results of this research indicated that US treatment effectively enhanced germination performance and seedling growth of sesame seeds especially at suboptimal temperatures. This increase in germination performance could have a positive impact on the success of early field establishment and canopy closer leading to better plant establishments and influence weed managements. So, this technique can be useful for alleviating the adverse effect of suboptimal temperature on seed germination, therefore, it can be very important in earlier canopy closing and finally improve yield.

5 REFERENCES

Aladjadjiyan A. 2002. Study of the influence of magnetic field on some biological characteristics of *Zea mays* L. *Journal of Central European Agriculture* 3: 89-94

Aladjadjiyan A. 2007. The Use of Physical Methods for Plant Growing Stimulation in Bulgaria. *Journal of Central European Agriculture* 8: 369-380

Aladjadjiyan A. 2012. Physical factors for plant growth food quality. In: food production, approaches, challenges and tasks. Intech

Arin L and Kiyak Y. 2003. The effects of pre-sowing treatments on emergence and seedling growth in tomato seed (*Lycopersicon esculentum* Mill.) under several stress conditions. *Pakistan Journal of Biological Science* 6: 990–994, doi: 10.3923/pjbs.2003.990.994

Ashraf M and Foolad MR. 2005. Pre-sowing seed treatment: A shotgun approach to improve germination, plant growth and crop yield under saline and non-saline conditions. *Advances in Agronomy* 88: 223–271, doi: 10.1016/S0065-2113(05)88006-X

Bailly C. 2004. Active oxygen species and antioxidants in seed biology. *Seed Science Research* 14, 93–107, doi: 10.1079/SSR2004159

Bailly C, Benamar A, Corbineau F, and CÔME D. 1996. Changes in malondialdehyde content and in superoxide dismutase, catalase and glutathione reductase activities in sunflower seeds as related to deterioration during accelerated aging. *Physiologia Plantarum* 97: 104–110, doi: 0.1111/j.1399-3054.1996.tb00485.x

Bailly C, Bogatek-Leszczynska R, Côme D, and Corbineau F. 2002. Changes in activities of antioxidant enzymes and lipoxygenase during growth of sunflower seedlings from seeds of different vigour. *Seed Science Research* 12: 47–55, doi: 10.1079/SSR200197

Bennet M. 2011. Sesame seed: A Handbook for Farmers and Investors. 29/08/11 .Available at www.agmrc.org/media/cm/sesame_38F4324EE52CB.pdf

Bewley JD and Black M. 1994. Seeds. Physiology of development and germination (2nd edition). New York, Plenum Press

Bochicchio A, Coradeschi MA, Zienna P, Bertolini M, and Vazzana C. 1991. Imbibitional injury in maize seed independent of chilling. *Seed Science Research* 1: 85-90, doi: 10.1017/S0960258500000702

Bradford KJ. 2002. Applications of hydrothermal time to quantifying and modeling seed germination and dormancy. *Weed Science* 50: 248-260, doi: 10.1614/0043-1745(2002)050[0248:AOHTTQ]2.0.CO;2

Chen Y, Liu Q, Yue Xz, Meng Zw, and Liang L. 2013. Ultrasonic vibration seeds showed improved resistance to cadmium and lead in wheat seedling. *Environment science pollution research*, DOI 10.1007/S11356-012-1411-1

Crowe J.H., Hoekstra F.A., and Crowe L.M., 1989. Membrane phase transitions are responsible for imbibitional damage in dry pollen. *Proceedings of the National Academy of Sciences* USA 86: 520–523, doi: 10.1073/pnas.86.2.520

Fateh E, Noroozi H, Farbod M, and Gerami F. 2012. Assessment of Fennel (*Foeniculum vulgare*) seed germination characteristics as influenced by ultrasonic waves and magnetic water. *European Journal of Experimental Biology* 2: 662-666

Florez M, Carbonell V, and Martínez E. 2007. Exposure of maize seeds to stationary magnetic fields: Effects of germination and early growth. *Environmental and Experimental Botany* 59: 68-75, doi: 10.1016/j.envexpbot.2005.10.006

Goussous SJ, Samarah N.H, Alqudah AM, and Othman MO. 2010. Enhancing seed germination of four crop species using an ultrasonic technique. *Experimental Agriculture* 46: 231–242, doi: 10.1017/S0014479709991062

Hoekstra FA, Crowe JH, and Crowe LM. 1992. Germination and ion leakage are linked with phase transitions of membrane lipids during imbibition of *Typha latifoliapollen*. *Physiologia Plantarum* 84: 29–34, doi: 10.1111/j.1399-3054.1992.tb08760.x

Langham DR. 2007. Phenology of sesame. In: Janick J., and Whipkey A. [ed.], *New Crops and New Uses*, 210-275. ASHS Press, Alexandria, VA

Moloi MJ and Westhuizen AJ. 2006. The reactive oxygen species are involved in resistance responses of wheat to the Russian wheat aphid. *Journal of Plant Physiology* 163: 1118–1125, doi: 10.1016/j.jplph.2005.07.014

Nijsse J, Walther P. and Hoekstra FA. 2004. Cold-induced imbibition damage of lettuce embryos: a study using cryo-scanning electron microscopy. *Seed Science Research* 14: 117-126, doi: 10.1079/SSR2004161

Olsen LJ and Harada JJ. 1995. Peroxisomes and their assembly in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology* 46: 123–146, doi: 10.1146/annurev.pp.46.060195.001011

Predieri S, Norman HA, Krizek DT, Pillai P, Mirccki RM, and Zimmerman RH. 1995. Influence of UV-B radiation on membrane lipid composition and ethylene evolution in 'Doyenne d' Hiver' pear shoots grown in vitro under different photosynthetic photon fluxes. *Environmental Experimental Botany* 35: 151-160., doi: 10.1016/0098-8472(95)00003-2

Probert R.J. 2000. The role of temperature in the regulation of seed dormancy and germination. In: Fenner M. [ed.] *Seeds: The Ecology and Regeneration in Plant Communities*. 261–291. CAB International, Wallingford, doi: 10.1079/9780851994321.0261

Puntarulo S, Galleano M, Sanchez RA, and Boveris A. 1991. Superoxide anion and hydrogen peroxide metabolism in soybean embryonic axes during germination. *Biochimica et Biophysica Acta* 1074: 277–283, doi: 10.1016/0304-4165(91)90164-C

Sharma A, Bhushan JHA, and Dubey RA. 2011. Oxidative stress and antioxidative defense systems in plants growing under abiotic stresses. In: Pessarakli M, [ed.], Handbook of plant and crop stress. 89-138. CRC press

Weiss EA. 2000. Oil Seed Crop. 2nd Edition Blackwell Longman Group Ltd. USA

Yaldagard M, Mortazavi SA, and Tabatabaie F. 2008a. Influence of ultrasonic stimulation on the germination of barley seed and its alpha-amylase activity. *African Journal of Biotechnology* 7: 2465–2471

Yaldagard M, Mortazavi SA, and Tabatabaie F. 2008b**.** Application of ultrasonic waves as a priming technique for accelerating and enhancing the germination of barley seed: Optimization of method by the Taguchi approach. *Journal of the Institute of Brewing* 114: 14–21, doi: 10.1002/j.2050-0416.2008.tb00300.x

Yeh Y, Chiu MKY, Chen CL, and Sung JM. 2005. Partial vacuum extends the longevity of primed bitter gourd seeds by enhancing their anti-oxidative activities during storage. *Science Horticulture* 104: 101-112, doi: 10.1016/j.scienta.2004.08.006

COBISS Code 1.01

DOI: 10.14720/aas.2015.105.2.04
Agrovoc descriptors: *Pisum sativum,* peas, phosphorus, phosphate fertilizers, rhizobacteria, Pseudomonas fluorescens, plant growth, plant growth stimulants, growth, crop yield

Agris category code: f01, f04

Effects of phosphorus fertilizer rate and *Pseudomonas fluorescens* strain on field pea (*Pisum sativum* subsp. *arvense* (L.) Asch.) growth and yield

Bahram SALEHI[1] and Hashem AMINPANAH[*1]

Received October 24, 2014; accepted August 04, 2015.
Delo je prispelo 24. oktobra 2014, sprejeto 04. avgusta 2015.

ABSTRACT

A field experiment was conducted at Rezvanshahr, Guilan province, Iran, to evaluate the effects of phosphorus fertilizer rate and *Pseudomonas fluorescens* strains on growth and yield of field pea (*Pisum sativum* L.). The experimental design was a randomized complete block in a factorial arrangement with three replicates. Factors were phosphorus fertilizer rates (0, 25, 50, 75, and 100 kg P_2O_5 ha^{-1} as triple superphosphate), and seed inoculation with *P. florescen*s strains [control (non-inoculated), inoculated with strain R41, and strain R187). Analysis of variance showed that plant height, seed yield, pod number per m^2, 100-seed weight, biological yield, harvest index, and leaf P concentration were significantly influenced by phosphorus fertilizer rate and *P. florescen*s strain. At the same time, phosphorus fertilizer rate × *P. fluorescens* strain interaction was significant only for 100-seed weight. On the other hand, seed number per pod was significantly affected neither by phosphorus fertilizer rate nor by pseudomonas strains. Result showed that seed yield was significantly increased from 1099 ± 67 to 1898 ± 118 kg ha^{-1} as P_2O_5 application rate increased from 0 to 75 kg ha^{-1}, and thereafter relatively remained constant. There was no significant difference in seed yield between plants raised from inoculated seeds with *P. fluorescens,* strain R187 (1664 ± 97 kg ha^{-1}) and those raised from inoculated seeds with *P. fluorescens,* strain R41 (1669 ± 104 kg ha^{-1}). At the same time, plants raised from inoculated seeds with *P. fluorescens* (both strains) produced greater grain yield compared to those raised from uninoculated seeds (1370 ± 80 kg ha^{-1}). Based on the results of this study, P_2O_5 application at the rate of 75 kg ha^{-1} and inoculation with pseudomonas bacteria are recommended for obtaining the greatest seed yield in field pea.

Key words: phosphorus, plant growth-promoting rhizobacteria, *Pisum sativum*

IZVLEČEK

UČINKI GNOJENJA S FOSFORJEM IN DODATKOV SEVOV BAKTERIJE *Pseudomonas fluorescens* NA RAST IN PRIDELEK POLJSKEGA GRAHA (*P. sativum* subsp. *arvense* (L.) Asch.)

Z namenom ovrednotenja vplivov gnojenja z različnimi odmerki fosfornih gnojil in dodatkov sevov bakterije *Pseudomonas fluorescens* na rast in pridelek poljskega graha (*P. sativum* subsp. *arvense* (L.) Asch.) je bil izveden poljski poskus v provinci Rezvanshahr, Guilan, Iran. Načrt poskusa je bil naključni bločni faktorski poskus s tremi ponovitvami. Faktorji v poskusu so bili gnojenje s fosforjem (0, 25, 50, 75 in 100 kg P_2O_5 ha^{-1}, kot trojni superfosfat) in inokulacija semen s sevi bakterije *P. florescen*s (kontrola (ne inokulirano), inokulirano s sevom R41 in sevom R187). Analiza variance je pokazala, da so na parametre kot so višina rastlin, pridelek zrnja, število strokov na m^2, masa 100-semen, biološki pridelek, žetveni indeks in vsebnost P značilno vplivala gnojenja s fosforjem in inokulacija s sevi bakterije *P. florescen*s, vendar je imelo hkratno gnojenje s fosforjem in inokulacija s sevi bakterije *P. fluorescens* značilen vpliv le na maso 100-semen. Po drugi strani se število semen na strok ni značilno spremenilo niti z različnimi odmerki fosforja niti z dodatki sevov bakterij. Rezultati so pokazali, da se je pridelek zrnja značilno povečal od 1099 ± 67 na 1898 ± 118 kg ha^{-1}, ko se je uporaba P_2O_5 povečala iz 0 na 75 kg ha^{-1}, in je potem ostal relativno konstanten. Med rastlinami, katerih semena so bila inokulirana s sevom bakterije *P. fluorescens,* R187 (1664 ± 97 kg ha^{-1}) in tistimi, katerih semena so bila inokulirana s sevom *P. fluorescens,* R41 (1669 ± 104 kg ha^{-1}) ni bilo značilnih razlik v pridelku zrnja, vendar je bil pridelek zrnja inokuliranih rastlin pri obeh sevih večji od neinokuliranih rastlin (1370 ± 80 kg ha^{-1}). Na osnovi izsledkov te raziskave lahko za doseganje večjih pridelkov poljskega graha priporočamo gnojenje s fosforjevimi gnojili v odmerku 75 kg, P_2O_5 ha^{-1} s hkratno inokulacijo s sevi zgoraj omenjenih bakterij.

Ključne besede: fosforjeva gnojila, rast-stimulirajoče rizobakterije, *Pisum sativum*

[1] Department of Agronomy and Plant Breeding, Rasht Branch, Islamic Azad University, Rasht, Iran; corresponding author: aminpanah@iaurasht.ac.ir

1 INTRODUCTION

Pisum sativum subsp. *arvense* (L.) Asch., the field pea which is also known as the garden pea, is one of the most important pulse crop. World wide, green peas is produced on 2.25 million ha, with an estimated of total production of 18.5 million tons and dry peas is produced on 6.76 million ha, with an estimated of total production of 10.4 million tons in 2012 (FAO, 2012). Field pea is a cool-season crop which is usually cultivated in early-November and harvested in late-May in northern Iran. Field pea has a high nutritive value, and is high in fiber, protein, vitamins (folate and vitamin C), minerals (iron, magnesium, phosphorus and zinc), and lutein (Urbano et al., 2003).

Phosphorus is one of essential nutrients for plant growth and development. Phosphorus regulates protein synthesis in plants, because it is a component of the complex nucleic acid structure. Phosphorus is important in cell division and development of new tissue. Also, phosphorus plays a vital role in plant energy reactions, photosynthesis, respiration, genetic transfer, seed and fruit production, and nutrient transport in plants (Raghothama and Karthikeyan, 2005). Phosphorus is also a component of phytin, a major storage form of P in seeds, phospholipids, and ATP. Moreover, phosphorus promotes root growth and stimulates tillering and often hastens maturity. The responses of leguminous crops to P fertilizer are mainly determined by the soil P available, but are not related to soil organic matter, total N, total P, soil $CaCO_3$ contents, and soil N available (Li et al., 2011). Results show that, when the available P in the soil was less than 10 mg kg^{-1}, P fertilizer gave good effect and application of P fertilizer was required, while above 15 mg P kg^{-1} the application of P fertilizer alone had no consistent effect (Lin et al., 1964). Li and Li (1992) reported that leguminous crops had different response to P application rate compared to cereal crops. They reported that wheat yield was significantly increased from 2040 to 4491 kg ha^{-1} as P rate increased from 0 to 97.5 kg ha^{-1}. In contrast, the highest pea yield was at the P rate of 78 kg P ha^{-1}, and thereafter decreased. One of the reasons proposed for explaining the difference in responses to P fertilizer is the higher P demand of legumes than non-legumes.

Although P is abundant in soils in both organic and inorganic forms (Khan et al., 2009), the amount of available forms to plants is generally low, because the majority of soil P is found in insoluble forms, while the plants absorb it only in soluble ions ($H_2PO_4^-$ and HPO_4^{2-}) (Bhattacharyya and Jha, 2012). To overcome the P deficiency in soils, phosphorus fertilizers must be frequently added to agricultural fields, but regular application of phosphate fertilizers is not only costly but is also environmentally undesirable, as is possible cause of eutrophication. This has led to search for an economically viable and eco-friendly option for improving crop production in low P soils. Legume root is colonized by numerous rhizospheric microorganisms, and these organisms have definite influence on the survival and nodulation ability of seed inoculated rhizobia (Dashti et al., 1998; Davison, 1988). Phosphate solubilizing microorganisms could convert insoluble phosphates into available forms for plant via the process of acidification, chelation, exchange reactions, and production of gluconic acid (Chung et al., 2005; Gulati et al., 2010), and hence a viable substitute to chemical phosphorus fertilizers (Khan et al., 2006). Of the various phosphate solubilizing microorganisms, *Pseudomonas flurescen*s is considered as one of the most significant phosphate solubilizing bacteria, which not only provide P to the plants, but also produce siderophore, antibiotic, and phytohormones such as indole-acetic acid (Leinhos and Nacek, 1994). Some PGPR strains such as pseudomonas (Grimes and Mount, 1984) enhance legume growth, nodulation, and nitrogen fixation when coinoculated with rhizobia. Fluorescent pseudomonads are also known to produce salicylic acid, which acts as local and systemic signal molecules in inducing resistance in plants (De Meyer and Hofte, 1997). Benhamou et al. (1996 a, b) found that *Pseudomonas fluorescens* induced accumulation of lignin in pea roots. *Pseudomonas spp*. can form gluconic acid through the oxidative glucose metabolism (Gyaneshwar et al., 2002). Sharma et al. (2003) point out that in 10 μM Fe–citrate along with *Pseudomonas* strain GRP3 treatment, chlorophyll a, chlorophyll b and total chlorophyll contents increased significantly by 34, 48 and 39 %, respectively, compared to the control. In a field experiment, van Elsas (1986)

reported significant increase in wheat seedling growth after inoculation with pseudomonas and bacillus. Inoculation of plants with PGPR can enhance the drought tolerance (Figueiredo et al., 2008), which can be attributed to the production of IAA, cytokinins, antioxidants and ACC deaminase. Sgroy et al., (2009) declared that several PGPR strains such as *Bacillus subtilis*, *B. licheniformis*, *B. pumilus*, and *Pseudomonas putida* have crucial roles in cell elongation, increasing ACC deaminase activity, and plant growth promotion. Inoculation of *Pseudomonas fluorescens* significantly increased total root length, surface area and volume in tomato and cucumber (Saravanakumar et al., 2007).

Reduced application rates of chemical fertilizers through inoculation with plant growth-promoting rhizobacteria were proposed by researchers. Adesemoye et al. (2009) found that supplementing 75 % of the recommended fertilizer rate with inoculants produced plant growth, yield, and nutrient (nitrogen and phosphorus) uptake that were statistically equivalent to the full fertilizer rate without inoculants. At the same time, Shaharoona et al. (2008) reported that N use efficiency increased in response to inoculation with *Pseudomonas fluorescens* at all fertilizer levels in wheat, causing 115 %, 52 %, 26 %, and 27 % increase over the uninoculated (control) at N, P, and K application rates of 25 %, 50 %, 75 %, and 100 % recommended doses, respectively.

This experiment was conducted to evaluate the response of field pea growth and yield to different phosphorus fertilizer rate and *Pseudomonas fluorescens* strains.

2 MATERIALS AND METHODS

A field experiment was conducted at Rezvanshahr, Guilan province, Iran, from early-November 2013 to late-May 2014. Some soil properties of the experimental field were presented in table 1. The experimental design was a randomized complete block in a factorial arrangement with three replicates. Factors were phosphorus fertilizer rates (0, 25, 50, 75, and 100 kg P_2O_5 ha^{-1} as triple superphosphate which was applied as side placement), and *Pseudomonas florescens* strains [control (non-inoculated), seed inoculation with strain R41, and strain R187]. These strains were obtained from Soil and Water Research Institute, Karaj, Iran. Some properties of florescent Pseudomonads strains were presented in table 2. Before the inoculation, the Gum Arabic (10 %) was applied to the seeds. Then, the seeds of field pea (*P. sativum* subsp. *arvense* 'Utrillo') were inoculated with *P. florescens* strain R41, or strain R187 in the proportion of 10 g of peat (10^8 cells/g peat) kg^{-1} seed according to Ferreira et al. (2010). The inoculated seeds were dried in sunshade for five hours and then were planted in seven 3-m rows spaced 30 cm apart at a density of 10 seeds m^{-2} (30 seeds in each row) on 5 November. N and K fertilizers were applied just before final land preparation as recommended doses i.e. 15 kg N ha^{-1} (as starter in the form of urea), and 50 kg K_2O ha^{-1} (as potassium sulphate). Weeds were controlled manually during the experiment. Plants were harvested on 28 May 2014.

Plant height was measured from the soil surface to the top of the main stem at harvest stage. Ten randomly selected plants were harvested from each plot at ground level for measuring number of pod per plant, number of seeds per pod, and 100-seed weight. Aboveground biomass from 1 m^2 of each plot was oven-dried at 70 °C for 96 h, and weighted for biological yield determination. Seed yield was determined by hand-harvesting the crop plants from 2.5 m^2 per plot and was adjusted to 160 g kg^{-1} seed moisture content. In each plot, field pea leaves were oven-dried at 70 °C for 48 h and grounded to pass through a 1-mm sieve and P concentration were measured using the spectrophotometric method of Lowry and Lopez (1946). Phosphorus concentration was expressed as the percent of leaf dry weight.

Analyses of variance were conducted using SAS procedures (SAS Institute, 2004) based on a factorial trial and randomized complete block design. For *Pseudomonas fluorescens* strains factor, the F-ratios were found to be significant for plant height , pod number per m^2, seed number per pod, biological yield and harvest index, so means separations were conducted using fisher's protected

LSD at the 5 % probability level. For P rate factor, the F-ratios were found to be significant for plant height, pod number per m^2, seed number per pod, biological yield and harvest index, so linear or quadratic regressions with standard error of the mean were used to describe the relationship between P application rate and these dependent variables. For traits such as 100-seed weight and leaf P concentration, the interaction between *Pseudomonas fluorescens* strain and P rate was significant. So, for each inoculation level, linear regressions with standard error of the means were used to describe the relationship between P application rate and these dependent variables.

Table 1: Some soil properties (0-30 cm) of experimental field prior to sowing

OC (%)	pH	Sand (%)	Silt (%)	Clay (%)	Texture	EC (ds m^{-1})	Total N (%)	Available P (mg kg^{-1})	Available K (mg kg^{-1})
3.25	7.1	54.4	17.2	28.4	sand loam	0.36	0.11	3.2	215

Table 2: Some bacteria properties using for this experiment (Soil and Water Research Institute, Karaj, Iran)

Bacteria	ACC-deaminase production	Phosphorus solubilizing activity	IAA production (mg l^{-1})	Siderophore production (halo diameter/colony diameter)
*P. **fluorescens*** strain R187	+	+	5.8	0.5
*P. **fluorescens*** strain R41	+	+	8.9	0.51

3 RESULTS AND DISCUSSION

3.1 Plant height

Analysis of variance showed that the main effects of phosphorus fertilizer rate and *Pseudomonas fluorescens* strain were significant for plant height, but the interaction between them was not significant (Table 3). For both *Pseudomonas fluorescens* strains and for the control, plant height was significantly increased from 72.9 ± 1.02 cm to 86.4 ± 1.71 cm as P_2O_5 rate increased from 0 to 75 kg ha^{-1}, and thereafter remained statistically constant (Figure 1). Phosphorus plays a vital role in cell division and elongation and, therefore, lower plant height in phosphorus-deficient plants may be due to the reduction in cell division and elongation (Kavanova´ et al., 2006). Averaged across P fertilizer rates, the plants raised from seed inoculated with *Pseudomonas fluorescens* (both strains) was significantly taller than uninoculated ones; however there was no significant difference in plant height between plants raised from seed inoculated with *Pseudomonas fluorescens* strain R41 and strain R187 (Table 4). Pseudomonas bacteria are considered as one of the most significant phosphate solubilizing bacteria which provide P to the plants. Promotion effect of high P level and pseudomonas bacteria on plant height was probably due to better development of root system and nutrient absorption (Hussain et al., 2006). In addition, Dey et al (2004) observed that inoculation of plant growth promoting rhizobacterial isolates *Pseudomonas fluorescens* PGPR1 and *Pseudomonas fluorescens* PGPR2 significantly enhanced the plant height of peanut (*Arachis hypogaea* L.).

Table 3: Mean squares of ANOVA for plant high (H), seed yield (Y), pod number per m^2 (PN), seed number per pod (SP), 100-seed weight (SW), biological yield (BY), harvest index (HI) and leaf P concentration (LPC) as affected by phosphorus rate and *Pseudomonas fluorescens* strain

S.O.V	df	H	Y	PN	SP	SW	BY	HI	LPC
R	2	7^{ns}	88109^{ns}	1125^{ns}	0.1^{ns}	0.2^{ns}	391806^{ns}	1^{ns}	0.00002^{ns}
Phosphorus rate (P)	4	471^{**}	950549^{**}	4123^{*}	2.4^{ns}	37.9^{**}	2480016^{**}	114^{**}	0.00321^{**}
Pseudomonas fluorescens strains (Ps)	2	79^{*}	440924^{**}	4176^{*}	1.7^{ns}	20.2^{**}	2346986^{**}	13^{*}	0.00462^{**}
P × Ps	8	3^{ns}	30163^{ns}	431^{ns}	0.1^{ns}	1.6^{**}	214903^{ns}	4^{ns}	0.00039^{**}
Error	28	22	47994	1256	1.1	0.4	168554	4	0.00009
CV (%)	-	6	14	7	16	3	7	6	3

*, ** represent significance at 0.05 and 0.01probability level, respectively.
ns represents no significant difference

Table 4: Averages of plant high (H), seed yield (Y), pod number per m^2 (PN), seed number per pod (SP), biological yield (BY), and harvest index (HI) response to *Pseudomonas fluorescens* strains as average across P rates

Traits / **Pseudomonas strains**	H	Y	PN	SP	BY	HI
Control	79± 1.9	1370 ± 80.0	553± 9.6	6.0± 0.4	4714± 150.0	29.0± 0.7
Pseudomonas fluorescens Strain41	83± 2.1	1669± 104.0	553± 9.6	6.5± 0.3	5420± 176.0	30.8± 0.7
Pseudomonas fluorescens Strain187	83± 1.9	1664± 97.0	524± 9.3	6.7± 0.4	5375± 167.0	30.6± 0.8
LSD (0.05)	3	164	26	0.8	307	1.4

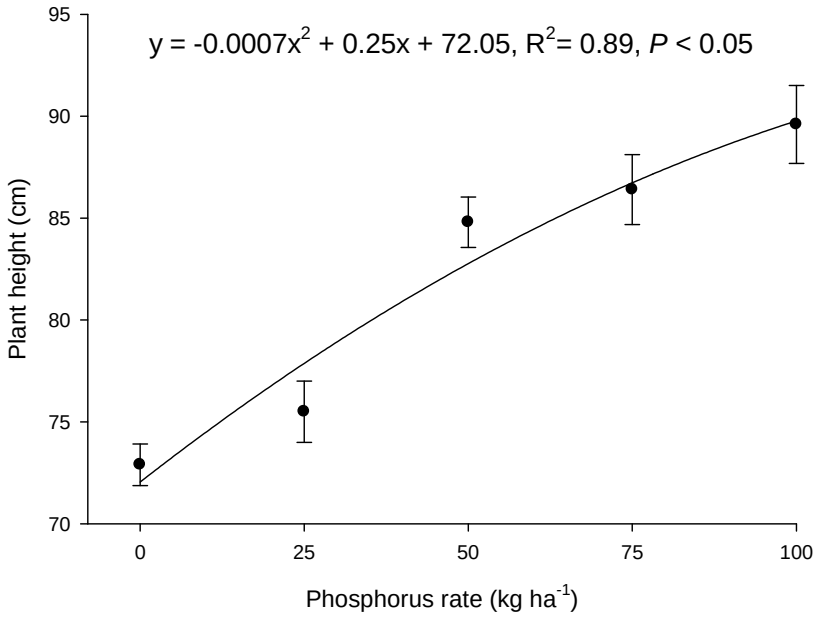


Figure 1: Effect of phosphorus fertilizer rate on plant height as average across *Pseudomonas fluorescens* strains. Vertical bars represent ± 1 SE of means

3.2 Seed yield

The main effects of phosphorus fertilizer rate and *Pseudomonas fluorescens* strain were significant for seed yield, while *Pseudomonas fluorescens* strain × phosphorus fertilizer rate interaction was not significant (Table 3). A quadratic equation ($Y = -0.049\ X^2 + 12.96\ X + 1103.77$, $R^2 = 0.98$) provided a good description of the relationship between seed yield and P application rate. Seed yield was significantly increased from 1099 ± 67 to 1898 ± 118 kg ha^{-1} as P_2O_5 application rate increased from 0 to 75 kg ha^{-1}, and thereafter relatively remained constant when averaged across *Pseudomonas fluorescens* strains (Figure 2). Regardless of P application rate, there was no significant difference in grain yield between plants raised from seed inoculated with *Pseudomonas fluorescens* strain R187 (1664 ± 97 kg ha^{-1}) and those raised from seed inoculated with *Pseudomonas fluorescens* strain R41 (1669 ± 104 kg ha^{-1}). At the same time, plants raised from seed inoculated with *Pseudomonas fluorescens* (both strains) produced greater grain yield compared to uninoculated (1370 ± 80 kg ha^{-1}) ones (Table 4). Pseudomonas bacteria not only could convert insoluble phosphates into available forms for plant via the process of acidification, chelation, exchange reactions, and production of gluconic acid (Chung et al., 2005; Gulati et al., 2010), but also could produce siderophore (Dey et al., 2004), ACC-deaminase enzyme (Dey et al., 2004; Shaharoona et al., 2008) and phytohormones such as indole-acetic acid (Leinhos and Nacek, 1994; Dey et al., 2004). At the same time, leaf growth depression under phosphorus deficiency is well documented (Assuero et al., 2004; Kavanova´ et al., 2006). Meanwhile, leaf number reduces by P deficiency. Therefore, the reduction in leaf surface area in phosphorus-deficient plants reduces light interception and photosynthesis assimilates, which in turn reduces plant dry matter. Also, the metabolism of N is inhibited with an inadequate supply of P, while the supply of N is necessary to allow crops to use P (Li and Zhao, 1990).

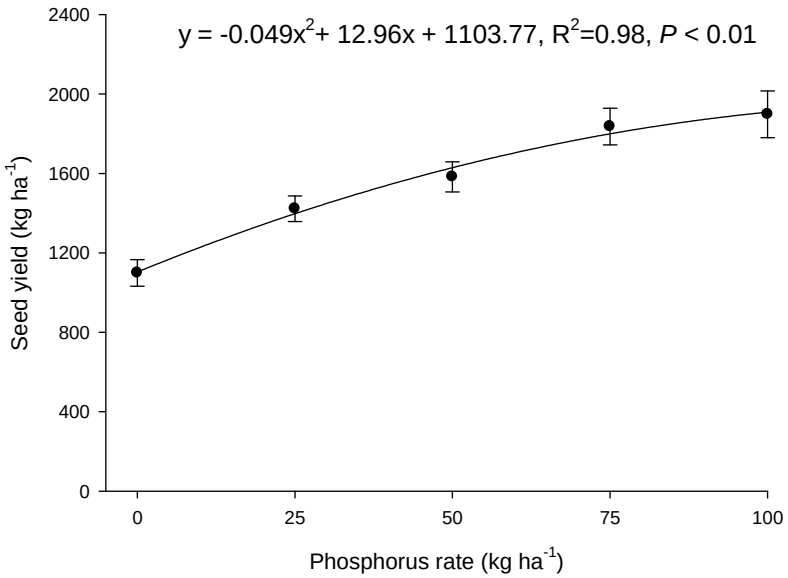


Figure 2: Effect of phosphorus fertilizer rate on seed yield as average across *Pseudomonas fluorescens* strains. Vertical bars represent ± 1 SE of means

3.3 Pod number per m^2

Analysis of variance showed that the main effects of phosphorus fertilizer rate and *Pseudomonas fluorescens* strain were significant for pod number per m^2, but the interaction between them was not significant (Table 3). Regardless of *Pseudomonas fluorescens* strain, pod number per m^2 was increased from 516 ± 12.0 to 574 ± 12.5 pod m^{-2} as P_2O_5 application rate increased from 0 to 100 kg ha^{-1} (Figure 3). Pod number per m^2 was significantly higher at inoculated plots (553 ± 9.6

pod m^{-2} for both strains) than uninoculated ones (524 ± 9.3 pod m^{-2}) as averaged across P application rates (Table 4). The increase in pod number per m^2 at high P level may be due to the positive P effects on increasing the flower formation and improving the fruit setting. Dey et al. (2004) reported that pod number in peanut (*Arachis hypogaea* L.) was significantly increased by inoculation with *Pseudomonas fluorescens* strain.

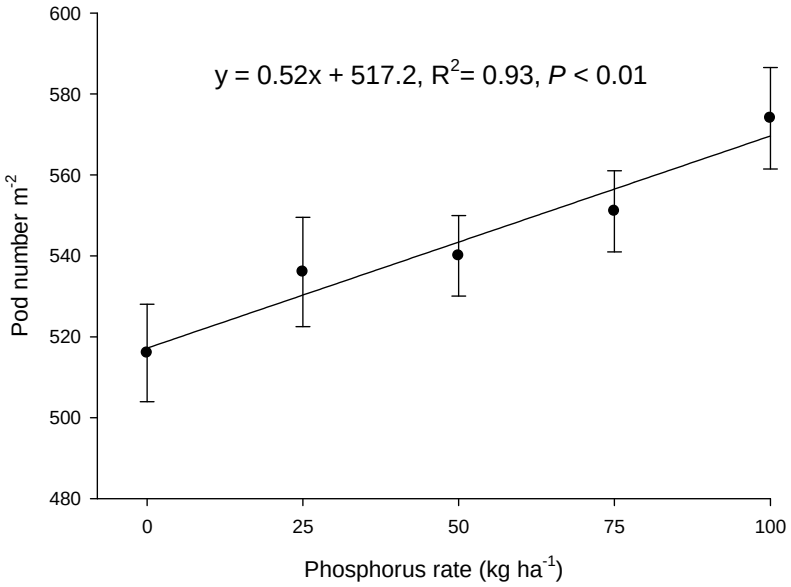


Figure 3: Effect of phosphorus fertilizer rate on pod number per m^2 as average across *Pseudomonas fluorescens* strains. Vertical bars represent ± 1 SE of means

3.4 Seed number per pod

Analysis of variance showed that seed number per pod was not significantly affected by phosphorus fertilizer rate and *Pseudomonas fluorescens* strains. At the same time, *Pseudomonas fluorescens* strains × phosphorus fertilizer rate interaction was not significant (Table 3 and 4).

3.5 100- Seed weight

Phosphorus fertilizer rate and *Pseudomonas fluorescens* strains had significant effect on 100-seed weight. Moreover, a significant phosphorus fertilizer rate × *Pseudomonas fluorescens* strain interaction ($P < 0.01$) was found for 100-seed weight (Table 3). For uninoculated treatment and strain R41, 100-seed weights were linearly increased as phosphorus fertilizer rate increased from 0 to 75 kg P_2O_5 ha^{-1}, and thereafter remained relatively constant (Figure 4). In contrast, for strain R187, 100-seed weight was linearly increased as phosphorus fertilizer rate increased from 0 to 100 kg P_2O_5 ha^{-1} (Figure 4). Better growth and development of crop plants due to phosphorus supply and nitrogen uptake might have increased the supply of assimilates to seed, which ultimately gained more weight (Ali et al., 2004). This finding is similar to the results of Ali et al. (2004), who reported that the highest 1000-seed weight for chickpea was obtained with seed inoculation and 90 kg ha^{-1} phosphorus application, while the lowest 1000-seed weight was obtained with uninoculation and zero applied phosphate. In addition, Dey et al. (2004) reported that, in most cases, seed inoculated plants produced significantly greater 100-seed mass over control plants.

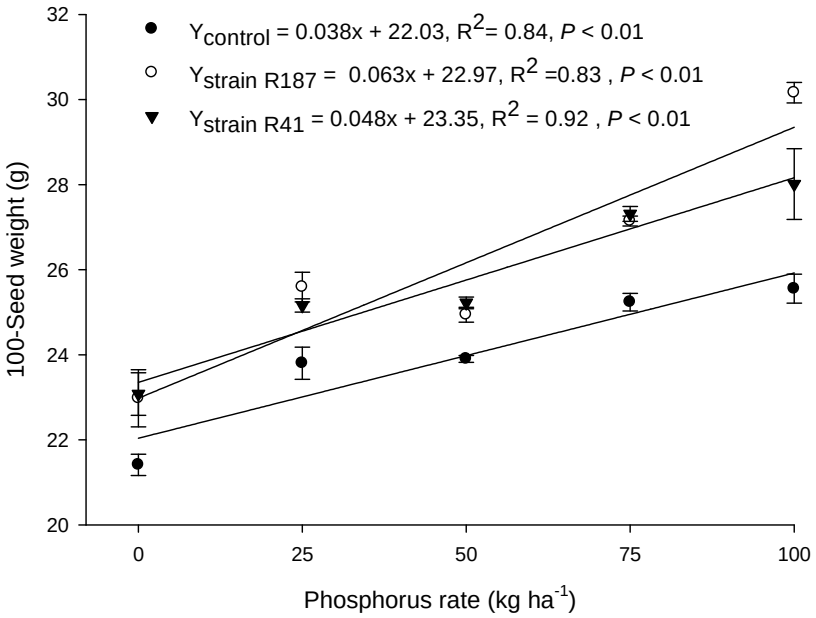


Figure 4: Phosphorus fertilizer rate × *Pseudomonas fluorescens* strains interaction effect on 100-seed weight. Vertical bars represent ± 1 SE of means

3.6 Biological yield

Main effects of *Pseudomonas fluorescens* and phosphorus fertilizer rate were significant for biological yield, while *Pseudomonas fluorescens* strain × phosphorus fertilizer rate interaction was not significant (Table 3). The relationship between P application rate and biological yield was well fitted by a quadratic equation ($y = -0.12x^2+25.16x+4438.28$, $R^2=0.96$). Regardless of *Pseudomonas fluorescens* strain, biological yield was significantly increased from 4483 ± 165 to 5726 ± 151 kg ha^{-1} as P_2O_5 application rate increased from 0 to 75 kg ha^{-1}, and thereafter statistically remained constant (Figure 5). This finding is similar to the result of Ali et al. (2004), who reported that the highest and the lowest biological yield for chickpea was obtained with 90 kg ha^{-1} and zero phosphorus application, respectively. Averaged across P fertilizer rates, biological yields were significantly increased by 15 and 14 % for plants raised from seed inoculated with *Pseudomonas fluorescens* strain R41 and those raised from seed inoculated *Pseudomonas fluorescens* strain R187, respectively, compared to plants raised from uninoculated seeds (Table 4). Consistent with this result, Ali et al. (2004) reported that seed inoculated chickpea plants produced greater biological yield than uninoculated ones. In addition, our findings also agree with those of Dey et al. (2004) who found greater haulm yield in seed inoculated plants of peanut (*Arachis hypogaea* L.) compared to control plants. Phosphorus is a component of ATP the "energy unit" of plants. ATP, forms during light reactions of photosynthesis, is then available as an energy source for dark reactions, which sugars are used as building blocks to produce other cell structural and storage components. At the same time, phosphorus increases leaf area index through increases in leaf number per plant, leaf cell division and elongation, which in turn increases radiation interception and plant photosynthesis rate and, therefore, increases plant biomass accumulation. Moreover, pseudomonas bacteria improve plant growth through increasing phosphorus availability to the plant as well as producing siderophore (Dey et al., 2004), ACC-deaminase enzyme (Dey et al., 2004; Shaharoona et al., 2008) and phytohormone such as indole-acetic acid (Leinhos and Nacek, 1994; Dey et al., 2004).

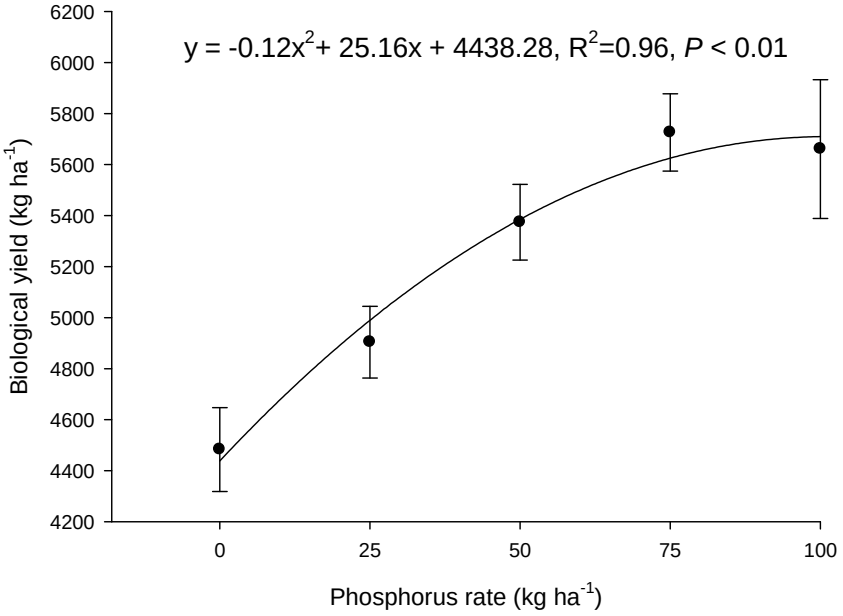


Figure 5: Effect of phosphorus fertilizer rate on biological yield as average across *Pseudomonas fluorescens* strains. Vertical bars represent ± 1 SE of means

3.7 Harvest index

Harvest index was significantly influenced by *Pseudomonas fluorescens* strain and phosphorus fertilizer rate (Table 3). However, the interaction between pseudomonas strain and phosphorus fertilizer rate was not significant (Table 3). Averaged across *Pseudomonas fluorescens* strains, harvest index was significantly increased from 24 ± 0.8 % to 32.5 ± 0.7 % as P_2O_5 application rate increased from 0 to 75 kg ha^{-1}, and thereafter slightly increased (Figure 6). Regardless of P application rate, plants raised from uninoculated seeds had lower HI than those raised from inoculated seeds, but no significant difference in HI was observed between those raised from inoculated seeds with *Pseudomonas fluorescens* strain R187 and strain R41 (Table 4). These indicate that plant produced higher biomass and advocated higher dry weight to seeds under high P application rate and seed inoculation with pseudomonas strains compared to low P application rate and uninoculated conditions. Similarly, Roy et al. (1995) concluded that HI for gram (*Cicer arietinum* L.) was increased by inoculation.

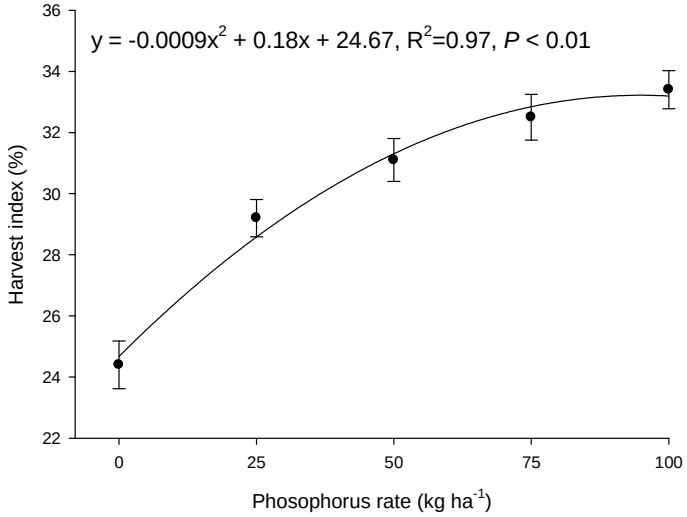


Figure 6: Effect of phosphorus fertilizer rate on harvest index as average across *Pseudomonas fluorescens* strains. Vertical bars represent ± 1 SE of means

3.8 Leaf P concentration

Phosphorus fertilizer rate, *Pseudomonas fluorescens*, and phosphorus fertilizer rate × *Pseudomonas fluorescens* strain had significant effect on leaf P concentration (Table 3). Leaf P concentration was more rapidly increased for seed inoculated plants with strains R187 and R41 than control plant when P_2O_5 application rate increased from 0 to 100 kg ha^{-1}. At the same time, the highest leaf P concentration was recorded for seed inoculated plants with strains R187 and plants received 100 kg ha^{-1} phosphorus fertilizer (Figure 7). This finding is similar to the result of Dey et al. (2004), who reported that seed inoculation with some *P. fluorescen* strains significantly enhanced the total phosphorus content in shoot of peanut (*Arachis hypogaea* L.) compared to control (uninoculated) plants.

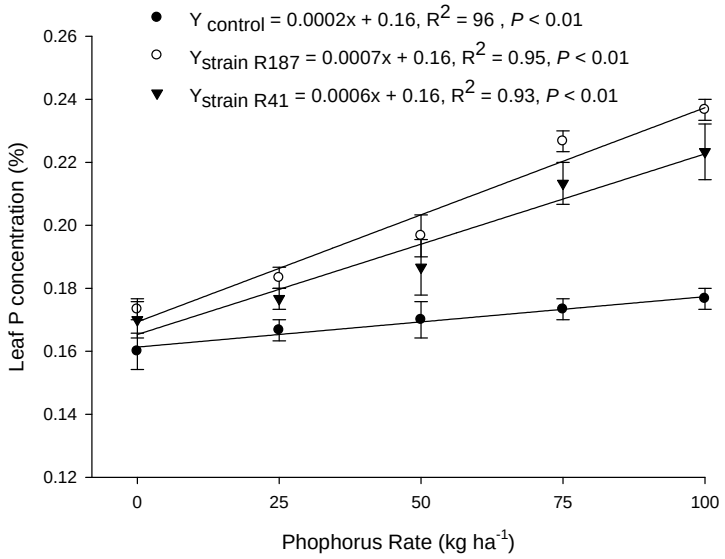


Figure 7: Phosphorus fertilizer rate × *Pseudomonas fluorescens* strains interaction effect on leaf P concentration. Vertical bars represent ± 1 SE of means

4 CONCLUSIONS

The experiment illustrated that seed yield was significantly increased from 1099 ± 67 to 1898 ± 118 kg ha^{-1} as P application rate increased from 0 to 75 kg ha^{-1}, and thereafter statistically remained constant. Plants raised from seeds inoculated with *Pseudomonas fluorescens* produced greater grain yield compared to those raised from uninoculated seeds. However, there was no significant difference in grain yield between plants raised from inoculated seeds with *Pseudomonas fluorescens* strain R187 and those raised from inoculated seeds with *Pseudomonas fluorescens* strain R41. Based on the results of this study, application of phosphorus at the rate of 75 kg ha^{-1} and seed inoculation with *Pseudomonas fluorescens* is recommended for obtaining the greatest seed yield in field pea.

5 REFERENCES

Adesemoye A.O., Torbert H.A., Kloepper J.W. 2009. Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microbial Ecology, 58:921–929, doi: 10.1007/s00248-009-9531-y

Ali H., Khan M.A., Randhawa S.A. 2004. Interactive effect of seed inoculation and phosphorus application on growth and yield of chickpea (*Cicer arietinum* L.). International Journal of Agriculture and Biology, 6, 1: 110–112

Assuero S.G., Mollier A., Pellerin S. 2004. The decrease in growth of phosphorus-deficient maize leaves is related to a lower cell production. Plant, Cell and Environment, 27: 887–895, doi: 10.1111/j.1365-3040.2004.01194.x

Benhamou N., Belanger R.R., Paulitz T., 1996b. Ultrastructural and cytochemical aspects of the interaction between Pseudomonas fluorescens and Ri T-DNA transformed pea roots: host response to colonization by *Pythium ultimum* Trow. Planta, 199: 105–117, doi: 10.1007/BF00196887

Benhamou N., Kloepper J.W., Quadt-Hallmann A., Tuzun S., 1996a. Induction of defense-related ultrastructural modifications in pea root tissues inoculated with endophytic bacteria. Plant Physiology, 112: 919–929

Bhattacharyya P.N., Jha D.K. 2012. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World Journal of Microbiology and Biotechnology, 28:1327–1350, doi: 10.1007/s11274-011-0979-9

Chung H., Park M., Madhaiyan M., Seshadri S., Song J., Cho H., Sa T. 2005. Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. Soil Biology and Biochemistry, 37: 1970–1974, doi: 10.1016/j.soilbio.2005.02.025

Dashti N., Zhang F., Hynes R., Smith D.L. 1998. Plant growth promoting rhizobacteria accelerate nodulation and increase nitrogen fixation activity by field grown soybean [*Glycine max* (L.)Merr.] under short season conditions. Plant and Soil, 200:205–213, doi: 10.1023/A:1004358100856

Davison J. 1988. Plant beneficial bacteria. Natural Biotechnology, 6:282–286, doi: 10.1038/nbt0388-282

De Meyer G., Hofte M. 1997. Salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 induces resistance to leaf infection by *Botrytis cinerea* on bean. Phytopathology, 87: 58–593, doi: 10.1094/PHYTO.1997.87.6.588

Dey R., Pal K.K., Bhatt D.M., Chauhan S.M. 2004. Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. Microbiological Research, 159, 371–394, doi: 10.1016/j.micres.2004.08.004

Ferreira J.S., Baldani J.I., Baldani V.L.D. 2010. Selecao de bactérias diazotroficas em duas variedades de arroz. Acta Scientiarum Agronomy, 32: 179–185, doi: 10.4025/actasciagron.v32i1.732

Figueiredo M.V.B., Seldin L., Araujo F.F., Mariano R.L.R. 2011. Plant growth promoting rhizobacteria: fundamentals and applications. In: Maheshwari, D.K. (Ed.), Plant Growth and Health romoting Bacteria. Springer-Verlag, Berlin, Heidelberg, pp. 21–42

Food and Agricultural Organization (FAO). 2012. FAOSTAT statistics database [Online]. Available at http:// http://faostat.fao.org

Grimes H.D., Mount M.S. 1984. Influence of *Pseudomonas putida* on nodulation of *Phaseolus vulgaris*. Soil Biology and Biochemistry, 16:27–30, doi: 10.1016/0038-0717(84)90121-4

Gulati A., Sharma N., Vyas P., Sood S., Rahi P., Pathania V., Prasad R. 2010. Organic acid production and plant growth promotion as a function of phosphate solubilization by Acinetobacter rhizosphaerae strain BIHB 723 isolated from the cold deserts of the trans-Himalayas. Archive der Microbiology 192: 975–983, doi: 10.1007/s00203-010-0615-3

Gyaneshwar P., Kumar G.N., Parekh L.J., Poole P.S. 2002. Role of soil microorganisms in improving P nutrition of plants. Plant and Soil, 245:83–93, doi: 10.1023/A:1020663916259

Hussain N., Khan A.Z., Akbar H., Akhtar S. 2006. Growth factors and yield of maize as influenced by phosphorus and potash fertilization. Sarhad Journal of Agriculture 22, 4: 579–583

Kavanova´ M., Lattanzi F.A., Grimoldi A.A., Schnyder H. 2006. Phosphorus Deficiency Decreases Cell Division and Elongation in Grass Leaves. Plant Physiology, 141:766–775, doi: 10.1104/pp.106.079699

Khan M.S., Zaidi A., Wani P.A. 2006. Role of phosphatesolubilizing microorganisms in sustainable agriculture – a review. Agronomy for Sustainable Development, 27: 29–43, doi: 10.1051/agro:2006011

Leinhos V., Nacek O. 1994. Biosynthesis of auxins by phosphate solubilizing rhizobacteria from wheat (*Triticum aestivum*) and rye (*Secale cereale*). Microbiology Research, 149: 31–35, doi: 10.1016/S0944-5013(11)80132-1

Li S.X., Li S.Q. 1992. Responses of wheat, hairy vetch and pea to phosphate fertilizer. Acta University Agriculturae Boreali-occidentalia, 20: 74–78

Li S.X., Wang Z.H., Stewart B.A. 2011 Differences of Some Leguminous and Nonleguminous Crops in Utilization of Soil Phosphorus and Responses to Phosphate Fertilizers. Advances in Agronomy, 110: 126- 249, doi: 10.1016/B978-0-12-385531-2.00003-7

Li, S.X., Zhao, B.S. 1990. The effect of soil nitrogen supplying capacity on phosphate fertilizer efficiency for some legume crops and non-legume crops. Soil Fertility, 4: 19–23

Lin C.G., Li Z.P., Zhang Y.H., Zou Q.X. 1964. Study on improvement of P fertilizer effect in the calcareous brown soil areas of Shanxi Province. Chinese Journal of Soil Science, 1: 4–12

Lowry O., Lopez A. 1946. Determination of inorganic phosphate in the presents of labile phosphate esters. Journal of Biological Chemistry, 162: 421-426

Raghothama K.G., Karthikeyan A.S. 2005. Phosphate acquisition. Plant and Soil, 274: 37–49, doi: 10.1007/s11104-004-2005-6

Roy S.K., Rahaman S.M.L., Salahuddin A.B.M. 1995. Effect of Rhizobium inoculation and nitrogen on nodulation, growth and seed yield of gram (*Cicer arietinum* L.). Indian Journal of Agronomy, 65: 853–7

Saravanakumara D., Vijayakumarc C., Kumarb N., Samiyappan R. 2007. PGPR-induced defense responses in the tea plant against blister blight disease. Crop Protection, 26: 556–565, doi: 10.1016/j.cropro.2006.05.007

SAS. 2004. SAS Institute, version 9.1.3. Cary, NC, USA

Sgroy V., Cassan F., Masciarelli O., Del Papa M.F., Lagares A., Luna V. 2009. Isolation and characterization of endophytic plant growth-promoting (PGPB) or stress homeostasis-regulating (PSHB) bacteria associated to the halophyte Prosopis strombulifera. Applied Microbiology and Biotechnology, 85:371–381, doi: 10.1007/s00253-009-2116-3

Shaharoona, B., Naveed, M., Arshad, M., Zahir, Z.A., 2008. Fertilizer-dependent efficiency of Pseudomonads for improving growth, yield, and nutrient use efficiency of wheat (*Triticum aestivum* L.). Applied Microbiology and Biotechnology, 79: 147–155, doi: 10.1007/s00253-008-1419-0

Sharma A, Johri B.N., Sharma, AK., Glick B.R. 2003 Plant growth-promoting bacterium Pseudomonas sp. strain GRP3 influences iron acquisition in mung bean (*Vigna radiata* L. Wilzeck). Soil Biology and Biochemistry, 3: 887–894, doi: 10.1016/S0038-0717(03)00119-6

Urbano G., Aranda P., Gomez-Villalva E. 2003. Nutritional evaluation of pea (*Pisum sativum* L.) protein diets after mild hydrothermal treatment and with and without added phytase. Journal of Agricultural and Food Chemistry, 51: 2415–2420, doi: 10.1021/jf0209239

Van Elsas J.D., Van Overbeek L.S., Fouchier R. 1991. A specific marker, pat, for studying the fate of introduced bacteria and their DNA in soil using a combination of detection techniques. Plant and Soil, 138: 49–60, doi: 10.1007/BF00011807

COBISS Code 1.01

DOI: 10.14720/aas.2015.105.2.05
Agrovoc descriptors: soybeans, glycine max, environmental control, water, light, irrigation, irrigation scheduling, crop management, crop yield

Agris category code: f01, f06

Rate and duration of seed filling and yield of soybean affected by water and radiation deficits

Kazem GHASSEMI-GOLEZANI[1]*, Javad BAKHSHI[1], Bahareh DALIL[2]

Received July 09, 2015; accepted August 18, 2015.
Delo je prispelo 09. julija 2015, sprejeto 18. avgusta 2015.

ABSTRACT

Seed filling and yield of soybean under water and radiation deficits were investigated during 2011 and 2012. Treatments were irrigations (I_1, I_2, I_3 and I_4 for irrigation after 60, 90, 120 and 150 mm evaporation from class A pan, respectively) in main plots and light interceptions (L_1: 100 %, L_2: 65 % and L_3: 25 % sunlight) in sub-plots. Seeds per plant under I_1 and I_2 decreased, but under I_3 and I_4 increased as a result of radiation deficit. Maximum seed weight and seed filling duration of plants under 25 % light interception (L_3) were higher than those under full sunlight (L_1) and 65 % light interception (L_2). In contrast, plants under full sunlight had the highest seed filling rate, particularly under water stress. Seed filling duration under severe light deficit (L_3) was about 9 days longer than that under full sunlight (L_1), leading to 15.8 % enhancement in maximum seed weight. Decreasing seed yield of soybean under well watering and mild water stress and improving it under moderate and severe water deficit due to low solar radiation are directly related with changes in seed filling duration and consequently in seed weight and number of seeds per plant under these conditions.

Key words: seed filling, shading, soybean, water deficit, yield

IZVLEČEK

POMANJKANJE VODE IN SVETLOBE VPLIVATA NA HITROST IN TRAJANJE POLNENJA SEMEN IN PRIDELEK SOJE

Vpliv pomanjkanja vode in svetlobe na polnenje semen in pridelek soje je bil preučevan v poljskem poskusu v letih 2011 in 2012. Obravnavanja so bila različni režimi namakanja (I_1, I_2, I_3 in I_4 kot namakanje po 60, 90, 120 in 150 mm evaporacije iz razreda A) na glavnih poskusnih ploskavah in različni svetlobni režimi (L_1: 100 %, L_2: 65 % in L_3: 25 % delež svetlobe) na podploskvah. Število semen na rastlino se je pri obravnavanjih I_1 in I_2 zmanjšalo, a se je pri obravnavanjih I_3 in I_4 povečalo kot posledica pomankanja svetlobe. Največja masa semen in najdaljše trajanje polnenja semen sta bila večja pri rastlinah, ki so rastle pri 25 % osvetlitvi (L_3) kot pri rastlinah, ki so rastle na polni (L_1) in 65 % (L_2) osvetlitvi.V nasprotju s tem, so imele rastline pri polni osvetlitvi največjo hitrost polnenja semen, še posebej ob sušnem stresu. Trajanje polnenja semen je bilo pri večjem pomanjkanju svetlobe (L_3) za 9 dni daljše kot pri polni osvetlitvi (L_1), kar je vodilo k 15.8 % povečanju maksimalne mase semen. Zmanjšanje pridelka semena soje pri polnem zalivanju ali blagem sušnem stresu in njegovo povečanje pri zmernem in velikem vodnem deficitu je bilo posledica manjše osvetlitve, kar je neposredno povezano s spremembami v trajanju polnenja semen in posledično s spremembami v masi semen in številu semen na rastlino v teh razmerah.

Ključne besede: polnenje semen, senčenje, soja, vodni deficit, pridelek

[1] Department of Plant Eco-physiology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran; *corresponding author: golezani@gmail.com

[2] Department of Agriculture, Payame Noor Universtiy, Tehran, Iran

This research article based on a work for PhD degree.

1 INTRODUCTION

Soybean is an important seed legume due to its high protein (35 %), oil and carbohydrate contents (21 % and 34 %, respectively). Nitrogen fixing ability (17-127 kg/ha/year) is another advantage of soybean plants (Messina 1997). Growth, development and yield of soybean are the result of a genetic potential interacting with environment. Soybean seed production may be limited by environmental stresses and minimizing these stresses will optimize seed yield (Mc Williams et al. 2004).

One of the important environmental stresses that affect crop production worldwide is water stress. When the full crop requirements are not met, water deficit in the plant can develop to a point where crop growth and yield are affected. The need for water in soybean increases with plant development, peaking during the flowering and seed filling phases (7-8 mm day^{-1}) and decreasing thereafter (Bertolli et al 2012). So, various growth stages of soybean respond differently to water stress (Egli and Bruening 2004). It has been observed that maximum reduction in yield, due to drought stress occurs during the pod set and seed filling period (Desclaux et al. 2000).

Radiation is another important factor affecting crop photosynthesis, development and yield (Zhao & Osterhuis 1998). Reduction of solar radiation imposes a limitation to biological productivity in plant, although the extent of the limitation varies with shade tolerance of the species and the nitrogen supply (Wong 1991). Shade, regardless of its source, reduces photosynthetically active radiation (PAR) and alters spectral quality, affecting plant photosynthesis (Bel et al. 2000). It has been reported (Kobata & Takami 1986) that inhibition of photosynthesis during the seed filling period, due to environmental stresses like shading, can result in a major reduction in seed yield of rice. At this time the shortage of available assimilate caused by shading during the early seed filling period, restricts final seed weight at the fully ripe stage, even if the shading is removed during the remainder of the seed filling period. In comparison, Nasrullahzadeh et al. (2007) showed that shading increases seed filling duration and seed weight of faba bean.

In most seed crops, individual seed weight is commonly analyzed as the product of the individual seed growth rate by the duration of seed filling. In legume seeds, the active filling period begins when the pod wall has approximately reached its final size. At the end of this phase, cell division stops, linear dry matter accumulation begins in cotyledons and continues until physiological maturity (Ney et al. 1993). Seed filling duration varies with changes in environmental conditions (Dumoulin et al. 1994). Thus, this research was arranged to evaluate variation in seed filling rate and duration and yield of soybean in response to different levels of radiation and irrigation.

2 MATERIALS AND METHODS

Two field experiments were carried out at the Research Farm of the University of Tabriz, Tabriz, Iran (Latitude 38º05´N, Longitude 14º17´E, Altitude 1360m above sea level) during the growing seasons of 2011 and 2012. The soil of the research area was sandy loam with an EC of 0.68 dS/m, pH of 8.1 and field capacity of 28.8 %. The experiments were arranged as split plot on the bases of randomized complete block design in three replicates. Factors were four irrigation treatments (I_1, I_2, I_3, I_4: irrigation after 60, 90, 120 and 150 mm evaporation from class A pan, respectively) in main plots and three levels of light interceptions (L_1: 100%, L_2: 65% and L_3: 25% sunlight) in sub plots. Seeds of soybean (Williams) were treated with Benomyl at a rate of 2 g/ kg and sown in prepared plots on May 2011 and 2012 at a depth of about 4cm. Seeding rate was 64 seeds m^{-2}. Plots were irrigated immediately after sowing, but subsequent irrigations were carried out according to the treatments. Hand weeding was done as required. Shading nets were spread over an iron frame (3m × 3m) 1.5 m above the soil to ensure good ventilation. These nets were large enough to fully cover the corresponding shaded plots immediately after seedling establishment up to

maturity. Averages of day length and solar radiation (full sunlight) during plant growth and development were 14.1 hours and 16.9 MJ/m^2/day, respectively.

During seed filling, plants were harvested at 8 stages with 5 days intervals. Seeds of each sample were oven-dried at 130ºC for 17 hours and then seed dry weight was determined. Maximum seed weight and seed filling duration were estimated, using a two piece regression model:

$$w = \begin{cases} a + bt & t < tm \\ a + bt & t \geq tm \end{cases}$$

Where *W* is seed weight, *a* is the intercept, *b* is the slope, *t* is days after anthesis and *tm* is the end of seed filling period (time of mass maturity). Subsequently, seed filling rate (*SFR*) was calculated as:

$$SFR = MSW/SFD$$

Where *MSW* is maximum seed weight and *SFD* is seed filling duration.

At maturity, 10 plants from each plot were harvested and seeds per plant were counted, and then mean number of seeds for each plot was calculated. Finally, 1m^2 in the middle of each plot was harvested and seeds detached from the pods and seed yield per unit area was determined. Statistical analysis was performed with MSTATC and SAS soft-wares and Excel soft-ware was used to draw the figures. Duncan test was applied to compare means of each trait at 5 % probability.

3 RESULTS

Combined analyses of variance (Table1) showed significant effects of year on seeds per plant, rate and duration of seed filling and seed yield, but not on maximum seed weight. Maximum seed weight, seed filling duration and seed filling rate were not significantly affected by irrigation intervals, while the effects of irrigation on seeds per plant and seed yield per unit area were significant. Rate and duration of seed filling and maximum seed weight were significantly influenced by radiation deficiency. Interaction of irrigation × light interception was also significant for seeds per plant, seed filling rate and seed yield, but interaction of irrigation × year was only significant for seed yield (Table 1).

Table 1: Combined analyses of variance of the effects of different irrigation and shading treatments on soybean

Source	df	Seeds per plant	Maximum seed weight	Seed filling duration	Seed filling rate	Seed yield
Year (Y)	1	341.47**	2.31	5288.23**	47.87**	146148.51**
Rep (Y)	4	94.19	990.3	123.11	3.18	6324.38
Irrigation (I)	3	609.74*	110.68	13.29	0.832	46844.34**
Y * I	3	39.3	851.98	26.29	0.471	4605.87**
Ea	12	26.26	313.81	78.91	0.758	2442.17
Light (L)	2	29.53	3606.51*	718.09*	19.05*	241.67
I * L	6	126.73**	475.99	74.13	5.21*	8157.65*
L * Y	2	8.15	169.89	35.81	0.87	230.31
L * I * Y	6	6.04	217.57	90.45	1.17	1789.64
Eb	32	27.1	438.28	93.37	1.34	1601.53
C.V (%)	-	21.05	16	25.74	29.32	22.54

*'** Significant at p≤0.05 and p≤0.01, respectively

Number of seeds per plant in 2012 was higher than that in 2011 (Table 2). Seeds per plant under I_1 and I_2 decreased, whereas under I_3 and I_4 increased as a result of radiation deficit. The highest number of seeds per plant was obtained from well watered plants under full sunlight. In general, mean

numbers of seeds per plant of soybean under I_3 and I_4 were lower than that under I_1 and I_2 (Figure 1).

Maximum seed weight and seed filling duration of plants under 25% light (L_3) were higher than those under full sunlight (L_1) and 65% light (L_2). Mean of seed filling duration in 2011 was significantly higher than that in 2012, while seed filling rate in 2012 was significantly higher than that in 2011. Seed filling rate of plants under full sunlight was higher than that under shading treatments, with no significant difference between L_2 and L_3 (Table 2). Plants under full sunlight had the highest seed filling rate under all irrigation treatments. This superiority was more evident under water stress (Figure 2).

Table 2: Means of maximum seed weight, seed filling duration and rate of soybean under irrigation and radiation treatments in 2011 and 2012

Treatments	Seeds per plant	Maximum seed weight (mg)	Seed filling duration (day)	Seed filling rate (mg day^{-1})
Year				
2011	22.55b	130.66a	46.11a	2.83b
2012	26.91a	131.01a	28.96b	4.52a
Light interception				
L_1	25.4a	125.15b	32.82b	3.81a
L_2	25.34a	122.45b	37.67ab	3.25b
L_3	23.45a	144.91a	42.11a	3.44b

Different letters in each column indicate significant difference at $P \leq 0.05$.
L_1, L_2, L_3: 100 %, 65 % and 25 % light interception, respectively

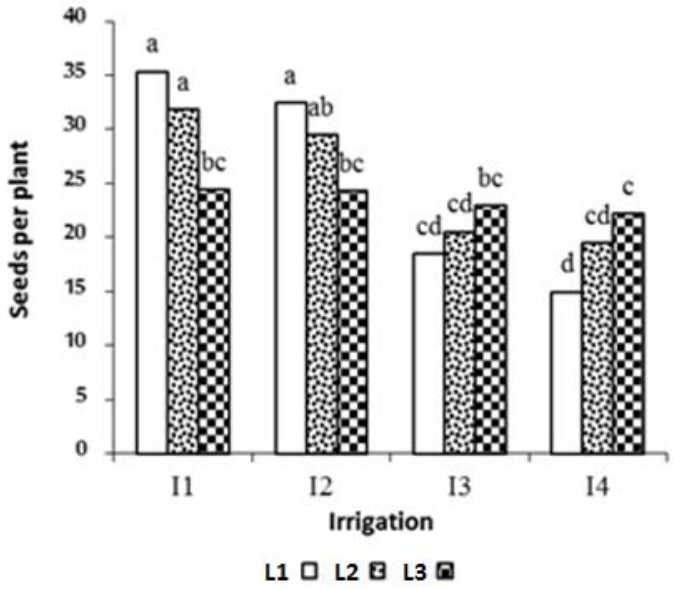


Figure 1: Seeds per plant under different irrigation and shading treatments. I_1, I_2, I_3, I_4: Irrigation after 60, 90, 120 and 150 mm evaporation, respectively. L_1, L_2, L_3: 100 %, 65 % and 25 % light interception, respectively

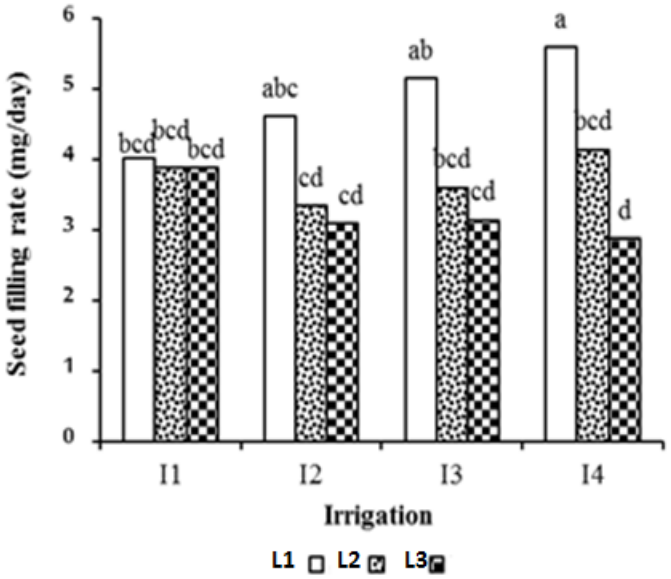


Figure 2: Seed filling rate of soybean under different irrigation and radiation treatments. I_1, I_2, I_3, I_4: Irrigation after 60, 90, 120 and 150 mm evaporation, respectively. L_1, L_2, L_3: 100 %, 65 % and 25 % light interception, respectively

Seed yield per unit area of plants under all irrigation treatments in 2011 was generally lower than that in 2012 (Figure 3). In general, seed yield under I_1 and I_2 was higher than that under I_3 and I_4 in all light interceptions. However, seed yield of plants under I_1 and I_2 decreased, but under I_3 and I_4 increased as a result of shading. The highest seed yield was recorded under well watering with full sunlight (Figure 4).

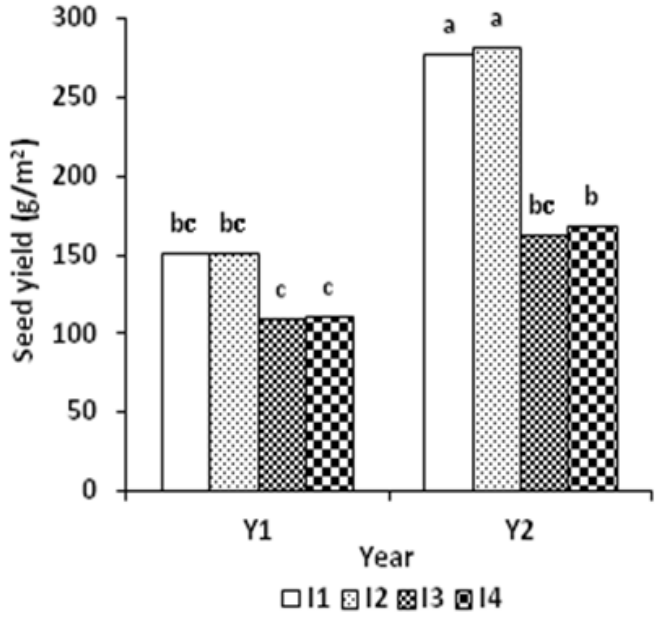


Figure 3: Seed yield of soybean under different irrigation treatments in 2011 (Y_1) and 2012 (Y_2). I_1, I_2, I_3, I_4: Irrigation after 60, 90, 120 and 150 mm evaporation, respectively

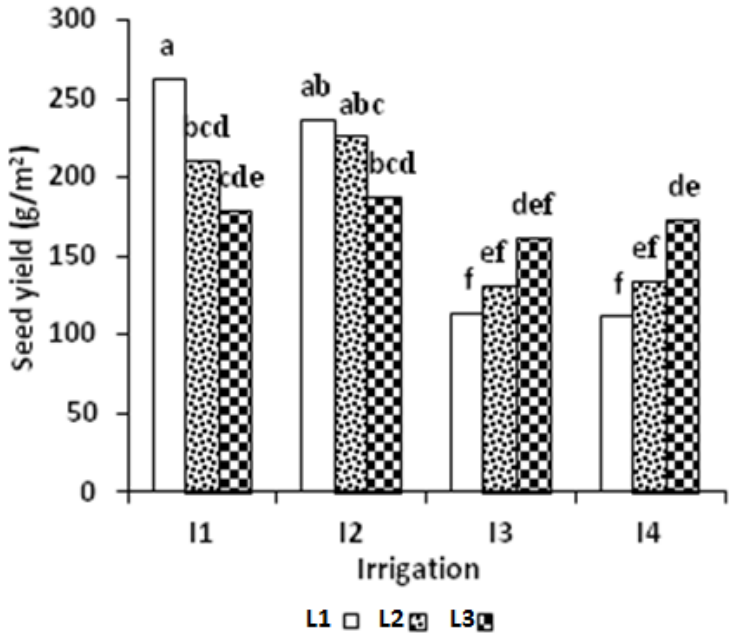


Figure 4: Seed yield of soybean under different irrigation and radiation treatments. I_1, I_2, I_3, I_4: Irrigation after 60, 90, 120 and 150 mm evaporation, respectively. L_1, L_2, L_3: 100 %, 65 % and 25 % light interception, respectively

4 DISCUSSION

Similar maximum seed weight in two years was the result of longer seed filling duration in 2011 and higher seed filling rate in 2012 (Table 2). It has been reported that seed filling rate (Ghassemi-Golezani et al. 2009) and seed filling duration (De Souza et al. 1997) are positively associated with maximum seed weight in soybean.

Although seed filling rate of plants under shade was lower than that under full sunlight, lower radiation increased seed filling duration (Table 2) as a result of decreasing temperature (Ghassemi-Golezani et al. 2013). Seed filling duration under low radiation (L_3) was about 9 days longer than that under full sunlight (L_1), leading to 15.8 % enhancement in maximum seed weight. Similarly, Nasrullahzadeh et al. (2007) reported that delaying physiological maturity under low light increases seed filling duration and consequently mean seed weight of faba-bean. Hadi et al. (2006) also showed that reduction in radiation could increase seed filling duration and mean seed weight of common bean. Seed weight is determined during the seed filling process and duration of seed filling is an important component of maturity (Monpara 2011).

Reduction in seed filling rate due to shading, particularly under severe water stress (Figure 2) may be associated with reduction in cotyledon cell division and cell number of soybean. It has been suggested that both cotyledon cell number and assimilate supply are important in determining seed growth rate (Egli et al. 1989). According to Egli and Bruening (2001), reduction in assimilate availability due to shading decreases seed filling rate and increases effective filling period in soybean. Anyway, variation in seed filling rate under different light interceptions had no significant effect on final seed weight of soybean (Table 2).

Increasing seed filling rate of non-shaded plants with increasing water stress (Figure 2) is the result of early senesces (Bahrani et al. 2009). It has been reported that under water deficit, particularly when there is access to carbohydrates either directly from the leaf photosynthesis or from those pre-stored in stems or leaves, seed filling rate increases (Ahuja et al. 2008). Yang et al. (2001) suggested that an altered hormonal balance in rice seeds by water stress during seed filling, especially a

decrease in GAs and an increase in ABA, enhances the remobilization of pre-stored carbon to the seeds and accelerates the seed filling rate.

Since there was no significant difference in maximum seed weight between two years (Table 2), higher seed yield in 2012 (Figure 3) resulted from greater number of seeds per plant under all irrigation treatments (Figure 1). However, decreasing seed yield of soybean under well watering and mild water stress and improving it under moderate and severe water deficit due to light deficit (Figure 4) are directly related with changes in seed filling duration and consequently in seed weight (Table 2) and number of seeds per plant (Figure 1) under these conditions.

5 CONCLUSION

Plants could be subjected to different levels of shading or solar radiation in intercropping, agroforestry and cropping under fruit trees. Water deficit may also occur in these conditions. Therefore, it is very important to understand the interactive effects of water and radiation deficits.

The results of this research clearly showed that shading could reduce the drought impact on soybean plants via enhancing seed filling duration, seed weight, seeds per plant and consequently seed yield per unit area due to reducing temperature. However, soybean is not an appropriate crop to grow under shade, when water supply is sufficient. Similar works on different crops, particularly on shade tolerant plants, can improve our knowledge about these interactions.

6 REFERENCES

Ahuja L.R., Reddy V.R., Saseendran S.A., Qiang Y. 2008. Current water deficit stress simulations in selected agricultural system models. In: Saseendran S.A., Ahuja L.R., Ma L. (Eds.). Response of crops to limited water, American Society of Agronomy, Madison, USA, pp. 1-38

Bahrani A., Heidari-Sharifabad H., Tahmasebi-Sarvestani Z., Moafporian G.H., Ayenehband A. 2009. Wheat response to nitrogen and post-anthesis water deficit. Proc Int Conf on CBEE, Singapore. pp. 33-34.

Bell G.E., Dannenberger T.K., McMaho M.G. 2000. Spectral irradiance available for turfgrass growth in sun and shade. Crop Sci., 40: 189-195, DOI: 10.2135/cropsci2000.401189x

Bertolli S.C., Rapchan G.L., Souza G.M. 2012. Photosynthetic limitations caused by different rates of water-deficit induction in *Glycine max* and *Vigna unguiculata*. Photosynthetica, 50: 329-336, DOI: 10.1007/s11099-012-0036-4

Desclaux D., Huynh T., Roumet P. 2000. Identification of soybean plant characteristics that indicate the timing of drought stress. Crop Sci., 40: 716-722, DOI: 10.2135/cropsci2000.403716x

De Souza P.I., Egli D.B., Bruening W.P. 1997. Water stress during seed filling and leaf senescence in soybean. Agron. J., 89: 807-812, DOI: 10.2134/agronj1997.00021962008900050015x

Dumoulin V., Ney B., Eteve G. 1994. Variability of seed and plant development in pea. Crop Sci., 34: 992–998, DOI: 10.2135/cropsci1994.0011183X003400040030x

Egli D.B., Bruening W.P. 2001. Source-sink relationship, seed sucrose levels and seed growth rates in soybean. Ann. Bot., 88: 235-242, DOI: 10.1006/anbo.2001.1449

Egli D.B., Bruening W.P. 2004. Water stress, photosynthesis, seed sucrose levels and seed growth in soybean. J. Agr. Sci., 142: 1-8, DOI: 10.1017/S0021859604004095

Egli D.B., Ramseur E.L., Zhen-Wen Y., Sullivan C.H. 1989. Source-sink alterations affect the number of cells in soybean cotyledons. Crop Sci., 29: 732-735, DOI: 10.2135/cropsci1989.0011183X002900030039x

Ghassemi-Golezani K., Bakhshi J., Zehtab-Salmasi S., Moghaddam M. 2013. Changes in leaf characteristics and seed yield of soybean (*Glycine max* L.) in response to shading and water stress. Int. J. Biosci., 3: 71-79.DOI: 10.12692/ijb/3.2.71-79

Ghassemi-Golezani K., Taifeh-Noori M., Oustan S.H., Moghaddam M. 2009. Response of soybean

cultivars to salinity stress. J. Food, Agri. Environ., 7: 401- 404

Hadi H., Ghasesemi–Golezani K., Rahimzadeh Khoei F., Valizadeh M., Shakiba M.R. 2006. Responses of common bean (*Phaseolus vulgaris* L.) to different levels of shade. J. Agro., 5: 595-599, DOI: 10.3923/ja.2006.595.599

Kobata T., Takami T. 1986. Changes in respiration, dry matter production and its partition in rice (*Oryza sativa* L.) in response to water deficit during seed filling period. Bot. Mag., 99: 379- 393, DOI: 10.1007/BF02488717

Ludlow M.M., Muchow R.C. 1990. A critical evaluation of traits for improving crop yields in water-limited environments. Adv. Agro., 43: 107-153, DOI: 10.1016/S0065-2113(08)60477-0

Messina M. (1997): Soyfoods: Their role in disease prevention and treatment. In: Liu K. (Ed.). Soybeans: Chemistry, technology and utilization, Chapman and Hall, New York, USA, pp. 442-466, DOI: 10.1007/978-1-4615-1763-4_10

Mc Williams D.A., Berglund D.R., Endres G.J. 2004. Soybean growth and management. North Dakota State University. University of Minnesota.

Monpara B.A. 2011. Seed filling period as a measure of yield improvement in bread wheat. J. Crop Imp., 38: 1-5.

Nasrullahzadeh S., Ghassemi–Golezani K., Javanshir A., Valizade M., Shakiba M.R. 2007. Effects of shade stress on ground cover and seed yield of faba bean (*Vicia faba* L.). J. Food Agri. Environ., 5: 337- 340.

Ney B., Duthion C., Fontaine E. 1993. Timing of reproductive abortion in relation to cell division, water content, and growth of pea seeds. Crop Sci., 33: 267–270, DOI: 10.2135/cropsci1993.0011183X003300020010x

Salimi S., Moradi S. 2012. Effect the correlation, regression and path analysis in soybean genotypes (*Glycine Max* L.) under moisture and normal condition. Int. J. Agro. Plant Prod., 3: 447-454.

Wong G.G. 1991. Shade tolerance of tropical forages: A review. In: Shelton HM, Stuart WW (Eds). Forages for plantation crops, Australian Center for International Agricultural Research, ACIAR Proceedings, Canberra, Australia, pp. 64-69

Yang J., Zhang J., Wang Z., Zhu Q., Wang W. 2001: Hormonal changes in the seeds of rice subjected to water stress during seed filling. Plant Physiol., 127: 315-23, DOI: 10.1104/pp.127.1.315

Zhao D., Oosterhuis D. 1998. Cotton responses to shade at different growth stages: Nonstructural carbohydrate composition. Crop Sci., 38: 1196-1203, DOI: 10.2135/cropsci1998.0011183X003800050014x

COBISS Code 1.01

DOI: 10.14720/aas.2015.105.2.06
Agrovoc descriptors: allelopathy, biological competition lettuce, *Lactuca sativa,* cannabis, *Cannabis sativa,* seed germination, germination, extracts

Agris category code: f01, f62

Allelopathic effect of medicinal plant *Cannabis sativa* L. on *Lactuca sativa* L. seed germination

Homa MAHMOODZADEH[1,*], Mohsen GHASEMI[2], Hasan ZANGANEH[1]

Received April 24, 2015; accepted July 20, 2015.
Delo je prispelo 24. aprila 2015, sprejeto 20. julija 2015.

ABSTRACT

In order to examine allelopathic effect of *Cannabis sativa* L. on germination capability and seedling growth of *Lactuca sativa* L., a study was performed in laboratory conditions. Treatments were set up in randomised block design in four replications for each of four concentration ranges of 25, 50, 75 and 100 % of aqueous extract made of shoot parts and 4 identical extract concentrations made of root of cannabis. Control variant was lettuce seed treated by distilled water. During the studies shoot and seminal root length of lettuce seedlings were measured after treatments with different concentrations of extracts made of root and shoot parts of cannabis, and the obtained values were compared with the control. The obtained results suggest that the extract from the shoot parts of cannabis in high concentrations of 75 and 100% had inhibiting effect to the germination indices while the extract from the root had no statistically significant effect on germination of lettuce seeds. Extract made of root part of cannabis showed also stimulatory effect to shoot and seminal root length of lettuce seedlings in extract concentrations of 50, 75 and 100 %.

Key words: allelopathy, lettuce, aqueous extract, cannabis, seed germination indices

IZVLEČEK

ALELOPATIČEN UČINEK KONOPLJE (*Cannabis sativa* L.) NA KALITEV SEMEN VRTNE SOLATE (*Lactuca sativa* L.)

V laboratorijskih razmerah je bil preučevan alelopatičen učinek navadne konoplje (*Cannabis sativa* L.) na kalitev semen in rast kalic vrtne solate (*Lactuca sativa* L.). Obravnavanja so bila izvedena kot naključni bločni poskus v štirih ponovitvah z vsako od štirih koncentracij, 25, 50, 75 in100 %, vodnega izvlečka poganjkov in korenin konoplje. V kontrolnem poskusu so bila semena vrtne solate tretirana na enak način z distilirano vodo. Poleg indeksa kalitve sta bili v poskusi merjeni še dolžina poganjkov in semenskih korenin kaleče solate. Rezultati so pokazali, da je imel izvleček poganjkov konoplje v večjih koncentracijah, 75 in 100 %, inhibitorni učinek na kalitveni indeks, medtemko izvlečki iz korenin niso imeli statistično značilnega vpliva na iste merjene parametre. Izvleček iz korenin konoplje je imel stimulativni učinek na dolžino poganjkov in semenskih korenin vrtne solate pri koncentracijah 50, 75 in 100 %.

Ključne besede: alelopatija, vrtna solata, vodni izvleček, konoplja, kalitveni indeks

1 INTRODUCTION

Hundreds of medicinal plant species are being used in modern medicines. They have been used as remedy for different diseases e.g. fever, malaria, cough, flu, asthma, colds, chest diseases, skin itch, acne, headache, jaundice, nausea, ulcer, tumors, typhus, stomach pain, heart attack, chills, inflammation, herpes, hepatitis, swelling, etc (Ishaque and Shahni, 1998). Most of them have been collected from wild sources. There is an increasing demand for medicinal plants-based

[1] Department of Biology, Faculty of Science, Mashhad Branch, Islamic Azad University, Mashhad, Iran; corresponding author: homa_mahmoodzadeh@yahoo.com

[2] Department of Agriculture, Mashhad Branch, Islamic Azad University, Mashhad, Iran

drugs and pharmaceuticals in the world market. However, pharmaceutically active compounds can also behave as allelochemicals. These allelopathic compounds can also be used as natural herbicides and other pesticides (Einhelling, 1995).The term "allelopathy" was proposed for expressing the harmful, stimulatory, enhanced and beneficial effects that one plant species has on another through the formation of chemical retardants escaping into the environment (Molisch, 1937). Allelochemicals are plant metabolites present in plants as end products and by-products. These chemicals are present in different parts of plants like stem, leaves, roots, flowers, inflorescence, fruits and seeds. Out of these plant parts, leaves seem to be the most consistent producers of these allelochemicals. These allelochemicals are often released from the plants by volatilization, leaching, exudation and decomposition from plant residues (Molisch, 1937). Major allelochemicals found in plants with documented allelopathic activity are phenolic compounds (Chon et al., 2002) that have stimulatory effects on seed germination and seedling growth of plants. This is generally accepted in the literature that phenolic compounds at low concentrations are stimulatory to germination and plant growth (Hegab et al., 2008; Ghareib et al., 2010), but higher concentrations resulted in a sharp germination reduction. The well-known terpenoids include menthol, camphor, thujone and the cannabinoids are found in *Cannabis sativa* (Ameh et al., 2010). Allelopathics are often due to synergistic activity of allelochemicals rather than to single compounds. The additive or synergistic effects become significant even at low concentrations of the extracts (Einhelliing, 1995). The concept of allelopathy was further supported and developed by Bonner (1950), Grummer and Beyer (1960), Evenari (1961), Whittaker (1970), Pitman and Duke (1978) and Fischer *et al*. (1978). According to Lavabre (1991), allelopathic effects are controversial and still poorly understood. In allelopathy, a major tool for research is bioassay which controls laboratory conditions, high sensitivity gives reproducible results, and takes relative short time to perform. There are many ways to evaluate the herbal aqueous extracts of allelopathic activities. These are hydroponic culture methods, Ratoon screening method, Plant box method (Lee *et al*., 2003), a plastic tray with 6 holes (Fujii *et al*., 2004), Dish pack method - a new bioassay for volatile allelopathy (Fujii *et al.*, 2005), Sandwich method (Fujii *et al*., 2003) and Filter paper (Barbosal *et al*., 2008). The filter paper is a suitable method because it can tolerate the moderate temperature during incubation (25 °C) in the laboratory. The aqueous extracts remain stable for longer period of time. Millipore filter paper is used to make the method sterile. The reason for the use of filter paper in techniques is that, it is easily available and free from contamination. It is easily handled and a good media for germination, it has high flow rate for movement of extracts and porosity (Gill et al., 2009). Allelopathic effect of medicinal species against other plants is well studied (Wahab *et al*., 1967; Rice, 1971; Han *et al*., 2008 and Li *et al*., 2009). *Cannabis sativa* is known for centuries as an effective weeds suppressing crop plant in Iran. Weeding role of this plant is mainly attributed to its high competitiveness for water, food and light, as it overshadows the soil quickly after the initial growth phase and therefore suppresses weed growth (Ranalli, 1999). In general, crop rotation and related practices should aim to give the competitive advantage to the crops in rotation against weeds. Only few studies concerned the rotation effects of *Cannabis sativa*, as an annual crop, fits well into crop rotation and can serve to the control of pests, as it is not related to conventional food crop species (Ranalli, 1999), hence the main purpose of the present study is to evaluate the allelopathic activity of medicinal plant, *Cannabis sativa* L. ssp. *sativa* on lettuce (*Lactuca sativa* L.*)*.

2 MATERIALS AND METHODS

2.1 Plant material and extraction procedure

In the period 2013-2014, at farms near Mashhad, parts of *C. sativa* L. ssp. *sativa* ('TN-96-35') were collected. Plant material was divided into shoot, i.e. stem plus leaf and root. Shoot and root parts were pulverized, after which separate extracts were made from each of these parts of of *C. sativa* L. Ten g of fresh shoot or root parts were mixed with 100 ml distilled water in a blender. The

homogenate was filtered through tissue paper after 10 minutes and the filtrate was centrifuged at 3000 × g for 20 min and the supernatant was used as stock solution. Extract of shoot and and root part of *C. sativa* was made in a range of concentrations of 25, 50, 75 and 100 % of stock solution

2.2 Seed germination test

Filter paper in Petri dishes, of 150 x 25 mm in size was moistened by 5 ml of the obtained extracts, and tested lettuce seed was germinated on it. Control was moistened by distilled water. Lettuce seed (*Lactuca sativa*'Longifolia') surface was sterilized before adding of extracts according to Elemar and Filho (2005). Each concentration of *C. sativa* extracts was made in four replications. Samples were laid in thermostatic device at 22 ± 2°C for 7 days. Laboratory tests were set up in randomized block design in four replications. Each Petri dish contained 25 lettuce seeds, i.e. 100 seeds per treatment. Germination tests were performed according to the rule issued by the International Seed Testing Association. The number of germinated seeds was noted daily for 7 days. Seeds were considered as germinated when their seminal root reached at least 1 mm length. In this study, we used following germination parameters: Germination percentage (GP, %), Germination rate (GR), Relative germination percentage (RGP), Mean germination time (MGT), Germination index (GI) and Weighted germination index (WGI). Final percentage germination (GP) for each treatment was calculated after seven days. The germination index (GI) is based on number of seeds that germinated and the germination rate. These parameters were also calculated from the formulas proposed by (Figueroa and Armesto, 2001; Bu et al., 2007; Wu and Du 2007).

$$GP = 100 \times GN / SN \quad (1)$$

GN is the total number of germinated seeds, SN is the total number of seeds tested

$$RGP = GP\ treatment / GP\ control \times 100 \quad (2)$$

$$GI = (\Sigma(N\text{-}i) \times G_i) \times 100)/(N \times GN) \quad (3)$$

GI is a synthetic measure designed to reflect the synthetical germination ability including germination rate and germination numbers. Where i is the number of days since the day of sowing and Gi is the number of seeds germinated on day i.

A weighted germination index (WGI) as described by Bu et al. (2007) was calculated with maximum weight given to the seeds germinating early and less to those germinating late

$$WGI = [N \times n_1 + (N\text{-}1) \times n_2 + (N\text{-}Z) \times n_3 + \ldots] / N \times N' \quad (4)$$

where n1, n2, …, n60 are the number of seeds that germinated on first, second, and subsequent days until the 60th day, respectively; N is total days of experiment; N´ is the total number of seeds placed in incubation

$$GR = \Sigma G_i / \Sigma n_i G_i \quad (5)$$

Where i is the number of days since the day of sowing and Gi is the number of seeds germinated on day i

Vigor index = germination % × seedling length (root + shoot). (6)

After an incubation period of 7 days, shoot and seminal root length of seedlings were measured using a ruler.

2.3 Data analysis

Significant differences for all statistical tests were evaluated at the level of $p \leq 0.05$ with ANOVA. All data analyses were conducted using SPSS for Windows, Version 13.0.

3 RESULTS

Based upon conducted studies, data on effect of extracts made of shoot and root parts *of C. sativa* to germination indices and seedling growth of *L.sativa* were obtained. Extracts made from shoot parts in concentrations of 75 and 100 % showed significant effects on germination indices and seedling growth of lettuce including: germination percent, relative germination percent, germination rate, mean germination time, weighted germination index and seminal root length, while all concentrations of shoot extracts of *C. sativa* showed no significant effect on germination index,

vigor index and shoot length. Measured values of lettuce seed germination treated by extract of the shoot parts of *C. sativa* confirmed that extract showed allelopathic – inhibitory effect to the germination indices and growth of seminal root in comparison to the obtained control values.

The obtained results for extract concentration of 75 % made of shoot parts of *C. sativa* showed that germination percent and relative germination percent were 90 % which was less in comparison to the value of control of 100 %; for the medium extract concentration of 100 % determined germination percent was 76.67 %. For mean germination time, at higher concentrations were also established lower values. For extract concentration of 75 %, MGT was 4.8 days, whereas in the highest concentration of 100 %, MGT of lettuce seeds was the lowest, i. e. 4.53 in comparison to control variant that was 5 days. For concentration of 100 % of extract made of shoot parts of *C. sativa*, the average value of WGI was 0.84, and control was 1, for concentration of extract of 75 %, the established value of WGI was 0.93,while control was 8.09. Concentration of 75 % and 100 % resulted in GR of 0.45 and 0.38 $Nday^{-1}$, respectively, with control value of 0.5 $Nday^{-1}$. For variant of 75 % of extract from the shoot parts of *C. sativa*, lettuce seminal root length was 2.3 cm. while control was 3.3 mm, the application of highest concentration of extract made of shoot part of 100 % resulted in average seminal root length of 2.1 cm. Duncan test showed statistically significant difference ($P \leq 0.05$) between the most germination indices of lettuce seed only in the case of the extract made of the shoot parts of *C. sativa* in concentration of 75 and 100 % (Table 1).

Table 1: Statistical data (mean ± standard error) on effects of the shoot parts of *Canabis sativa* L. to germination of lettuce seeds

Shoot extract (%)	GP(%)	GR(Nd^{-1})	RGP	MGT(day)	GI	WGI	VI	Shoot length(cm)	Seminal root length(cm)
0	100± 0	0.5± 0	100± 0	5± 0	33.3± 0	1 ±0	506.67± 110	2.1 ±0.65	3.3± 0.26
25	96.67± 5.7	0.48± 0.02	96.67 ±5.7	4.93± 0.11	32.2± 1.9	0.97± 0.04	575.6± 89	2.86± 0.7	3.06± 0.11
50	90± 10	0.45± 0.05	90± 10	4.8± 0.2	29.9± 3.3	0.93± 0.07	457.3± 57.9	2.4± 0.45	2.7± 0.46
75	*90± 0	*0.45± 0	*90± 0	* 4.8 ±0	33.3± 0	*0.93± 0	483± 99	2.8± 0.36	*2.3 ±0.6
100	*76.67± 15.2	*0.38± 0.07	*76.67 ± 15.2	*4.53 ±0.3	26.6± 6.6	*0.84± 0.1	361± 58.9	2.6± 0.26	*2.1± 0.32

*Marked differences are significant at $p<0.05$

Bioassay revealed that for extract of the root of *C. sativa* there were no statistically significant deviations between values of lettuce seed GP, RGP, MGT, GI, VI from control values. For variant of 25, 75 and 100 % of extract from the root parts of *C.sativa*, WGI of lettuce seeds was 0.97, while control was 1. The application of highest concentration of extract made of root part of 100 % resulted in average GR of 0.46 $Nday^{-1}$, whereas the average GR in control was 0.5 $Nday^{-1}$. Length of lettuce seminal root in treatment with the 75 and 100 % extracts made of the root part of *C. sativa* confirmed stimulatory effect in comparison to the control variant. Extract made of root part of *C. sativa* also did show stimulatory effect to shoot length in extract concentrations of 50 % (Table 2).

Table 2: Statistical data on effects of the root parts of *Canabis sativa* L. to germination of lettuce seeds

Root extract(%)	GP(%)	GR(Nd^{-1})	RGP	MGT(day)	GI	WGI	VI	Shoot length(cm)	Seminal root length(cm)
0	100± 0	0.5± 0	100± 0	5± 0	33.3± 0	1± 0	506.67± 110	2.1± 0.65	3.3± 0.26
25	96.67 ±5.7	0.48± 0.02	100± 0	4.93± 0.11	32.2± 1.9	*0.97± 0.04	382.6± 105	2.1 ±0.55	3.3± 0.47
50	96.67 ±5.7	0.5 ±0	100± 0	5± 0	33.3± 0	1± 0	322± 42.3	2.1± 0.52	2.9± 0.3
75	96.67 ±5.7	0.48 ±0.02	96.67 5.7±	4.93± 0.11	32.2 ± 1.9	*0.97± 0.04	314± 16.9	*3.36± 0.46	*4.3 ±0.85
100	93.33± 5.7	*0.46 ±0.02	96.67 5.7±	5.26± 0.64	31.1 ± 1.9	*0.97± 0.04	197.3± 62	2.6± 0.57	*4.3± 0.51

*Marked differences are significant at $p<0.05$

4 DISCUSION

In the study, allelopathic effect of the medicinal plant, *C. sativa* was established for germination indices and early growth of lettuce. Extracts made of shoot parts of *C. sativa* showed inhibitory effects to lettuce seed germination indices except from GI and VI. Extract made of the shoot parts, in concentration of 75 and 100 % significantly affected mentioned factors, while the lower two concentrations did not show a significant effect. Extracts made of shoot parts of *C. sativa* did not show statistically significant effect to shoot growth in any concentration. Extracts made of shoot part of *C. sativa* in concentrations of 75 and 100 % showed statistically significant effect to the growth of seminal root, while concentrations of 25 and 50 % had no statistically significant effect.

The seeds imbibed with shoot extracts of *C. sativa* delayed and inhibited germination in comparison to control. The inhibitory effect of different concentrations of shoot extract of *C. sativa* on seed germination might be due to imbalance in metabolism regulated by various enzyme activities (Oyun, 2006). The structure of plasma membrane might have become denatured by the phytotoxins present in shoot extract of *C. sativa* when the seeds were soaked in extract. Positive correlation was observed between the increase in the concentration of extract and the inhibition of germination percentage. Allelochemicals are known to inhibit the metabolic processes i.e. cellular respiratory ability of the target plant and energy transfer which is responsible for ATP synthesis (Demos *et al*., 1974). The phytotoxins present in shoot extracts of *C. sativa* might have arrested the protease, α - amylase activity resulting in inhibition of protein and starch breakdown which reduces the germination process.

Extract made of root part of *C. sativa* had stimulatory effect to shoot and seminal root length in extract concentrations of 50, 75 and 100 %. The similar phenomenon was observed by other authors (Sun *et al*., 2006) analyzing the allelopathic effect of *Solidago canadensis* L. on rape. Anjum *et al.* (2010) performed laboratory experiments to examine the allelopathic potential of some medicinal plants on the germination and growth of lettuce. Using sandwich method, *Albezzia lebbeck* (L.) Benth. and *Broussonetia papyrifera* (L.) Vent. have strong inhibitory effects on the seminal root and hypocotyls growth of lettuce. However, stimulatory effects were recorded with *C. sativa* and *Parthenium hysterophorus* L. at 5mg leaves concentrations (Anjum *et al.*, 2010).

Extract from the shoot parts of *C. sativa* has a higher inhibitory effect to lettuce germination and seedling growth than extract from roots. Contents of biologically active compounds in shoot and root parts of *C. sativa* are probably different both qualitatively and quantitatively.

5 CONCLUSIONS

Higher content of allelochemicals present in extract of shoot parts of *C. sativa* may be responsible for the inhibition of metabolic activities of lettuce in the laboratory bioassay tests

The study revealed reduced germination capability of lettuce seeds after treatment by extract made of the shoot parts of *C. sativa* which was supported by calculated statistical significance that indicated existence of allelopathic effect. Extract made from the roots of *C. sativa* did not have allelopathic effect to the growth of lettuce and seed germination.

6 REFERENCES

Ameh S.J., Obodozie O., Inyang U. S., Abubakar M.S., Garba M. 2010. Current phytotherapy - A perspective on the science and regulation of herbal medicine. Journal of Medicinal Plants Research. 4(2): 72-81

Anjum A.U., Hussain Z., Yousaf F.K. and Umer A. 2010. Evaluation of allelopathic action of some selected medicinal plant on lettuce seeds by using sandwich method. J. Med. Plants Res. 4: 536-541

Barbosal G.E., Vânia R.P., Sérgio T.M. 2008. Allelopathic evidence in *Brachiaria decumbens* and its potential to Invade the Brazilian Cerrados. Rua do Matão, Travessa 14; Cidade Universitá. 51(4):825-831

Bonner J. 1950. The role of toxic substances in interaction of higher plants. Bot. Rev. 16: 51-65, doi: 10.1007/BF02879785

Bu H.Y., Chen X.L., Wang Y.F. 2007. Germination time, other plant traits and phylogeny in an alpine meadow on the eastern Qinghai-Tibet plateau. Community Ecology. 8: 221 – 227, doi: 10.1556/ComEc.8.2007.2.8

Chon S.U., Choi S.K., Jung S., Jang H.G., Pyo B.S., Kim S.M. 2002. Effects of alfalfa leaf extracts and phenolic allelochemicals on early seedling growth and root morphology of alfalfa and barnyard grass. Crop Prot. 21: 1077-1082, doi: 10.1016/S0261-2194(02)00092-3

Demos E. K., Woolwine M., Wilson R. H., McMillan, C. 1975. The effect of ten phenolic compounds on hypocotyl growth and mitochondrial metabolism of mungbean. American Journal of Botany. 62:97-102, doi: 10.2307/2442083

Einhelling F.A. 1995. Characterization of mechanisms of Allelopathy Modeling and experimental approaches. In: cheng Idergit HH and Dakshini KMM. (eds), allelopathy, organism, processes and applications. American Chemical Society, Washington, pp 132-141

Elemar V., Filho V. 2005. Allelopathic effects of aconitic acid on wild poinsettia (*Euphorbia heterophylla*) and morningglory (*Ipomoea grandifolia*). Brazilian Society on Weed Science Congress, 40(1):217

Evenari M. 1961. Chemical influence of other plants (allelopathy Handbuch der pflanzen-Physiol. 16: 691-736

Figueroa J.A., Armesto J.J. 2001. Community-wide germination strategies in a temprate rainforest of southern chile: ecological and evolutionary correlates. Austrulian Journal of Botany 49: 411 – 425, doi: 10.1071/BT00013

Fischer R.F., Woods R.A., Glavicic M.R. 1978. Allelopathic effects of goldrod and ashes on young sugar maple. Canadian J. Res. 8: 1-9, doi: 10.1139/x78-001

Fujii Y., Parvez S.S., Parvez M.M., Ohmae Y., Iida, O. 2003. Screening of medicinal plant species for allelopathic activity using Sandwich method. Weed Biol. Manage. 3: 233-241, doi: 10.1046/j.1444-6162.2003.00111.x

Fujii Y., Shibuya T., Nakatani K., Itani T., Hiradate S.M., Parvez M. 2004. Assessment method for allelopathic effect from leaf litter leachates. Weed Biol. Manage. 4: 19-23, doi: 10.1111/j.1445-6664.2003.00113.x

Ghareib H.R.A., Abdelhamed M.S., Ibrahim O.H. 2010. Antioxidative effects of the acetone fraction and vanillic acid from *Chenopodium murale* on tomato plants.Weed Biol. Manage. 10: 64–72, doi: 10.1111/j.1445-6664.2010.00368.x

Gill G., Anoliefo L.S., Iduoze U.V. 2009. Allelopathic effects of aqueous extract from Siam Weed on the growth of Cowpea. Department of Botany, University of Benin, Benin City, Nigeria 3rd . edi. pp. 3-20

Han C.M., Pan K.W., Wu N., Wang J.C., Li W. 2008. Allelopathic effect of ginger on seed germination

and seedling growth of soybean and chive. Sci. Hortic. 116(3): 330-336, doi: 10.1016/j.scienta.2008.01.005

Hegab M.M., Khodary S.E.A., Hammouda O., Ghareib H. R. 2008. Autotoxicity of chard and its allelopathic potentiality on germination and some metabolic activities associated with growth of wheat seedlings. Afr. J. Biotechnol. 7: 884–892

Inderijt I., Duke S.O. 2003. Ecophysiological aspects of allelopathy. Planta 217: 529-539, doi: 10.1007/s00425-003-1054-z

Ishaque M., Shahan M.N.I. 1998. Survey and domestication of wild medicinal plants of Sindh. Survey report. KAKC, Islamabad Pakistan pp. 2-3

Lavabre E.M. 1991. Weed control, McMillan Education Ltd. London. pp. 1-10

Lee S.B., Kim K.H., Hahn S.J., Chung I.M. 2003. Evaluation of screening methods to determine the allelopathic potential of rice varieties against *Echinochloa crus-galli* Beauv. var. *oryzicola* Ohwi, Allelopathy J. 12: 37-52

Li H.Y., Pan K.W., Liu Q., Wang J.C. 2009. Effect of enhanced ultraviolet-B on allelopathic potential of *Zanthoxylum bungeanum*. Sci. Hortic. 119(3): 310-314, doi: 10.1016/j.scienta.2008.08.010

Molisch H. 1937. Der einfluss einer pflanze auf die andere Allelopathie. The role of chemical inhibition in Vegetational. pp. 99-106

Oyun M. B. 2006. Allelopathic potentialities of *Gliricidia sepium* and *Acacia auriculiformis* on the germination and seedling vigour of maize. American Journal of Agricultural and Biological Sciences. 1(3): 44 – 47, doi: 10.3844/ajabssp.2006.44.47

Pitman A.R., Duke W.B. 1978. Allelopathy in agroecosystem. Annu. Rev. Phytopathol. 16: 431-451, doi: 10.1146/annurev.py.16.090178.002243

Ranalli P. 1999. Agronomical and physiological advances in hemp crops. In:Advances in Hemp Research Haworth Press Binghamt, NY, USA, pp.61-84

Rice E.L. 1971. Possible role of *Ambrosia psilostachya* patterning and succession in old fields. Am. Midland Naturalist. 86: 344-357, doi: 10.2307/2423628

Rice E. L. 1984. Allelopathy. II edition. New York: Academic Press. 421

Sun B., Tan J., Wan Z., Gu F., Zhu M. 2006. Allelopathic effects of extracts from *Solidago canadensis* L. against seed germination and seedling growth of some plants. J. Environ. Sci. 18:304-309

Wahab A., Niel A.S., Rice E.L. 1967. Plant inhibition by Johnson grass and its possible significance in old field succession. Bull. Torrey Bot Club, 94: 486-487, doi: 10.2307/2483566

Whittaker R.H. 1970. The biochemical ecology of higher plants. In Sondheimer, E. and B. Simeone (eds.), Chemical Ecology, Academic Press, N.Y., USA, pp. 43-70, doi: 10.1016/b978-0-12-654750-4.50009-8

Wu G.L., Du G.Z. 2007. Germination is related to seed mass in grasses (Poaceae) of the eastern Qinghai – Tibetan plateau, China. Nordic Journal of Botany 25: 361 – 365, doi: 10.1111/j.0107-055X.2007.00179.x

COBISS Code 1.01

DOI: 10.14720/aas.2015.105.2.07
Agrovoc descriptors: rice, oryza, nitrogen fertilizers, rhizobacteria, azotobacter, *Azospirillum,* plant growth stimulants, growth, crop yield

Agris category code: f01, fo4, f62

Grain, milling, and head rice yields as affected by nitrogen rate and bio-fertilizer application

Saeed FIROUZI[2]

Received May 29, 2014; accepted August 22, 2015.
Delo je prispelo 29. maja 2014, sprejeto 22. avgusta 2015.

ABSTRACT

To evaluate the effects of nitrogen rate and bio-fertilizer application on grain, milling, and head rice yields, a field experiment was conducted at Rice Research Station of Tonekabon, Iran, in 2013. The experimental design was a factorial treatment arrangement in a randomized complete block with three replicates. Factors were three N rates (0, 75, and 150 kg ha^{-1}) and two bio-fertilizer applications (inoculation and uninoculation with Nitroxin, a liquid bio-fertilizer containing *Azospirillum* spp. and *Azotobacter* spp. bacteria). Analysis of variance showed that rice grain yield, panicle number per m^2, grain number per panicle, flag leaves area, biological yield, grains N concentration and uptake, grain protein concentration, and head rice yield were significantly affected by N rate, while bio-fertilizer application had significant effect on rice grain yield, grain number per panicle, flag leaves area, biological yield, harvest index, grains N concentration and uptake, and grain protein concentration. Results showed that regardless of bio-fertilizer application, rice grain and biological yields were significantly increased as N application rate increased from 0 to 75 kg ha^{-1}, but did not significantly increase at the higher N rate (150 kg ha^{-1}). Grain yield was significantly increased following bio-fertilizer application when averaged across N rates. Grains N concentration and uptake were significantly increased as N rate increased up to 75 kg ha^{-1}, but further increases in N rate had no significant effect on these traits. Bio-fertilizer application increased significantly grains N concentration and uptake, when averaged across N rates. Regardless of bio-fertilizer application, head rice yield was significantly increased from 56 % to 60 % when N rate increased from 0 to 150 kg ha^{-1}. Therefore, this experiment illustrated that rice grain and head yields increased with increasing N rate, while bio-fertilizer application increased only rice grain yield.

Key words: nitrogen fertilizer, plant growth-promoting rhizobacteria, rice yield and yield components

IZVLEČEK

VPLIV GNOJENJA Z RAZLIČNIMI ODMERKI DUŠIKOVIH GNOJIL IN BIO-GNOJIL NA PRIDELEK ZRNJA IN MLEVSKE LASTNOST RIŽA

Z namenom vrednotenja učinkov gnojenja z različnimi odmerki dušika in uporabe bio-gnojil na pridelek riža in njegove mlevske lastnosti je bil izveden poljski poskus na Rice Research Station of Tonekabon, Iran, v letu 2013. Načrt poskusa je bil faktorski naključni bločni poskus s tremi ponovitvami. Preučevani dejavniki v poskusih so bili tri gnojenja z različnimi odmerki dušika (0, 75, in 150 kg ha^{-1}) in uporaba dveh bio-gnojil (z ali brez inolukacije z bio-gnojilom Nitroxin, tekoče bio-gnojilo, ki vsebuje bakterije iz rodov *Azospirillum* spp. in *Azotobacter* spp.). Analiza variance je pokazala, da je imelo gnojenje z dušikom značilen učinek na pridelek zrnja riža, število latov na m^2, število zrn na lat, površino najvišjega lista (zastavarja), biološki pridelek, privzem in vsebnost N v zrnju, vsebnost beljakovin v zrnju in pridelek oluščenega riža, uporaba bio-gnojil pa je imela značilen vpliv le na pridelek zrna riža, število zrn na lat, površino najvišjega lista, biološki pridelek, žetveni indeks, privzem in vsebnost N v zrnju in vsebnost beljakovin v zrnju. Rezultati raziskave so pokazali, da sta se pridelek zrnja riža in njegov biološki pridelek povečala ne glede na uporabo bio-gnojil, ko se je gnojenje z dušikom povečalo z 0 na 75 kg ha^{-1}, vendar gnojenje z večjimi odmerki N (150 kg ha^{-1}) ni imelo značilnega vpliva na povečanje teh dveh parametrov. Pridelek zrnja se je značilno povečal pri uporabi bio-gnojil pri vseh odmerkih dušika. Vsebnost in privzem N v zrnje sta se značilno povečala pri povečanju gnojenja z dušikom iz 0 na75 kg ha^{-1}, vendar nadaljna povečanja gnojenja z N niso imela značilnega vpliva na ta parametra. Uporaba bio-gnojil je značilno povečala vsebnost in privzem N v zrnju pri vseh odmerkih dušika. Ne glede na uporabo bio-gnojil, se je pridelek oluščenega riža značilno povečal iz 56 % na 60 %, ko se je gnojenje z N povečalo z 0 na 150 kg ha^{-1}. Izsledki te raziskave kažejo, da se je pridelek zrnja riža in oluščenega riža povečal z večjim gnojenjem z dušikom, uporaba bio-gnojil pa je povečala le pridelek zrnja.

Ključne besede: dušikova gnojila, rast stimulirajoče rizobakterije, pridelek riža in njegove komponente

[1] Department of Agronomy and Plant Breeding, Rasht Branch, Islamic Azad University, Rasht, Iran; P.o.Box: 41335-3516; E-mail: firoozi@iaurasht.ac.ir

1 INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food crop for about 3 billion world's population. In many countries, rice provides more than 70 % of human caloric intake. In Iran, much of the population consumes rice at least once a day (Kanawapee et al., 2011). Iran's total rice production stands at 2.2 million tonnes per annum, while its annual consumption is about three million tonnes (Rezazadeh et al., 2013). So, Iran has to import about 800 thousand tonnes of rice from some rice producing countries. Consequently, increasing the rice production in the country is an essential.

Plant nutrients, which come primarily from chemical fertilizer, are essential for crop production. In agriculture, nitrogen is an essential element for crop growth and development. Nitrogen is a basic constituent of chlorophyll, proteins and all enzymes are involved in photosynthesis, especially Rubisco which alone accounts for more than 75 % of the total leaf nitrogen (Hak et al., 1993). Li et al. (2012) and Manzoor et al. (2006) declared that rice grain yield was significantly increased with increasing nitrogen fertilizer application rate. At the same time, nitrogen has been known as an important factor influencing rice milling quality (Perez et al., 1996). Dilday (1988) reported that the head rice yield decreased 7 to 22 % and 2 to 6 % in 'Lemont' and Newbonnet' rice cultivars, respectively, when no nitrogen applying compared to applying 200 kg N ha^{-1}. Perez et al. (1996) indicated that the head rice yield was significantly increased as N rate increased from 0 to 225 kg ha^{-1}.

A lot of studies noted that the nitrogen use efficiency is relatively low in paddy fields. This indicates that a major portion of applied nitrogen is wasted in paddy fields. Nitrogen losses occur through denitrification, volatilization, and leaching which may cause the air and water pollutions (Brown et al., 2012). Therefore, reducing the chemical nitrogen rate by applying the bio-fertilizers may be a solution. Bio-fertilizers are substances which comprise of living microorganisms that stimulate the plant growth by increasing the supply or availability of primary nutrients to the plant and the synthesis of growth-promoting substances. Hence, bio-fertilizers can be expected to reduce the use of chemical fertilizers. Plant growth-promoting rhizobacteria like as *Azospirillum* and *Azotobacter* can supply nutrients for crops through nitrogen fixation and produce phytohormones like auxins, cytokinins, gibberellins and ethylene (Keyeo et al., 2011). Therefore, this experiment was conducted to evaluate the effects of nitrogen rate and bio-fertilizer application on rice grain yield, yield components and head rice yield.

2 MATERIALS AND METHODS

2.1 Experimental site, design, and plant growth conditions

A field experiment was conducted at Rice Research Station of Tonekabon, Iran, in 2013. The soil texture was loamy with 1.5 % organic matter content, pH 7.7, total N 0.9 g kg^{-1}, available P 5.0 mg kg^{-1}, and available K 140.0 mg kg^{-1}. Three N rates (0, 75, and 150 kg ha^{-1}) and two bio-fertilizer applications (inoculation and uninoculation with Nitroxin, a commercial liquid bio-fertilizer containing *Azospirillum* spp. and *Azotobacter* spp. bacteria), organized into a randomized complete block design with a 3 × 2 factorial treatment arrangement and three blocks, were applied to plot area. The colony forming unit (cfu) of *Azotobacter* and *Azospirillum* were 10^9 cells per gram of carrier material. Bio-fertilizer (Nitroxin) was applied at two stages according to the manufacture recommendation, i.e. seed dipping and seedling root dipping methods. Plot size was 3 m by 4 m. Consistent with the lowland paddy field practices in north of Iran, rice seeds ('Tarom') were sown in a nursery seedbed on 18 April and seedlings from these seeds were transplanted on 14 May at a hill spacing of 0.20 by 0.20 m, with three seedlings per hill. Fertilizer N (as urea) was split-applied, with 34 % just before final land preparation, 33 % at panicle initiation, and 33 % at flowering stage. Also, all plots received 100 kg P ha^{-1} as triple superphosphate and 75 kg K ha^{-1} as potassium sulfate just before final land preparation. Additionally, all weeds were

removed manually whenever necessary. Plots were harvested on 21 August.

2.2 Plant sampling

At the soft dough stage, five randomly chosen plants were removed from each plot and the flag leaves area (cm^2) was determined with an area meter (Model LI-3100, LI-COR, Lincoln, NE, USA). At maturity stage, rice grain yield (based on 14 % humidity) was determined from 2.5 m^2 per plot. Moisture content of grains was measured using a digital grain moisture meter (Model GMK-303R5-Korea) and grain yield per pot was calculated as ((100 - moisture content of the sample) × fresh grain weight)/86 to convert the sample to 14 % moisture content. Yield components, that is, number of panicles per square meter, number of filled grains and 1000-grain weight, were determined from 12 plants (excluding the border ones) sampled randomly from each plot. To determine aboveground biomass, a 1 m^2 sample from each plot was randomly chosen, clipped at the ground level, threshed, dried at 70°C for three days, weighed, and expressed as the dry weight of above-ground plant per hectare. Harvest index was the proportion (percentage) of filled grain weight to total above-ground dry biomas. For measuring grain N concentration, rice grains were grounded to pass through a 1-mm sieve. N concentration was determined using micro-Kjeldahl method as described by Pregl (1945) and was expressed as the percent of grain dry weight. N uptake in grain was calculated by multiplying grain dry weight by grain N concentration. Grain protein concentration was calculated as 6.25 × nitrogen content measured by the micro Kjeldahl technique.

Paddy samples for milling quality evaluation were harvested from the 2.5 m^2 per plot, threshed by a simple motorized thresher and dried up to 8 %, wet basis (w.b.) using the laboratory dryer (Memmart Model 600, Germany) set at 45 ˚C. Paddy moisture content was determined using the digital grain moisture meter (Model GMK-303R5-Korea). After drying process, 200 g of dried paddy from each treatment were dehulled by a laboratory rubber roll huller (SATAKE Co. Ltd, Japan) and then was milled using a laboratory rice whitener (McGill Miller, USA). Mass of each milled rice sample was weighed by a laboratory scale with an accuracy of 0.01 g, separated into broken and head rice kernels by sizing with a laboratory rotary sieve (SATAKE TRG058, Japan) and then broken and whole kernel fractions were weighed. Head rice yield was determined as the mass of head milled rice after milling, divided by the mass of dried paddy prior to milling process (Zhao and Fitzgerald, 2013). Milling yield was also measured as the mass ratio of total milled rice to the un-hulled dried paddy prior to milling process.

2.3 Data analysis

Data were analyzed by analysis of variance (ANOVA) procedure using SAS program (SAS Inst. 2004) and means were compared using Fisher's Protected LSD at the 0.05 level of significance. Pearson correlation coefficients were calculated using correlation analysis to assess the interrelationships between the different measured parameters.

3 RESULTS AND DISCUSSION

3.1 Yield and yield components

The main effects of nitrogen rate and bio-fertilizer application were significant, but there was no significant interaction of nitrogen rate and bio-fertilizer application for grain yield (Table 1). Regardless of bio-fertilizer application, rice grain yield was significantly increased from 4251.4±346.5 to 5016.2±349.3 kg ha^{-1} (18 %) as N application rate increased from 0 to 75 kg ha^{-1}, but did not significantly increase at the higher N rate (150 kg ha^{-1}) (Table 2). Grain yield was significantly increased by bio-fertilizer application when averaged across N rates (Table 2). Pedraza et al. (2009) reported that inoculation of rice plants with *Azospirillum* significantly increased grain yield. Mukhopadhyay et al. (2013) also reported that the highest grain yield for rice was obtained when bio-fertilizer was applied with 60 % of recommended N rate. Induction of longer roots with increased number of root hairs and root laterals is a growth response attributed to IAA production by *Azospirillum* and *Azotobacter*, which increase grain yield in inoculated plants

(Keyeo et al., 2011). Panicle number per m^2 was significantly affected only by nitrogen rate, while grain number per panicle was significantly influenced by both nitrogen rate and bio-fertilizer application. Regardless of bio-fertilizer application, the highest (323±25.9 panicle) and the lowest (248.2±18.1 panicle) panicle number per m^2 was observed in plots receiving 0 and 150 kg N ha^{-1}, respectively (Table 2). Averaged across bio-fertilizer applications, grain number per panicle was significantly increased from 96.4±3.7 to 107.6±4.3 when nitrogen application rate increased from 0 to 150 kg ha^{-1} (Table 2). These results indicated that nitrogen application increased sink size by increasing both panicle number per m^2 and grain number per panicle, while bio-fertilizer inoculation increased sink size only by increasing grain number per panicle. Similarly to these results, Weerakoon et al. (2005) also reported that panicle number per m^2 and grain number per panicle increased with increases in N rate. On the other hand, 1000-grain weight was significantly affected neither by nitrogen rate nor by bio-fertilizer application (Table 1 & 2). Contrary to this result, Weerakoon et al. (2005) reported that 1000-grain weight increased with increasing in N rate. Regardless of N rate, grain number per panicle was significantly increased by 11 % when bio-fertilizer was applied. Similar result was reported by others (Mukhopadhyay et al., 2013; Isawa et al., 2010). Bio-fertilizer application increased panicle number per m^2 and 1000-grain weight by 17 % and 4 %, respectively, but the increases were not statistically significant. In contrast, Mukhopadhyay et al. (2013) and Isawa et al. (2010) found that panicle number per m^2 and 1000-grain weight for rice were significantly increased following bio-fertilizer application. The ANOVA also showed that the interaction between nitrogen rate and bio-fertilizer application were not significant for all of yield components (Table 1). This indicates that all yield components had similar responses to N rate with or without bio-fertilizer application. Rice grain yield was positively correlated with panicle number per m^2, grain number per panicle, 1000-grain weight, flag leaves area, biological yield, harvest index, grain N concentration, and grain N uptake at $P < 0.01$ level (Table 3).

Table 1: Mean squares of ANOVA for grain yield (Y), panicle number per m^2 (PN), grain number per panicle (GN), 1000-grain weight (ThGW), flag leaves area (FLA), biological yield (BY), harvest index (HI), grain N concentration (GNC), grain N uptake (GNU), grain protein concentration (GPC), milling yield (MY), and head rice yield (HRY) as affected by nitrogen rate and bio-fertilizer application

S.O.V	Df	Y	PN	GN	ThGW	FLA	BY	HI	GNC (%)	GNU	GPC	MY	HRY
R	2	43778 ^{ns}	23.4^{ns}	128*	1.39 ^{ns}	7647157 ^{ns}	589981^{ns}	1.4 ^{ns}	0.002^{ns}	29 ^{ns}	0.01^{ns}	9.6^{ns}	12.1 **
Nitrogen (N)	2	1885091**	37.4*	191**	11.14^{ns}	54259227**	7098144**	7.2 ^{ns}	0.21**	1729**	8.3 **	1.9^{ns}	19.8 **
Bio-fertilizer (B)	1	5221834**	37.9^{ns}	638**	3.65 ^{ns}	20723922*	17608156**	25.6*	0.41**	4402**	16.1 **	0.2^{ns}	0.05 ^{ns}
N×B	2	349822 ^{ns}	1.2^{ns}	19 ^{ns}	0.02 ^{ns}	2085236^{ns}	1007564 ^{ns}	3.2 ^{ns}	0.017^{ns}	188 ^{ns}	1.1 ^{ns}	1.6 ^{ns}	0.28 ^{ns}
Error	10	294163	9.4	30	10.60	4583681	886630	4.2	0.02	156	0.8	0.9	1.49
CV (%)	-	11	16	5	13	22	7	5	10	15	10	2	2

*, ** represent significance at 0.05 and 0.01probability level, respectively
ns represents no significant difference

3.2 Biological yield and harvest index

Both nitrogen rate and bio-fertilizer application had significant effects ($P < 0.01$) on biological yield, while harvest index was significantly influenced only by bio-fertilizer application (Table 1). Moreover, no significant interactions between nitrogen rate and bio-fertilizer application were

found for both biological yield and harvest index (Table 1). Regardless of bio-fertilizer application, biological yield increased from 10653.3±648.6 to 12159.1±637.3 kg ha^{-1} (14.1 %) when N rate increases from 0 to 75 kg ha^{-1}, with no further increase in biological yield as N rate increased from 75 to 150 kg ha^{-1} (Table 2). Harvest indices were similar for all N rates when averaged across bio-fertilizer applications (Table 2). Thus, the increase in grain yield was due to an increase in total biomass production rather than harvest index. Mukhopadhyay et al. (2013) reported that harvest index increased following bio-fertilizer application.

Table 2: Grain yield (Y), panicle number per m^2 (PN), grain number per panicle (GN), 1000-grain weight (ThGW), flag leaves area (FLA), biological yield (BY), harvest index (HI), grain N concentration (GNC), grain N uptake (GNU), grain protein concentration (GPC), milling yield (MY), and head rice yield (HRY) response to nitrogen rate and bio-fertilizer application

Traits / Factors	Y (kg ha^{-1})	PN (No. m^2)	GN (No. panicle)	ThGW (g)	FLA (cm^2)	BY (kg ha^{-1})	HI (%)	GNC (%)	GNU (kg ha^{-1})	GPC (kg ha^{-1})	MY (g 100g)	HRY (%)
Nitrogen rates (kg ha^{-1})												
0	4251.4±346.5^b	248.2±18.1^b	96.4±3.7^b	22.7± 1.0^a	6216.1± 224.9^b	10653.3± 648.6^b	39.6±0.9^a	1.29±0.1^b	55.6±6.9^b	8.0±0.4^b	69.8±0.6^a	56.8±0.7^a
75	5016.2±349.3^a	306.8±23.7^{ab}	100.3±2.9^b	23.5± 0.9^a	9937.4± 1132.0^a	12159.1± 637.3^a	40.9±1.0^a	1.59±0.0^a	81.6±11.3^a	9.9±0.7^a	69.9±0.7^a	58.5±0.7^b
150	5342.8±237.3^a	323.0±25.9^a	107.6±4.3^a	25.4± 1.4^a	12168.2± 1239.2^a	12753.4± 402.7^a	41.8±0.7^a	1.63±0.1^a	87.5±6.3^a	10.2±0.5^a	70.8±0.5^a	60.5±0.6^c
LSD (0.05)	697.03	61.9	7.1	4.1	2754.03	1210.7	2.6	0.19	16.4	1.2	1.2	1.5
Bio-fertilizer												
application	5409.0±192.6^a	315.0±20.5^a	107.0±2.8^a	24.3±1.0^a	10513.2± 1286.8^a	12855.9± 345.9^a	42.0±0.6^a	1.65±0.1^a	90.0±7.1^a	10.3±0.3^a	70.3±0.5^a	58.6±0.8^a
No application	4332.3±258.9^b	269.3±19.0^a	95.0±2.4^b	23.4±0.9^a	8366.9± 870.7^a	10878.1± 495.0^b	39.6±0.8^b	1.35±0.0^b	59.0±5.0^b	8.4±0.5^b	70.1±0.5^a	58.9±0.6^a
LSD (0.05)	568.8	51.0	6.02	3.4	2140.0	989.0	2.1	0.15	13.4	1.0	0.9	1.3

3.3 Flag leaves area

There were significant effects of nitrogen rate and bio-fertilizer application on flag leaves area, while the interaction effect of nitrogen rate and bio-fertilizer application was not significant (Table 1). Regardless of bio-fertilizer application, flag leaves area was significantly increased from 6216.1±224.9 to 12168.2±1239.2 cm^2 when N rate increased from 0 to 150 kg ha^{-1} (Table 2). Averaged across N rates, flag leaves area was significantly increased following bio-fertilizer application by 25 % (Table 2). Lemaire et al. (2008) declared that N shortage reduced leaf expansion in C_3 cereals plants. Insufficient N availability during rice growth stage declined both flag leaf size and number and, therefore, reduced total net photosynthesis (Zong and Shangguan, 2014), which in turn decreased rice grain yield. Besides, Toth et al. (2002) suggested that lower rates of photosynthesis under low N supply are often attributed to reduction in chlorophyll content and Rubisco activity. Flag leaves area was positively correlated with grain and biological yields, yield components height, grain N concentration and uptake, and head rice yield at $P < 0.01$ level, but not correlated with HI, and milling yield (Table 3).

Table 3: Correlation coefficients for measurements of rice as influenced by N rate and bio-fertilizer application

	Y	PN	GN	ThGW	FL	BY	HI	GNC	GNU	GPC	MY
PN	0.51*	1									
GN	0.55**	0.49*	1								
ThGW	0.54**	0.01ns	0.22ns	1							
FL	0.57**	0.51*	0.60**	0.47ns	1						
BY	0.97**	0.52**	0.58**	0.50*	0.56**	1					
HI	0.86**	0.42ns	0.35ns	0.52*	0.44ns	0.73**	1				
GNC	0.75**	0.65**	0.70**	0.42ns	0.65**	0.74**	0.62**	1			
GNU	0.92**	0.60**	0.67**	0.52*	0.66**	0.89**	0.78**	0.94**	1		
GPC	0.75**	0.65**	0.70**	0.42ns	0.65**	0.74**	0.62**	0.99**	0.94**	1	
MY	0.01ns	-0.14ns	0.21ns	-0.24ns	0.07ns	0.08ns	-0.09ns	0.01ns	0.01ns	0.01ns	1
HRY	0.51*	0.15ns	0.26ns	0.22ns	0.64**	0.42ns	0.29ns	0.69 **	0.68 **	0.69 **	0.54**

*, ** represent significance at 0.05 and 0.01probability level, respectively
ns represents no significant difference
Y, Grain yield; PN, panicle number per m^2; GN, grain number per panicle; ThGW, 1000-grain weight; FLA, Flag leaves area; BY, biological yield; HI, harvest index; GNC, grain N concentration; GNU, grain N uptake; MY, milling yield; and HYR, head rice yield

3.4 Grain N concentration, grain N uptake, and grain protein concentration

The main effects of nitrogen rate and bio-fertilizer application were significant ($P < 0.01$) for grain N concentration, grain N uptake, and grain protein concentration, but there were no significant interactions of nitrogen rate × bio-fertilizer application for all of them (Table 1). Regardless of bio-fertilizer application, grain N concentration, grain N uptake, and grain protein concentration had similar responses to N rate. Grain N concentration, grain N uptake, and grain protein concentration were significantly increased as N rate increased up to 75 kg ha^{-1}, but further increases in N rate had no significant effect on these traits (Table 2). Regardless of N rate, grain N concentration, grain N uptake, and grain protein concentration were significantly increased when bio-fertilizer was applied (Table 2). The higher grain N concentration resulting from inoculation may be attributed to increased N uptake by a larger root surface area associated with additional root hairs and lateral root development and/or to biological nitrogen fixation (Biswas et al., 2000). Cong et al. (2009) and Pedraza et al. (2009) also reported that rice grain N uptake was significantly increased with applying bio-fertilizer.

3.5 Milling yield and head rice yield

The ANOVA results showed that nitrogen rate and bio-fertilizer application as well as the interaction between them had no significant effects on milling yield (Table 1). Head rice yield was significantly affected by nitrogen rate, while bio-fertilizer application and the interaction between nitrogen rate and bio-fertilizer application were not significant (Table 1). Averaged across bio-fertilizer applications, head rice yield was significantly increased from 56.8±0.7 % to 60.5±0.6 % when N rate increased from 0 to 150 kg ha^{-1} (Table 2). The positive influence of nitrogen on head rice yield is contributed to this fact that N fertilization increases the packing of protein matrix between endosperm starches in rice grains and grain protein makes it more resistant to cracking and breakage during milling process (Blumenthal et al., 2008). Leesawatwong et al. (2005) also noted that the head rice yield was positively correlated with relative abundance of the storage protein in the lateral section of the endosperm of rice kernels. The positive effect of nitrogen on head rice yield also was reported by Perez et al. 1996, and Dilday (1988). Head rice yield was positively correlated with grain yield, flag leaves area, grain N concentration and uptake, grain protein concentration, and milling yield, but not correlated with yield components, biological yield, and harvest index (Table 3).

4 CONCLUSIONS

This experiment illustrated that rice grain and head yields increased with increasing N rate, while bio-fertilizer increased only rice grain yield. Regardless of bio-fertilizer application, rice grain yield was significantly increased from 4251.4±346.5 to 5016.2±349.3 kg ha^{-1} as N application rate increased from 0 to 75 kg ha^{-1}, but did not significantly increase at the higher N rate (150 kg ha^{-1}). Grain yield was significantly increased by bio-fertilizer application when averaged across N rates. Regardless of bio-fertilizer application, head rice yield was significantly increased from 56.8±0.7 % to 60.5±0.6 % when N rate increased from 0 to 150 kg ha^{-1}.

5 ACKNOWLEDGEMENTS

The author is grateful for the funding supplied by the Rasht branch, Islamic Azad University and Rice research institute of Iran for providing research facilities.

6 REFERENCES

Biswas J.C., Ladha J.K., Dazzo F.B., Yanni Y.G., Rolfe B.G. 2000. Rhizobial inoculation influences seedling vigor and yield of rice. Agron. J. 92: 880–886, doi: 10.2134/agronj2000.925880x

Blumenthal J.M., Baltensperger D.D., Cassman K.G., Mason C.S., Pavlista A.D. 2008. Importance and effect of nitrogen on crop quality and health. Published in Nitrogen in the Environment: Sources, Problems, and Management, Second edition, edited by J. L. Hatfield and R. F. Follett (Amsterdam: Elsevier, 2008). Copyright © 2008 Elsevier, doi: 10.1016/b978-0-12-374347-3.00003-2

Brown J.R., Blankinship J.C., Niboyet A., van Groenigen K.J., Dijkstra P., LeRoux X. 2012. Effects of multiple global change treatments on soil N2O fluxes. Biogeochemistry 109, 85–100, doi: 10.1007/s10533-011-9655-2

Cong P.T., Dung T.D., Hien T.M., Hien N.T., Choudhury A.T.M.A., Kecskés M.L., Kennedy I.R. 2009. Inoculant plant growth-promoting microorganisms enhance utilisation of urea-N and grain yield of paddy rice in southern Vietnam. Eur. J. Soil Biol. 45, 52–61, doi: 10.1016/j.ejsobi.2008.06.006

Dilday R.H. 1988. Effect of nitrogen fertilizer on milling quality of rice (Oryza Sativa). Proceedings Arkansas Academy of Science. 42:26-27

Hak R., Rinderle-Zimmer U., Lichtenthaler H.K., Natr L. 1993. Chlorophyll a fluorescence signatures of nitrogen deficient barley leaves. Photosynthetica 28: 151–159

Isawa T., Yasuda M., Awazaki H., Minamisawa K., Shinozaki S., Nakashita H. 2010. Azospirillum sp. strain B510 enhances rice growth and yield. Microbes Environ. 1: 58–61, doi: 10.1264/jsme2.ME09174

Kanawapee N., Sanitchon J., Srihaban P., Theerakulpisut P. 2011. Genetic diversity analysis of rice cultivars (Oryza sativa L.) differing in salinity tolerance based on RAPD and SSR markers. Electronic Journal of Biotechnology, 14(6): No. 4, pp: 17

Keyeo F., Ai'shah O.N., Amir H.G. 2011. The effects of nitrogen fixation activity and phytohormone production of diazotroph in promoting growth of rice seedlings. Biotech. 10: 267-273, doi: 10.3923/biotech.2011.267.273

Leesawatwong M., Jamjod S., Kuo J., Dell B., Rerkasem B. 2005. Nitrogen fertilizer increases seed protein and milling quality of rice. Cereal Chemistry 82: 588–593, doi: 10.1094/CC-82-0588

Lemaire G., van Oosterom E., Jeuffroy M.H., Gastal F., Massignam A. 2008. Crop species present different qualitative types of response to N deficiency during their vegetative growth. Field Crops Res. 105, 253–265, doi: 10.1016/j.fcr.2007.10.009

Li Y., Chen X., Shamsi I.H., Fang P., Lin Y. 2012. Effects of Irrigation Patterns and Nitrogen Fertilization on Rice Yield and Microbial Community Structure in Paddy Soil. Pedospher, 22 (5): 661–672, doi: 10.1016/S1002-0160(12)60051-4

Manzoor Z., Awan T.H., Zahid M.A., Faiz F.A. 2006. Respons of rice crop (SUPER BASMATI) to different nitrogen levels. J. Anim. Pl. Sci. 16(1-2): 52-55

Mukhopadhyay M., Datta J.K., Garai T.K. 2013. Steps toward alternative farming system in rice. Europ. J. Agronomy 51:18– 24, doi: 10.1016/j.eja.2013.06.005

Pedraza R.O., Bellone C.H., de Bellone S.C., Sorte P.M.F.B., Teixeira K.R.S. 2009. Azospirillum inoculation and nitrogen fertilization effect on grain yield and on the diversity of endophytic bacteria in the phyllosphere of rice rainfed crop. Europ. J. Soil Boil. 45: 36–43, doi: 10.1016/j.ejsobi.2008.09.007

Perez C.M., Juliano B., Liboon S., Alcantara J.M., Cassman K.G. 1996. Effects of late nitrogen fertilizer application on head rice yield, protein content, and grain quality of rice. Cereal Chem. 73: 556–560

Pregl F. 1945. Quantitative Organic Microanalysis. 4th ed. J.A. Churchill Ltd. London, p.126-129

Rezazadeh T., Aghaiypour Kh., Heidari Z. 2013. The significance of food safety in trade and banning the importation of GMO products into Iran. Croat. J. Food Sci. Technol. (2013) 5 (2) 92-95.

SAS 2004. SAS Institute, version 9.1.3. Cary, NC, USA.

Toth, V.R., Meszkaros I., Veres S., Nagy J. 2002. Effects of the available nitrogen on the photosynthetic activity and xanthophyll cycle pool of maize in field, J. Plant Physiol. 159: 627–634, doi: 10.1078/0176-1617-0640

Weerakoon W.M.W., Ingram K.T., Moss D.N. 2005. Atmospheric CO2 concentration effects on N partitioning and fertilizer N recovery in field grown rice (Oryza sativa L.). Agric. Ecosyst. Environ. 108: 342–349, doi: 10.1016/j.agee.2004.12.014

Zhao X., Fitzgerald M. 2013. Climate Change: Implications for the Yield of Edible Rice. PLOS ONE, 8(6): e66218. pp:9

Zong Y., Shangguan Z. 2014. Nitrogen deficiency limited the improvement of photosynthesis in maize by elevated CO2 under drought. J. Integ. Agric. 13(1): 73-81, doi: 10.1016/S2095-3119(13)60349-4

COBISS Code 1.01

DOI: 10.14720/aas.2015.105.2.08
Agrovoc descriptors: sunflower, *Helianthus annuus, plant breeding,* genetic control, hybrids, hybridization, lipid content, yields

Agris category code: f30

Genetic analysis of agro-morphological traits in promising hybrids of sunflower (*Helianthus annuus* L.)

Maryam GOLABADI[*1], Pooran GOLKAR[2], Mohammad Reza SHAHSAVARI[3]

Received July 16, 2015; accepted August 17, 2015.
Delo je prispelo 16. julija 2015, sprejeto 17. avgusta 2015.

ABSTRACT

The main objective underlying sunflower breeding programs is to develop high-yielding productive F_1 hybrid cultivars. This study was conducted to investigate the genetic control of some agro-morphological traits of new sunflower F_1 hybrids. For this purpose, fourteen inbred lines of sunflower were crossed with three male sterile inbred lines. Their hybrids (14 hybrids) were then evaluated against three control cultivars. The data thus obtained were analyzed using the nested model (North Carolina Design I) as a completely randomized block design (CRBD) with four replications. Analysis of variance showed that the hybrids were significantly different in all the traits studied, except for head and stem diameters. From among the hybrids evaluated, Cms19 × Rn1-81 was found to have the highest seed yield and oil content. Cluster analysis classified the hybrids into four different groups. Genetic analysis showed that days to maturity, seed weight, and oil content (%) were under the additive gene action. Breeding strategies based on selection could be suggested for the improvement of these traits. Head angle, head diameter, seed yield, and oil yield were under the dominance gene action; breeding based on hybridization methods is, therefore, proposed for these traits. Finally, both additive and dominance gene actions were observed to play important roles in the genetic control of plant height and stem diameter.

Key words: additive, dominance effect, evaluation of hybrids, oil content, sunflower, yield

IZVLEČEK

GENETSKA ANALIZA AGRO-MORFOLOŠKIH LASTNOSTI PRI OBETAJOČIH KRIŽANCIH NAVADNE SONČNICE (*Helianthus annuus* L.)

Glavni cilj žlahniteljskih programov navadne sončnice je razvoj visoko produktivnih F_1 hibridnih sort. V raziskavi smo preučevali genetsko kontrolo nekaterih agro-morfoloških lastnosti nekaterih novih F_1 hibridov navadne sončnice. V ta namen smo opravili križanja 14 inbridiranih linij navadne sončnice s tremi moško sterilnimi inbridiranimi linijami. Dobljeni križanci (14 hibridov) so bili ovrednoteni glede na tri kontrolne sorte. Tako pridobljeni podatki so bili analizirani z vgnezdenim modelom (North Carolina Design I) v bločni zasnovi s štirimi ponovitvami. Analiza variance je pokazala, da so bili križanci značilno različni v vseh preučevanih lastnostih, z izjemo premera koška in premera stebla. Izmed ovrednotenih križancev je imel križanec Cms19 × Rn1-81 največji pridelek semena in največjo vsebnost olja. Klastrska analiza je razvrstila križance v štiri skupine. Genetska analiza je pokazala, da so bile lastnosti, kot so število dni do zrelosti, masa semena in vsebnost olja (%), pod aditivno gensko kontrolo. Za izboljšanje teh lastnosti priporočamo žlahniteljsko strategijo zasnovano na selekciji. Lastnosti kot so naklon koška, premer koška, pridelek semena in pridelek olja so bile dominantno dedovane, zato za te lastnosti priporočamo žlahtnenje na osnovi križanja. Pri lastnostih kot sta višina stebla in njegov primer smo opazili pomembni vlogi tako aditivnega kot dominantnega delovanja genov.

Ključne besede: aditivno dominantni učinki genov, vrednotenje križancev, navadna sončnica, pridelek, vsebnost olja

[1] Department of Agronomy and Plant Breeding, Collage of Agriculture, Isfahan Branch (Khorasgan), Islamic Azad University, P.O.Box:81595-158, Iran, *corresponding author E-mail: golabadim@gmail.com

[2] Institute of Biotechnology and Bioengineering, Isfahan University of Technology, Isfahan, 84156-83111, Iran

[3] Isfahan Agriculture and Natural Resources Center, Isfahan, Iran

1 INTRODUCTION

Sunflower (*Helianthus annuus* L.) is widely grown in tropical, semi-arid, and arid regions such as Iran (Weiss, 2000). It is an important oil crop of high quality oil, good adaptation, and high seed yield (Razi and Asad, 1999; Hu et al., 2010). Sunflower oil contains approximately 12 % saturated fat and 64 % linoleic acid (an omega-6 essential fatty acid) (Weiss, 2000). The genotypes possessing self-incompatibility and cytoplasmic male sterility (CMS) offer the potential for hybrid production (Fick, 1987). Currently, the highest levels of sunflower cultivars belong to hybrid varieties grown in developed countries. The production of hybrid varieties of sunflower in Iran is accomplished by using CMS and restoring fertility (RF) lines. Hybrids of sunflower are more stable and highly self-fertile with a high yield performance and greater uniformity at maturity (Kaya and Atakisi, 2004). In some cases, the hybrid genotypes have been found to be superior in terms of seed yield to their parental lines. Shahsavari et al. (2010) observed significant differences in days to maturity, plant height, stem diameter, head diameter, seed weight, seed yield, and oil yield among twenty genotypes of sunflower including new hybrids and parental genotypes. Haq et al. (2006) also reported significant differences among sunflower hybrids with respect to their plant height, stem diameter, oil percentage, seed yield, and seed weight. Similarly, Razi and Assad (1999) reported significant differences in growth period, head diameter, seed weight, as well as oil and seed yields among the different genotypes of sunflower they studied. The creation of new sunflower hybrids with a high genetic potential for seed yield requires information about their mode of inheritance.

Various genetic mating designs have been used to generate improved plants. All such designs are meant to realize the following four main objectives: 1) to obtain information about the genetic control of the traits under study, 2) to generate a breeding population for use as a basis in the selection and development of potential varieties, 3) to provide estimates of genetic gain, and 4) to gain information about the parents used in the breeding programs (Acquaah, 2012). North Carolina Designs (I, II, and III) were developed by Comstock and Robinson (1948). Design I (NCDI) is commonly used for estimating the additive and dominance variances (Acquaah, 2012). The main advantage of this design is its ability to supply a test of significance for the additive genetic variance (Hill et al., 1998). The design has also been successfully used in obtaining estimates of genetic variance in sunflower. Knowledge of genetic factors helps researchers select appropriate breeding methods. The genetic components and heritability of seed yield and its components in sunflower hybrids have been identified by different genetic designs such as NCD-II (Alza et al., 1997) and line × tester (Ghaffari et al., 2011).

The present study was designed to evaluate both the mean performance of some new F_1 hybrids and the genetic control of such traits as stem diameter, head angle, and days to maturity in sunflower via the NCD-I genetic design.

2 MATERIALS AND METHODS

This research was conducted at the experimental research farm of Agricultural Research Institute of Isfahan, at Kabutar Abad region (51º 51´ longitude and 32º 31 latitude) in 2012–2013. The North Carolina Design I was used to prepare a new genetic population of sunflower hybrids with improved traits. Three lines of cytoplasmic male sterility (CMS14, CMS19 and CMS 522), as the female parents, and 14 new restorer inbred lines (Rn1-144, Rn1-56, Rn-14, Rn-1-128, Rn1-152, Rn1-60, Rn-864, Rn-1-76, Rn-1-130, Rn1-149, Rn1-4, Rn1-81, Rn4 and Rn-1-77), as the male parents, were separately planted in one row 4 m long in a small crossing plot at two different planting dates in 2012. The CMS and restore lines were prepared by oil seed research branch of Agricultural Research Center of Isfahan, Iran. A distance of 60 cm was left between the rows. Some plants in every line were covered with sheer before flowering time. For hand pollination, liberal amounts of pollen were applied on three plants of every male sterile on every second day of the

flowering period for a total number of three times. The seeds of 14 F_1 hybrids with three control genotypes ('Azargol', 'Allstar', and 'Haysan-33') were evaluated in a completely randomized block design (CRBD) with four replications in 2013. Each experimental plot consisted of four lines in row of 5.5 m long each. The distances between and within the rows were set to 60 cm and 25 cm, respectively. The standard agronomic package of practices and suitable plant protection were adopted to raise healthy crops. Ten randomly selected plants were taken from the two middle rows of each experimental plot for evaluating their traits.

2.1 Traits studied

Seed and oil yields of each plot were calculated (kg/ha) after elimination of two margin lines. Seed oil (%) was determined using the seeds collected from ten random plants per plot by the NMR (Nuclear Magnetic Resonance, H20-18-25A) method. Plant height (cm) was measured as the distance between soil surface and stem attachment to the head. Head angle was defined as the receptacle orientation expressed at maturity as an angle in degrees. Analysis of variance was carried out using the SAS 9.1 software. Mean comparison was accomplished by the Least Significant Difference (LSD) test at a probability of 5% and cluster analysis was conducted using the Ward method with the SPSS software. Finally, the genetic population was subjected to genetic analysis using the SAS software. The variance components of NC-I are reported in Table 1.

Table 1: Analysis of variance and its components in North Carolina Design (I)

Source of Variation	D.F	Mean squares	Expected Mean Squares	Related covariance
Replication	r-1			
Female	f-1	M_3	$\sigma^2+r\,\sigma^2_{f/m}+rm\,\sigma^2_f$	σ^2+r (Cov F.S – Cov H.S)+ rm CovH.S)(
Female (male)	f(m-1)	M_2	$\sigma^2+r\,\sigma^2_{f/m}$	σ^2+r (Cov F.S – Cov H.S)
Error	mf-1) (r-1)	M_1	σ^2	σ^2

r, f, and m denote replication, female, and male, respectively

According to Table 1, dominance variance (σ^2_D) and additive variance (σ^2_A) would be calculated (as F=1) using the following Relation (Kempthorne, 1957):

$$\text{Cov (F.S)} = [\,(\frac{1+F}{2})\sigma^2_A + (\frac{1+F}{2})^2\sigma^2_D\,] \text{ and } \quad \text{Cov (H.S)} = (\frac{1+F}{4})\sigma^2_A$$

Broad-sense and narrow-sense heritability of the studied traits (Mahmud and Keramer, 1951) and degree of dominance (Kearsey and Pooni, 1996) were obtained using the following Relation:

$$H_b = \frac{\sigma^2_A + \sigma^2_D}{\sigma^2_A + \sigma^2_D + \sigma^2_e}, \qquad H_n = \frac{\sigma^2_A}{\sigma^2_A + \sigma^2_D + \sigma^2_e}, \qquad \bar{d} = \sqrt{2\delta^2_D / \delta^2_A}$$

3 RESULTS AND DISCUSSION

Analysis of variance showed significant differences among the entries for all the studied traits (Table 2). Moreover, female and female (male) mean squares were significant for all the traits (Table 2). This indicates the existence of high variations among the parental genotypes. The control genotypes showed significant differences in all the traits studied, except for seed diameter and oil content. Comparison of F_1 hybrids vs. controls showed significant differences between the means of F_1 hybrids and the control genotypes (Table 2). Previous studies have also reported significant differences in growth period, plant height, seed weight, oil yield, and seed yield on F_1 hybrids of sunflower (Razi and Assad, 1999; Haq et al., 2006; Arshad et al., 2007).

Table 2: Analysis of variance and genetic components for agronomic traits in 14 promising F_1 hybrids and 3 control lines of sunflower with NCD-1

		Mean squares								
S.O.V	D.F	DM	PH	HA	HD	SD	SW	SY	OY	OC
Rep.	3	8.46	690.33**	1421.6**	7.238	6.94**	154.74**	800496*	96358**	1.77
Entries	16	89.1**	915.5**	1203.2**	7.87*	3.8**	345.6**	1855859.9**	341647**	14.5**
Female	2	388.9**	776.35**	2059.6*	14.19**	7.4**	659.67**	1255622**	203312**	45.2**
Female (Male)	11	40.8**	160.2**	653.27**	5.72*	1.5*	90.3**	344780*	80274*	9.5**
Control	2	76.58**	1982.33**	3543.7**	14.33**	0.02	1195.88**	7284571.5	1411360**	2.46
Control *vs.* F_1	1	45.81**	7368.43**	842.21	5.95	29.45**	969.18**	8820790.9	1353993**	31.5**
Residual	48	5.31	72.6	269.62	2.8	0.58	24.31	105113	38774	2.1
Genetic estimates										
σ^2_A		12.43	21.22	50.23	0.30	0.21	20.33	32530	4394.2	1.38
σ^2_D		2	10.9	70.79	0.57	0.12	6.33	43651.7	8177.8	0.38
$\bar{d}$		0.56	0.99	1.67	1.95	1.08	0.78	1.63	1.92	0.74
h^2_b		91.57	64.44	64.22	60.57	69.81	81.43	75.34	56.46	77.10
h^2_n		78.86	43.10	26.65	31.28	43.86	62.10	31.74	19.1	60.43

**, *: Significant at 1% and 5%, respectively; ns: not significant

Abbreviations: DM: days to maturity, PH: Plant height, HA: Head angle, HD: Head diameter, SD: Stem diameter, SW: Seed weight, SY: Seed yield, OY: Oil yield, OC: Oil content.

$\bar{d}$ =average of dominance, h^2_b= broad-sense heritability, h^2_n: Narrow-sense heritability

3.1 Genetic components analysis

The genetic components of the studied traits were calculated and reported in Table 2. The variance of female (σ^2_f) (CMS lines) and female (male) ($\sigma^2_{f/m}$) (restorer fertility lines) were significant for all the studied traits, implying the important effects of both additive and dominance gene actions on the genetic control of the traits investigated. The variance among the CMS lines (female) for all the traits were greater than that among the male lines (restorer), indicating the possible existence of some degree of maternal effects for the genetic control of the studied traits (Ghaffari et al., 2011). Alza et al. (1997) reported that female variance was significant for seed weight and oil content.

3.2 Days to maturity

According to Table 2, σ^2_A (12.43) is larger than σ^2_D (2) for days to maturity (Table 2). Comparison of the variance components, the value of dominance ratio (0.56), and the high narrow-sense heritability (78.86) indicate the importance of the additive gene action for the genetic control of days to maturity. In agreement with our findings, Saeidi et al. (2009) reported that additive genetic effects had a greater contribution to the genetic control of days to maturity than did the non-additive ones. Wehner (1984) reported that dominance variance was greater than additive variance for days to germination as a phenological trait. Ghaffari et al. (2011) reported both additive and dominance gene actions to be effective in the genetic control of days to maturity.

3.3 Plant height

A major objective in sunflower breeding programs is to improve crops by reducing plant height. Genetic analysis in the present case showed that the value of σ^2_A (22) was larger than that of σ^2_D (10.9) for plant height (Table 2). Similar to the findings of Gaffari et al. (2011) and Ortegon et al. (1992), the dominance ratio (d=1) in the present study implied the importance of both additive and dominance gene actions in the genetic control of plant height. Also, Miller and Hammond (1991) reported that additive gene action played a more important role than the dominance one did.

3.4 Head angle and head diameter

Dominance variance (σ^2_D) was found to be greater than σ^2_A for head angle and head diameter (Table 2). This indicates the predominant roles of the dominance over the additive gene action in the genetic control of these traits. Gangappa et al. (1997) reported that non-additive gene action was predominant for the genetic control of head diameter, which is in agreement with our results. Contrary to our results, however, Ghaffari et al. (2011) reported additive ones to be important for the genetic control of head diameter. To the best of our knowledge, no study has been reported on the genetic control of head angle in sunflower.

3.5 Stem diameter

Increasing stem diameter is another major objective in sunflower breeding programs. Analysis of genetic components showed that σ^2_A (0.21) was greater than σ^2_D (0.12) for stem diameter (Table 2). Comparison of variance components, dominance ratio (1.08), and medium narrow-sense heritability (43.86) implied the importance of both additive and dominance gene actions in the genetic control of stem diameter. These results confirm those reported by Miller and Hammond (1991).

3.6 Seed weight

According to Table 2, σ^2_A (20.33) was greater than σ^2_D (6.33) for seed weight (Table 2). The dominance ratio (0.78) and the high narrow-sense heritability (62.10) obtained in this study showed the predominant role of the additive over the dominance gene action in the genetic control of seed weight. This is in agreement with the findings of Ghaffari et al. (2011). However, Bajaj et al. (1997) reported the dominance gene action to be important for the genetic control of seed weight, which is not in agreement with our results**.** The differences between the results could be explained by differences in the genotypes used and the environmental effects in each study.

3.7 Seed and oil yield

The magnitude of σ^2_D (as compared to σ^2_A), the dominance ratio, and the low value of narrow-sense heritability obtained in this study indicate the importance of the dominance gene action for the genetic control of the plant traits investigated (Table 2). The importance of dominance gene action for seed yield has also been reported elsewhere (Khani et al., 2005; Skoric et al., 2000; Ghaffari et al., 2011). Gomez et al. (1999) reported that line and line × tester mean squares were not significant for seed yield in sunflower. In agreement with our results, however, other studies performing line × tester analysis in sunflower have indicated that oil yield is governed by the non-additive gene action (Kadkol et al., 1984; Ghaffari et al., 2011).

3.8 Oil content (%)

Genetic analysis showed that σ^2_A (1.38) was greater than σ^2_D (0.38) (Table 2). The high values obtained for the narrow-sense heritability (60.43) and dominance ratio (0.74) indicate the importance of the additive gene action for the control of oil content. In agreement with our results, Saeidi et al. (2009) reported that additive gene action was more important than the non-additive effects for the genetic control of seed oil content. Contrary to our results, Andarkhor et al. (2013) reported the significant role of dominance gene action for the genetic control of oil yield.

3.9 Heritability of traits

Broad-sense heritability varied between 91.57 (days to maturity) and 56.46 (oil yield) (Table 2). This is while narrow-sense heritability varied between 78.88 (days to maturity) and 19.1 (oil yield) (Table 2). The low-medium values obtained for the narrow-sense heritability demonstrated the low contribution of the additive genetic variance to the total genotypic variance (Kearsey and Pooni, 1996).

3.10 Mean comparison of F_1 hybrids and controls

The mean comparison of the studied traits is presented in Table 3. Earliness is an important trait in most regions of sunflower cultivation in Iran, where sunflower is grown as the second crop following the last irrigation of a main crop or in rotation with cereal crops. Early maturing hybrids are, therefore, appropriate for cultivation in these areas. Also, early maturity enables sunflower genotypes to avoid environmental stresses, especially biotic (diseases and insects) or abiotic stresses (heat and drought) at the ripening stage. In this study, days to maturity was found to range between 93 (Cms14×Rn1-56) and 106.5 (Cms19×Rn1-77) days (Table 3). Depending on the specific genotype used and the environmental conditions, different ranges have been reported for days to maturity in sunflower hybrids (Razi and Assad, 1999; Ghaffari, 2003). In the present study, the Cms14×Rn1-144, Cms14×Rn1-56, Cms522/2×Rn14, Cms522/2×Rn864, and Cms19×Rn1-76 hybrids showed a shorter growth period than did the 'Allstar' hybrid (as the earliest hybrid in Iran) (Table 3). These promising hybrids could, therefore, be applied in breeding programs aimed at producing early maturity genotypes of sunflower. Plant height was found to range from 107 (Cms19×Rn1-77) to 156.3 cm (Hysun-33) in the hybrids (Table 3). Plant height was shorter in all the test hybrids than in the two control ('Haysan-33' and 'Azargol') ones. It may, therefore, be concluded that the hybrids investigated in this study exhibited reduced plant heights as compared to the control genotypes. Razi and Assad (1999) reported that plant height in their study ranged between 124.3 and 222.9 (cm) in sunflower.

Head diameter in the present study ranged between 19.9 cm in Cms19×Rn1-149 and 15.9cm in Cms14×Rn1-56 (Table 3). Based on these results, the hybrids were found superior to the control with respect to head diameter. Stem diameter ranged between 19.6 mm in Cms19×Rn1-60 to 15.9 mm in Cms19×Rn1-76 (Table 3). These results indicate a high variability in the hybrids with respect to stem diameter. Regarding seed weight, the values were observed to range between 75.9 ('Azargol') and 42.6 (Cms522/2×Rn864) (g) (Table 3). Finally, seed yield recorded the highest values in 'Azargol', 'Haysan-33', and 'Allstar' as compared to the other hybrids (Table 3). The highest seed yield (4104.17 Kg/ha) was recorded for Cms19×Rn1-81 (Table 3). However, no other significant differences, were observed between this hybrid and the control genotypes (Table 3). This finding is in agreement with those of Haq et al. (2006), Razi and Assad (1999), Ghaffari (2003), and Arshad et al. (2007). Generally speaking, the hybrid derived from the Cms-19×Rn1-81 cross was found to be the best for seed yield among the hybrids investigated in this study.

Improvement of seed yield and oil content forms a major goal in hybrid production programs. Oil yield is an important sunflower trait which justifies the economical production of specific hybrids since the oil from properly selected and modified genotypes offers a good potential for improving food products while it also has many industrial applications (Seiler, 2007). However, it is not easy to improve these traits due to the low heritability and high sensitivity of the plant to genotypic interactions with the environment (Marinkovic, 1992). The experiments in this study showed that oil yield ranged between 44.7% (Cms19×Rn1-81)

and 39.9% (Cms19×Rn1-76) (Table 3). The hybrids of Cms522/2×Rn1-4 and Cms19×Rn1-81 showed to be superior with respect to seed and oil yields (Table 3). Cms19×Rn1-77 was found to be the unsatisfactory hybrid in this study as it recorded the lowest seed and oil yields and the longest growth period. Some hybrids showed early maturity compared to 'Allstar' which is known as an early maturity genotype.

Cluster analysis, as a multivariate analysis, is widely used to describe genetic diversity based on similarities or differences among genotypes (Peeters and Martinelli, 1989). The genotypes investigated in this study were subjected to cluster analysis using the Ward method which yielded a classification of genotypes into four distinct groups (Figure 1), with 6 hybrids assigned to group 1, 6 to group 2, 4 to group 3, and 1 to group 4.

The mean comparison of different traits is presented in Table 4. Significant differences were observed among the groups in all the traits studied, except for stem diameter (Table 4). The differences in days to maturity, plant height, head angle, and head diameter observed among the groups were highly significant (Table 4).

The second group exhibited the highest values of seed yield, oil yield, seed weight, and plant height. The two control hybrids ('Azargol' and 'Haysan-33') were in this group as well. Based on the mean values obtained for all the traits in the hybrids investigated, the hybrids Cms19×Rn1-130, Cms19×Rn1-149, and Cms19×Rn1-81 along with the control 'Azargol' and 'Haysan-33' were found to have the best seed yields. Thus, these hybrids could be recommended for further evaluation from a seed yield improvement viewpoint. The third group was identified as the earliest maturity group. It also exhibited acceptable seed and oil yields. The genotypes in this group could be recommended for cases of limited planting season. The hybrid Cms19×Rn1-77, in group 4, recorded the highest number of days to maturity among the four groups evaluated and ranked the lowest with respect to plant height, head angle, seed weight, and seed yield (Table 4). The highest value for head diameter belonged to this hybrid as well. The hybrids from common parents (i.e., Cms 522/2 and Cms 14) were classified in the same groups, indicating identical effects of parents on the progeny and probably the existence of strong maternal effects in the parents. In contrast, those from the parent Cms-19 were placed in different groups, indicating less of similar parental effects on the progeny.

Generally speaking, the hybrids Cms-19×Rn1-81, Cms19×Rn1-130, and Cms19×Rn1-149 had the best conditions for seed and oil yields. On the other hand, Cms14×Rn1-56, Cms14×Rn1-144, and Cms19×Rn1-76 with suitable seed and oil yields were identified with earliness in growth period. Therefore, depending on the regional conditions, it seems better when selecting the best hybrids to make a tradeoff between yield and growth period. Alba *et al.* (2010) used cluster analysis for categorizing 11 sunflower hybrids based on 8 agronomic characters including oil yield with different sowing dates. The hybrids were classified by cluster analysis with respect to their performance into groups that could be differenced by means and stability.

Table 3: Mean comparison of different studied traits in promising hybrids and control genotypes of sunflower

Code	Genotype	DM	PH (cm)	HA	HD (cm)	SD (mm)	SW (g)	SY (Kg ha^{-1})	OY (Kg ha^{-1})	Oc(%)
1	Cms14×Rn1-144	93.5	122	10.7	17.5	18.2	72.8	3166	1269.5	40.7
2	Cms14×Rn1-56	93	112.7	26.5	15.9	17.9	53.9	3552	1454.7	40.9
3	Cms522/2×Rn14	94	137	44	17.5	18.4	52.6	3447	1493.6	43.4
4	Cms522/2×Rn1-4	102.2	122	39.2	16.5	18.1	45.4	3437	1538.5	44.7
5	Cms522/2×Rn864	93.7	129.25	44.5	16.5	17.7	42. 6	3044.5	1353.6	44. 5
6	Cms522/2×Rn4	97.7	138.5	53	16.5	17.7	44.6	3666.6	1530.2	41.7
7	Cms19×Rn1-28	100.7	149.25	54.7	18.1	16.6	59	3116.6	1326.8	42.6
8	Cms19×Rn1-52	101.2	147. 25	48	16.7	19.1	61.7	3839.5	1684.6	43.9
9	Cms19×Rn1-60	97.2	132.75	42.2	17.6	19.6	56.8	3668.7	1565.8	42.9
10	Cms19×Rn1-76	96.2	114.25	12	17.6	15.9	58. 8	3229.1	1292.4	39.9
11	Cms19×Rn1-130	103.5	149.75	50	17.5	16.3	62.2	3858. 3	1666.4	43.4
12	Cms19×Rn1-49	104	136	34	19.9	17.2	64.1	3924.9	1620.9	41.3
13	Cms19×Rn1-81	100.2	138.5	46.7	19.2	17.4	59.8	4104	1821.6	44.3
14	Cms19×Rn1-77	106.5	107	8	20	17.6	47.5	2287.4	944.4	41.6
15	Allstar	96.7	115.5	20.2	17	18.1	65.9	4358.3	1815.9	41.6
16	Hysun-33	104.7	156.25	42	20	19.2	61.2	4985.4	2035.1	40.8
17	Azargol	102.2	150	57.5	19.5	17.7	75.9	4986. 2	2132.3	42.9
	LSD (P=0.05)	3.19	11.8	22.7	2.31	1.05	6.83	449.3	272.9	2.08

Abbreviations: DM: days to maturity, PH: Plant height, HA: Head angle, HD: Head diameter, SD: Stem diameter, SW: Seed weight, SY: Seed yield, OY: Oil yield, OC: Oil content.

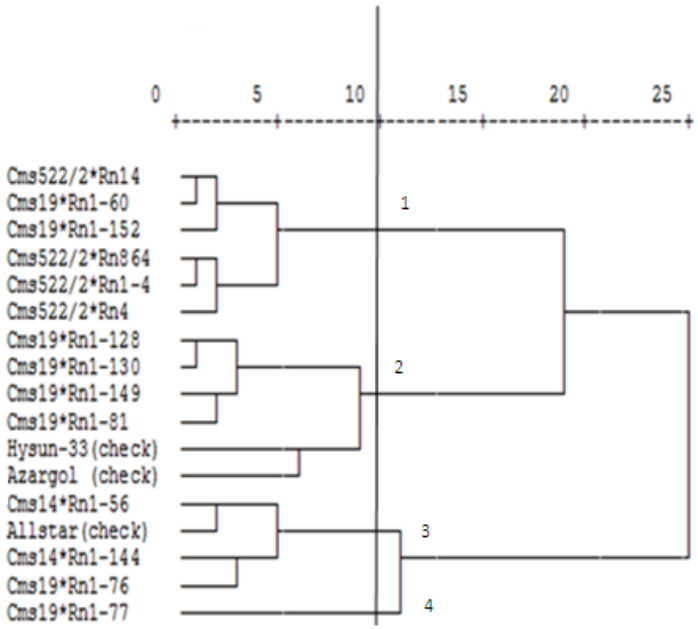


Figure 1: Cluster dendrogram of 17 sunflower genotypes based on Euclidean rescaled distances

The biplots, therefore, provide more useful information for sunflower breeders and PCA can be performed based on agronomic traits data for the determination of real hybrid performances.

Agronomic traits with favorable values of principal components were effectively used to discriminate entries for multivariate selection. Acute angle for seed yield and oil yield indicated a positive relationship between these traits (Figure 2). Seed weight, days to maturity, and head diameter were also closely associated (Figure 2). The associated traits can be, therefore, used for the selection of one of these traits, and this would provide the opportunity to implement a multi-trait selection process in sunflower breeding programs. Oil content was found not to be associated with seed yield or oil yield; rather, oil content exhibited strongly negative associations with oil yield and seed yield because of the obtuse angle of their vectors. Therefore, biplots provide more useful information in this case for sunflower breeders and PCA can be performed based on agronomic traits to determine the real performance of the hybrids. According to Figure 2, Cms19×Rn1-52, Cms19×Rn1-130, and Cms19×Rn1-81 hybrids were the superior ones with respect to plant height, oil content, and stem diameter. The hybrids Cms19×Rn1-76 and Cms19×Rn1-77 showed the least PC values, demonstrating the low breeding value of these hybrids for seed and oil improvement.

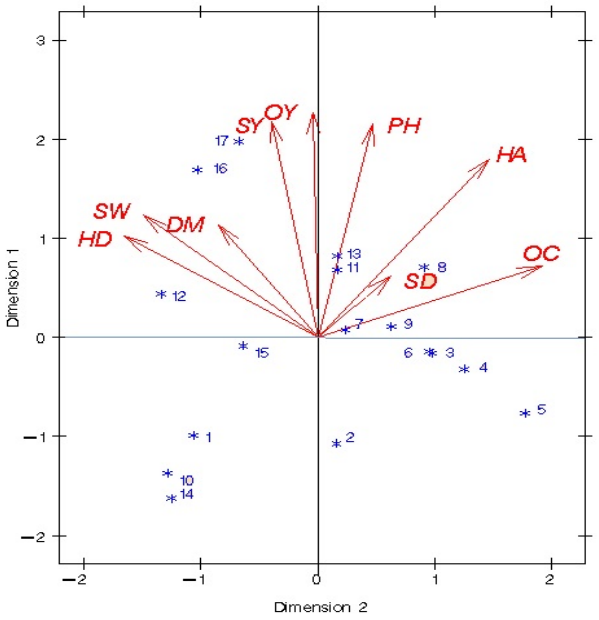


Figure 2: Biplots for the 1st and 2nd principal components used for mean agronomic traits in sunflower

Abbreviations: DM: days to maturity, PH: Plant height, HA: Head angle, HD: Head diameter, SD: Stem diameter, SW: Seed weight, SY: Seed yield, OY: Oil yield, OC: Oil content;

Table 4: Means comparison of groups from cluster analysis based of LSD test for sunflower hybrids

Trait¥	Mean squares	Means of groups			
		Group 1	Group 2	Group 3	Group 4
DM	0.33**	97.71b[c]	102.58[ab]	94.875[c]	106.5[a]
PH (cm)	605.63**	134.46[a]	146.62[a]	116.12[b]	107[b]
HA	1392.13**	45.17[a]	48.62[a]	17.37[b]	8[b]
HD (cm)	3.13**	16.89[b]	19.04[a]	17[b]	20[a]
SD (mm)	2.34ns	18.43[a]	17.4[a]	17.49[a]	17.6[a]
SW (g)	422.45*	50.62a[b]	63.69[a]	62.86[a]	47.55[b]
SY (kg/ha)	18831.78*	3517.43[a]	4162.63[a]	3576.56[a]	2287.47[b]
OY (Kg/ha)	5502*	1527.7[a]	1767.18[a]	1458.14[a]	944.37[b]
OC (%)	16.05*	43.53[a]	42.54[ab]	40.77[b]	41.36[ab]

**, *: significant at 1% and 5%, respectively.
¥: Abbreviations: DM: days to maturity, PH: Plant height, HA: Head angle, HD: Head diameter, SD: Stem diameter, SW: Seed weight, SY: Seed yield, OY: Oil yield, OC: Oil content.
Means followed by the same letter in each row are not significantly different at $P = 0.05$.

4 CONCLUSION

This paper exploited the North Carolina Design 1 analysis to investigate the genetic implications regarding seed yield, oil yield, and certain agronomic traits in different new F_1 hybrids of sunflower. Cluster analysis revealed significant differences among all the study groups for all the traits investigated, except for head diameter. The second group with six hybrids showed the highest values for grain yield, yield components, and oil yield. Therefore, the hybrids in this group may be recommended for application in breeding programs. Moreover, the predominance of additive gene action in explaining genetic variations observed in days to maturity, seed weight, and oil content (%) supports the statement that genetic improvement may be suggested through accumulation of favorable alleles from parents and using such proper methods as recurrent selection. The predominance of the dominance gene action in explaining the genetic variation observed in head angle, head diameter, seed oil, and seed yield supports the claim that hybridization breeding methods are required to improve these traits. The importance of both additive and dominance gene actions for the genetic control of plant height and stem diameter supports breeding methods based on both selection and hybridization for improving these traits.

5 REFERENCES

Acquaah G. 2012. Principles of plant genetics and breeding. 2nd ed. Wiley-Blackwell, Oxford, doi: 10.1002/9781118313718

Alba V., Polignano G.B., Montemurro C., Sabetta W., Bisignano V., Turi M., Ravaglia S., Troccoli A., Colecchia S. A., Alba E., Blanco E. A. 2010. Similarity Patterns and Stability of Environmental Response in sunflower Hybrids. International Journal of Agronomy. 2010:1-9, doi: 10.1155/2010/637928

Alza J. O., Fernandez-Martinez J. M. 1997. Genetic analysis of yield and related traits in sunflower (*Helianthus annuus* L.) in dry land and irrigated environments. Euphytica, 95(2): 243-251, doi: 10.1023/A:1003056500991

Andarkhor S.A., Rameeh V., Alitabar R. A. 2013. Estimation of genetic parameters for yield components and seed yield in sunflower using line × tester analysis. African Journal of Biotechnology, 12(25): 3978-3983

Arshad M., Kashif –Ilyas M., Ayub Khan M. 2007. Genetic divergence and path coefficient analysis for seed yield traits in sunflower (*Helianthus annuus* L.) hybrids. Pakistan Journal of Botany, 39(6): 2009-2015.

Bajaj R. K., Aujla K. K., Chahal G.S. 1997. Combining ability studies in sunflower. (*Helianthus annuus* L.). Crop Improvement, 34**:** 141-146.

Comstock, R.E., Robinson H.E. 1948. The components of genetic variance populations. Biometrics 4: 254-266, doi: 10.2307/3001412

Fick G.N. 1987. Sunflower. Pp 544-585. In: Rabbelen, G., Doweny, R.K. and Ashri, A.D.(eds). Oil crops of the World. McGrow Hill, U.S.A.

Gangappa E., Channakishnaiah K. M., Harini M. S., Ramesh S. 1997. Studies on combining ability in sunflower. Helia, 20(27):73-84

Ghaffari, M. 2003. Use of principle component analysis method for selection of superior three way cross hybrids in sunflower. Seed and Plant Improvement Journal, 19(4): 513-527

Ghaffari M., Farrokhi I., Mirzapour M. 2011. Combining ability and gene action for agronomic traits and oil content in sunflower (*Helianthus annuus* L.) using F_1 hybrids. Crop breeding Journal, 1(1): 73- 84

Gomez S. D., Bladini M., Charles D.A., Vannozzi G. P. 1999. Genetic variances and heritability of sunflower traits associated with drought tolerance. Helia, 22: 23 - 34

Haq A., Rashid A., Butt M. A., Akhter M. A., Aslam M. and Saeed A. 2006. Evaluation of sunflower (*Helianthus annuus* L.) hybrids for yield and yield components in central Punjab. Journal of Agricultural Research, 44(4): 277-284

Hill J., Becker H.C., Tigerstedt P. M. A. 1998. Quantitative and ecological aspects of plant breeding**.** Chapman and Hall, UK, London. P.275, doi: 10.1007/978-94-011-5830-5

Hu J., Seiler G., Kole C. 2010. Genetics, genomics and breeding of sunflower. CRC Press, doi: 10.1201/b10192

Kadkol G. P., Anand, J. J., Sharma, R. P. 1984. Combining ability and heterosis in sunflower. Indian Journal of Genetics and Plant Breeding, 44(3): 447-451

Kaya Y., Atakisi, I.K. 2004. Combining ability analysis of some yield characters of sunflower (*Helianthus annuus* L.). Helia, 27(41):75-84, doi: 10.2298/HEL0441075Y

Kearsey M. J., Pooni, H. S. 1996. The Genetical Analysis of Quantitative Traits. Chapman and Hall, London, doi: 10.1007/978-1-4899-4441-2

Kempthorne O. 1957. An introduction to genetic statistics. John Willey and Sons, Inc. New York.

Khani M., Daneshian J., Zeinali Khaneghah H. and Ghannadha, M.R. 2005. Genetic analysis of yield and its components using line ×tester cross design in sunflower inbred lines under the stress and non-stress drought conditions. Iranian Journal of Agricultural Science, 36 (2): 435-445

Mahmud I., Keramer H.H. 1951. Segregation for yield, height and maturity, following a soybean crosses. Agronomy Journal. 43: 605- 609, doi: 10.2134/agronj1951.00021962004300120005x

Marinkovic R. 1992. Path-coefficient analysis of some yield components of sunflower (*Helianthus annuus* L.). Euphytica, 60: 201-205

Miller J. F., Hammond J. J. 1991. Inheritance of reduced height in sunflower. Euphytica 53: 131-136, doi: 10.1007/BF00023793

Ortegon M., Escabedo A. A., Villarreal L. Q. 1992. Combining ability of sunflower lines and comparisons among Parent lines and hybrids. Proc. 13th Int. Sunflower Conf. (Pisa - Italy). PP: 1178

Peeters J. P., Martinelli, J. A. 1989. Hierarchical clustering analysis as a tool to manage variation in germplasm collection. Theoretical and Applied Genetics, 78: 42-48, doi: 10.1007/BF00299751

Razi H., Assad, M.T. 1999. Comparison of selection criteria in normal and limited irrigation in sunflower. Euphytica, 105: 83-90, doi: 10.1023/A:1003472212917

Saeidi G. H. A., Rezaei. A. M., Abbasi A., Farokhi, E. 2009. General and specific combining ability for agronomic and seed quality traits in some inbred lines of sunflower. Iranian Journal of Field Crop Science, 40 (2): 105 - 113

Seiler G. J. 2007. Wild annual Helianthus anomalus and H. deserticola for improving oil content and quality in sunflower. Industrial Crops and Products, 22(1): 95-100, doi: 10.1016/j.indcrop.2006.07.007

Shahsavari, M. R., Nikobin, M. and Karimi, M. 2010. Final evaluation of new hybrids of sunflowers. First National Symposium of Oil Grain Plants, Isfahan University of Technology, Isfahan, Iran.

Skoric D., Jocic S., Molnar I. 2000. General (GCA) and specific (SCA) combining abilities in sunflower. Proc. 15th Int. Sunflower Conference. Toulose France, PP: E23 - E27

Wehner T.C. 1984. Estimates of heritabilities and variance components for low temperature germination ability in cucumber. Journal of the American Society for Horticultural Science, 109: 664-667

Weiss E. A. 2000. Oil seed Crops. 2nd ed. Blackwell Science, Oxford. P.364

COBISS Code 1.01

DOI: 10.14720/aas.2015.105.2.09
Agrovoc descriptors: *Plant extracts, Artemisia*, *Eucalyptus camaldulensis, Rosmarinus officinalis,plant protection, insecticides,* botanical insecticides*, insecticidal properties, Myzus persicae, integrated control*

Agris category code: h01, h10

Insecticidal activity of three plants extracts against *Myzus persicae* (Sulzer, 1776) and their phytochemical screening

Billal NIA[1], Naama FRAH[2] and Imane AZOUI[3]

Received July 29, 2015; accepted August 20, 2015.
Delo je prispelo 29. julija 2015, sprejeto 20. avgusta 2015.

ABSTRACT

To reduce the use of synthetic pesticides and their negative effects on the environment, leaves extracts of *Artemisia herba-alba* Asso, *Eucalyptus camaldulensis* Dehnh and *Rosmarinus officinalis* L. were obtained with petroleum ether, ethanol and distilled water as solvents. These extracts were evaluated under laboratory conditions for their insecticidal effect against 3 to 4 days-old *Myzus persicae* individuals (Homoptera: Aphididae) at 1, 2.5, 5, and 10 %. We made observations after 24 hours. Etheric extract of all plants was effective and caused mortalities (100 %, 53 % and 60 % respectively) at the highest concentration. However, ethanolic and aqueous extracts did not show any significant insecticidal effect. The phytochemical screening showed the richness of etheric extract in terpenes. The results obtained suggest that we can make bioinsecticides based on leaves etheric extracts from these plants for use in integrated pest management.

Key words: *Artemisia herba-alba*, botanical insecticides*, Myzus persicae, Eucalyptus camaldulensis, Rosmarinus officinalis*

IZVLEČEK

INSEKTICIDNO DELOVANJE IZVLEČKOV TREH RASTLIN NA LISTNO UŠ *Myzus persicae* (Sulzer, 1776) IN NJIHOVA FITOKEMIČNA ANALIZA

Z namenom zmanjševanja negativnih učinkov sintetičnih pesticidov na okolje so bili narejeni izvlečki listov s petroletrom, etanolom in distilirano vodo iz naslednjih treh rastlin *Artemisia herba-alba* Asso, *Eucalyptus camaldulensis* Dehnh. in *Rosmarinus officinalis* L.. Insekticidni učinki teh izvlečkov so bili ovrednoteni v laboratorijskih razmerah na 3 do 4 dni starih osebkih listnih uši vrste *Myzus persicae* (Sulzer, 1776), Homoptera: Aphididae) v 1, 2.5, 5, in 10 % razredčitvah. Opazovanja so bila opravljena 24 ur po uporabi. Učinkoviti so bili izvlečki s petroletrom vseh rastlin, ki so povzročili smrtnost (100 %, 53 % in 60 %) pri največjih koncentracijah. Etanolni in vodni ekstrakti niso imeli značilnega insekticidnega delovanja. Fitokemična analiza ekstraktov je pokazala veliko vsebnost terpenov v izvlečkih, dobljenih s petroletrom. Rezultati raziskave nakazujejo, da lahko naredimo bioinsekticide iz analiziranih rastlin na osnovi izvlečkov s petroletrom in jih uporabimo v integriranem varstvu rastlin.

Ključne besede: *Artemisia herba-alba*, botanični insekticidi*, Myzus persicae, Eucalyptus camaldulensis, Rosmarinus officinalis*

1 INTRODUCTION

Chemical pest control employs potent chemical pesticides to reduce or eliminate pests and constitutes the major way in crop production throughout the world. In fact, the use of these conventional insecticides have over the years manifested a number of disadvantages, the most

[1] Mohamed Khider University, Faculty of Natural and Life Sciences, Department of Agronomy, 07000 Biskra, Algeria. E-mail: bnagro@yahoo.fr.

[2] Hadj Lakhdar University, Institute of Veterinary and Agricultural Sciences, Department of Agronomy, 05000 Batna, Algeria. E-mail: frahnaama@yahoo.fr

[3] Hadj Lakhdar University, Faculty of Sciences, Department of Natural and Life Sciences, 05000 Batna, Algeria. E-mail : azouiimane@hotmail.fr

important of which are the risks involved for human health and for the environment (Ofuya and Okuku, 1994). To reduce the use of synthetic pesticides on fruit and vegetable plantations, phytochemicals and plant extracts have long been a subject of research in an effort to develop alternatives to conventional insecticides but with reduced health and environmental impact (Dancewicz *et al*., 2011). Most plant species that are used in phytomedicine contain ingredients, which inhibit the development of insects, hinder their feeding (antifeedants) or act as repellents and confusants (Laznik *et al*., 2010).

The green peach aphid, *Myzus persicae* (Sulzer, 1776) is found throughout the world. In addition to attacking plants in the field, green peach aphid readily infests vegetables and ornamental plants grown in greenhouses (Capinera, 2011). Its management is generally based upon the use of synthetic insecticides (Ciarla *et al*., 2005). Unfortunately, there is little knowledge in the literature about the effect of a plant extracts such as *Artemisia herba-alba* Asso, *Eucalyptus camaldulensis* Dehnh. and *Rosmarinus officinalis* L. against aphids.

This study aims to determine the toxicity of *Artemisia herba-alba*, *Eucalyptus camaldulensis* and *Rosmarinus officinalis* leaves extracts against *Myzus persicae* by using several concentrations.

2 MATERIALS AND METHODS

2.1 Selection of plant species and preparation of leaves extracts

In April 2013, leaves of *Artemisia herba-alba*, *Eucalyptus camaldulensis* and *Rosmarinus officinalis* were collected from Batna in the East of Algeria. It is located at 35° 61′ N latitude and 6° 24′ E longitude with an elevation of 1048 meter above sea level.

We prepared the extracts according to the method presented by N'guessan *et al*. (2009) using various techniques. First, we dried samples at 40 °C into an oven before grinding them with an electric grinder. Crude extracts were obtained by successive extractions with three solvents ordered by increasing polarity. In this order, we used petroleum ether, ethanol and distilled water. For extraction with petroleum ether, we dissolved 80 g of powder obtained from leaves in 240 mL of petroleum ether. A manual agitation was done for 10 min. Then, we filtered the mixture. The filtrate obtained was named etheric filtrate 1. On residual marks, we added 240 mL of petroleum ether; after 10 min of agitation and filtration, we obtained the etheric filtrate 2. The same operation allowed getting the etheric filtrate 3. These three filtrates were grouped together and concentrated on a bath sand. These series of operations resulted in a concentrated solution called etheric extract. After exhaustion by petroleum ether, residual marks were dried. The powder was recovered in 240 mL of ethanol. Homogenization by manual agitation during 10 min allowed getting the ethanolic filtrate 1. The same operation was repeated and gave the ethanolic filtrate 2. The ethanolic filtrates were also grouped and concentrated on a bath sand to give ethanolic extract. To prepare the aqueous extract, we infused dried powder in 800 mL of distilled water during 15 min. After filtration, we obtained aqueous extract. Finally, 4 concentrations were prepared from these extracts: 1, 2.5,5 and 10 %.

2.2 Aphid collecting and rearing

We collected last stage larvae of *Myzus persicae* (Sulzer, 1776) in April 2014 from Biskra in the East of Algeria. It is located at 34° 52′ N latitude and 5° 45′ E longitude with an elevation of 120 meter above sea level. These larvae were found on *Malva sylvestris* L.

A mass rearing of the green peach aphid was started on broad beans (*Vicia faba* L.) in a greenhouse. Each plant was inoculated with apterous adult when emerging in the morning. Aphids were collected after 10 days by brushing them carefully from the leaves.

2.3 Bioassay test

To determine the insecticidal effect of etheric, ethanolic, and aqueous extracts of selected plants, 15 *M. persicae* apterous larvae (3 to 4 days-old)

were placed in a Petri dish containing three leaves of *V. faba* soaked in different concentrations (1, 2.5,5 and 10 %) of these extracts with three replications. The experiment was carried out in the laboratory. The mortality was determined after 24 hours from the beginning of exposure. When no leg or antennal movements were observed, insects were considered dead (Salari *et al*., 2010).

2.4 Phytochemical screening

We characterized the different chemical groups of plants extracts using standard procedures as described by Kayani *et al*. (2007), Benmehdi *et al.* (2012), and Rahim *et al*. (2012).

2.4.1 Test for alkaloids

0.2 g of each extract was warmed with 2 % H_2SO_4 for two minutes. Then, few drops of Dragendorff's reagent (solution of potassium bismuth iodide) were added. Presence of orange red precipitate indicated as positiveness for alkaloids.

2.4.2 Test for terpenoids (Salkowski test)

0.2 g of each extract was mixed in 2 mL of chloroform, and concentrated H_2S0_4 (3 mL) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

2.4.3 Test for saponins

0.2 g of each extract was shaken with 5 ml of distilled water. Then, it heated to boil. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.

2.5 Statistical analysis

The efficiency (E %) of different extracts was calculated according to the Schneider-Orelli formula:

$$E\,\% = [\,(b - k)/(100 - k)\,] \times 100,$$

in which: **b** = percentage of individuals in the treated sample, and **k** = percentage of individuals found dead in the witness sample (Tomescu *et al*., 2009).

A chi-square test was applied to estimate the correlation between mortality and concentrations. Afterwards, data were subjected to the logistic regression model to determine lethal concentrations. We used the statistical program Statistica 8 (StatSoft, Inc., Tulsa, OK) for all analyses.

3 RESULTS AND DISCUSSION

In the conducted experiment, effects of the *A. herba-alba*, *E. camaldulensis* and *R. officinalis* leaves extracts were recorded in the control of the green peach aphid (*Myzus persicae*) after 24 hours. The statistical analyses indicated that only etheric extracts of all plants showed a dependence between the mortality and different concentrations ($P < 0.01$) (Table 1).

Table 1: Mortality of *Myzus persicae* larvae with different types of plants extracts

Plant	Extract	χ^2	*P*
Artemisia herba-alba	Etheric extract	146	0.00
	Ethanolic extract	2.0	0.4
	Aqueous extract	4.0	0.1
Eucalyptus camaldulensis	Etheric extract	75.81	0.00
	Ethanolic extract	4.0	0.1
	Aqueous extract	2	0.4
Rosmarinus officinalis	Etheric extract	91.7	0.00
	Ethanolic extract	1	0.6
	Aqueous extract	1	0.6

We obtained the greatest percentages of mortality of the aphid, 93 % and 100 %, with the two highest concentrations (5 % and 10 %) of *A. herba-alba*. In the case of *E. camaldulensis*, the percentage of mortality was almost 53 % at 10% and 60 % for *R. officinalis* at the same concentration (Figure 1).

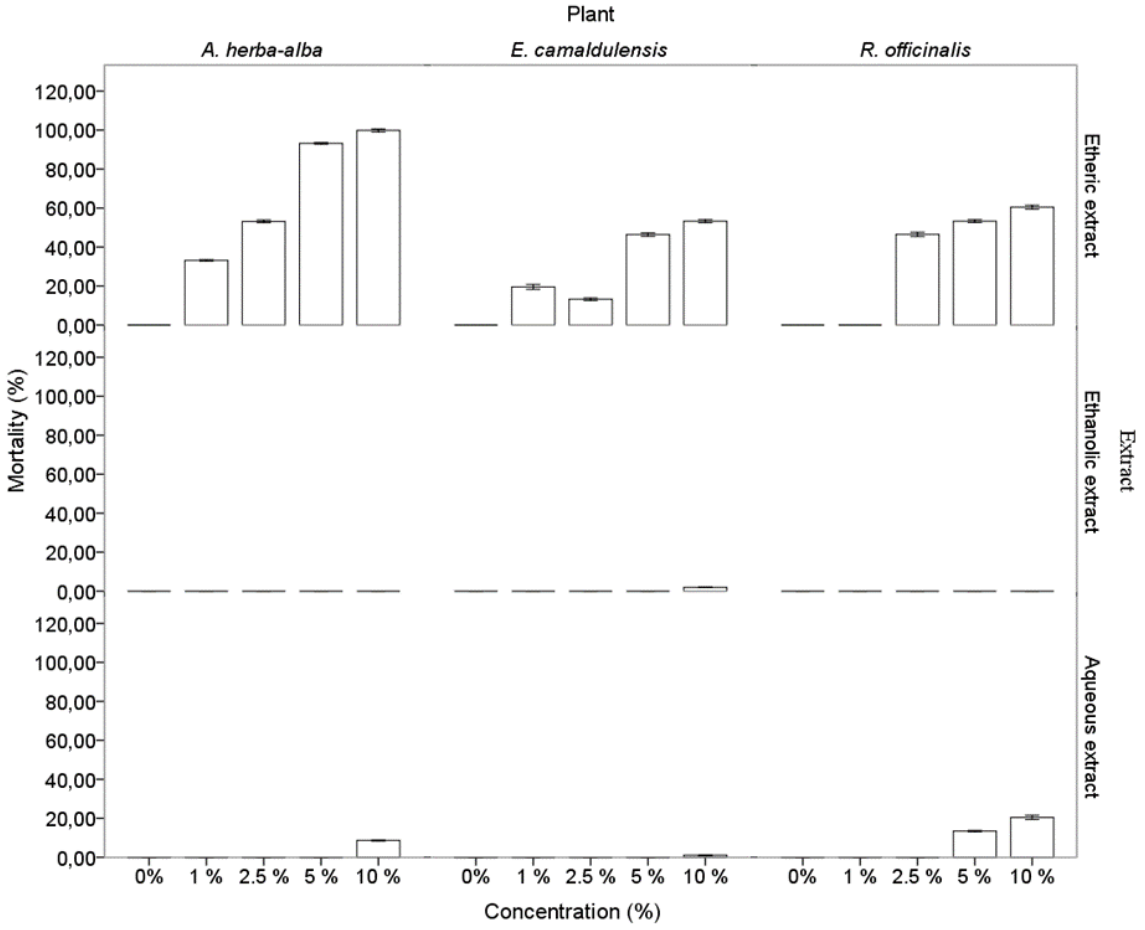


Figure 1: Average mortality (% ± standard error) of *Myzus persicae* larvae with leaves extracts from *A. herba-alba, E. camaldulensis and R. officinalis* with several concentrations after 24 hours of exposure

However, ethanolic extract of all plants did not show any dependence between mortality and concentrations (Table 1). The mortality of *M. persicae* obtained was 0% for all plants at a concentration of 5%, and almost 9% at 10 % when *E. camaldulensis* was used (Figure 1). Also, no dependence between the mortality and concentrations concerning aqueous extract of all plants ($P > 0.05$) (Table 1) was established. Nearly 8 % was obtained with *A. herba-alba* at 10 % and 13 % with *R. officinalis* at the same concentration (Figure 1).

From the Logit analyses, the LC_{50} (Lethal Concentration) was 2.07 % for *A. herba-alba,* 8.35 % for *E. camaldulensis* and 6.6 % in the case of *R. officinalis*. These data proved that *A. herba-alba* is more toxic than the other two plants etheric extracts (Table 2).

Table 2: Toxicity of etheric extracts on *M. persicae* larvae (LC_{50}'s [%] calculated by logistic regression)

Plant extract	B	Wald χ^2	*P*	Odds Ratio	LC_{50} (%)
A. herba-alba etheric extract	0.8	67.5	< 0.01	2.2	2.1 %
E. camaldulensis etheric extract	0.3	69.0	< 0.01	1.3	8.3 %
R. officinalis etheric extract	0.22	48.0	< 0.01	1.2	6.6 %

Soliman (2007) reported that *Artemisia herba-alba* oil gave a high toxicity with LC_{50} 0.023 % and caused 90.44 % reduction in the population on *Aphis gossypii* (Glover , 1877). Abdel-Shafy *et al*. (2009) evaluated the crude extracts with different solvents of *A. herba-alba* against the third instar larvae of *Chrysomyia albiceps* (Wiedemann, 1819). Results showed that all extracts had toxic effects on larvae. The settling of *M. persicae* on host plant leaves was strongly deterred by wormwood (*Artemisia absinthium* L.) (Dancewicz, 2008). *Artemisia seiberi* Besser oil extract was the most toxic to woolly apple aphid (*Eriosoma lanigerum* [Hausmann, 1802]) in terms of concentration and time responses compared with other tested oil extracts (Ateyyat *et al.*, 2012). Işık and Görür (2009) demonstrated that between 7 essential oils used against cabbage aphid (*Brevicoryne brassicae* [Linnaeus, 1758]), rosemary oil can be considered as an important aphidicide to control aphid population. It can be used also as an acaricide against the two-spotted spider mite (*Tetranychus urticae* Koch, 1836), causing complete mortality in the laboratory at concentrations that cause no phytotoxicity to host plants (Miresmailli, 2006). According to Rojht *et al*. (2012), the mortality rate of the *Acanthoscelides obtectus* (Say, 1831) adults after 7 days using ethanol extract of *R. officinalis* reached 91.2 % between the concentrations of 50 % and 100 %. The use of rosemary essential oils could significantly affect pest insects as well. In fact, the longevity, fecundity, and fertility of the cowpea weevils (*Callosobruchus maculatus* [Fabricius, 1775]), (Coleoptera, Bruchinae) were negatively affected by these oils (Douiri *et al*., 2014). Furthermore, 81.7 % adult mortality at 1 % concentration on sycamore lace bug (*Corythucha ciliata* [Say, 1832]) (Rojht *et al*., 2009). The highest mortality percentage against *Hyalopterus pruni* (Geoffroy, 1762) adults was achieved with extract of *E. camaldulensis* leaves, reached 92.6 % at 10 % after 48 days of treatment (Haji Younis, 2013). Elbanna (2006) recorded that 20 ml of the eucalyptus seed extract at concentration 1000 ppm caused 80 and 100 % mortality in larvae of *Culex pipiens* (Linnaeus, 1758) within 14 hours.

Phytochemical screening showed the presence of terpenoids in the etheric extract of all plants used in this study (Table 3).

Table 3: Phytochemical screening of *A. herba-alba*, *E. camaldulensis* and *R. officinalis* using different solvents

Plant species	Alkaloids			Terpenoids			Saponins		
	PE	E	W	PE	E	W	PE	E	W
A. herba-alba	-	-	-	+	+	+	-	-	+
E. camaldulensis	-	-	-	+	+	+	-	-	+
R. officinalis	-	-	+	+	+	+	-	-	+

(+) : positive, (-) : negative

Many works showed the richness in terpenoids of *A. herba-alba,* like monoterpene hydrocarbons (Behtari, 2012), oxygenated monoterpenes (Hudaib and Aburjai, 2006) and sesquiterpenes (Laid, 2008; Paolini, 2010). Qualitative and quantitative analyses of *A. herba-alba* and *Artemisia monosperma* Delile essential oils showed that both oils were mainly characterized by high concentration of total terpene compounds (75.281 and 78.69 % respectively) and low concentration of sesquiterpenes (24.357 and 21.023 % respectively) (Soliman, 2007). Miresmailli *et al*. (2006) and Verma (2012) pointed out the presence of monoterpenes in rosemary whereas Zhang *et al*. (2014) reported its richness in diterpenoid and triterpenoid glycosides. Terpenoids are abundant also in the foliage of *E. camaldulensis*, providing the characteristic smell as well as being valuable economically and influencing ecological interactions (Leicach, 2010; Külheim *et al*., 2015). Moreover, in *E. camaldulensis* oil, monoterpenes were prevalent while sesquiterpene hydrocarbons and oxygenated sesquiterpenes were less represented (Mediouni Ben Jemâa, 2012).

4 CONCLUSIONS

A. herba-alba, *E. camaldulensis* and *R. officinalis* leaves etheric extract were efficacious against *Myzus persicae*. The results obtained suggest that we can make bioinsecticides based on extracts from these plants for use in integrated pest management, which are a good alternative to conventional synthetic insecticides. This study is a first step and its purpose was to compare the effect of several plants leaves crude extracts against aphids. More studies will necessary to test the activity of each identified compounds against aphid species and other pests.

5 REFERENCES

Ateyyat M., Abu-Romman S., Abu-Darwish M., Ghabeish I. 2012. Impact of flavonoids against woolly apple aphid, *Eriosoma lanigerum* (Hausmann) and its sole parasitoid, *Aphelinus mali* (Hald.). J. Agr. Sci. 4 (2) : 227-236, doi: 10.5539/jas.v4n2p227

Abdel-Shafy S., El-Khateeb R.M., Soliman M.M.M., Abdel-Aziz M.M. 2009. The efficacy of some wild medicinal plant extracts on the survival and development of third instar larvae of *Chrysomyia albiceps* (Wied) (Diptera: Calliphoridae). Trop. Anim. Health Pro. 41:1741–1753, 10.1007/s11250-009-9373-0

Benmehdi H., Hasnaoui O., Benali O., Salhi F. 2012. Phytochemical investigation of leaves and fruits extracts of *Chamaerops humilis* L. J. Mater. Environ. Sci. 3 (2) : 320-237

Behtari B., Gholami F., Khalid K.A., Tilaki G.D., Bahari R. 2012. Effect of growth stages and altitude on *Artemisia herba-alba* Asso essential oil growing in Iran. Jeobp 15 (2): 307 – 313

Capinera J.L. 2011. Green Peach Aphid, *Myzus persicae* (Sulzer) (Insecta: Hemiptera: Aphididae). Entomology and Nematology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, United states of America

Ciarla M.V., Mareggiani G., Heit G., Puhl L. 2005. *Myzus persicae* (Homoptera: Aphididae) and *Capsicum annuum* (Solanaceae) volatiles: their effect on predators attraction Bol San Veg Plagas 31: 503-507

Dancewicz K., Gabryś B., Przybylska M. 2011. Effect of garlic (*Allium sativum* L.) and tansy (*Tanaceum vulgare* L.) extracts and potassic horticultural soap on the probing and feeding behaviour of *Myzus persicae* (Sulzer, 1776). Aphids and other hemipterous insects 17:129–136

Douiri L.F., Boughdad A., Alaoui M.H., Moumni M. 2014. Biological activity of *Rosmarinus officinalis*essential oils against *Callosobruchus maculatus* (Coleoptera, Bruchinae). J. Biol. Agri. Healtc. 4 (2): 5-14

Elbanna M.S. 2006. Larvaecidal effects of eucalyptus extract on the larvae of *Culex pipiens* mosquito. Int. J. Agri. Biol. 8 (6): 896–897.

Haji Younis G. 2013. The Effect of Some Extracts on the Stone Fruit Aphid "*Hyalopterus Pruni*" in Duhok Region. Int. J. Pure Appl. Sci. Technol. 18 (2): 39-44

Hudaib M.M., Aburjai T.A. 2006. Composition of the essential oil from *Artemisia herba-alba* grown in Jordan. J. Essent. Oil Res. 18: 301-304, doi: 10.1080/10412905.2006.9699096

Işık M., Görür G. 2009. Aphidicidial activity of seven essential oils against the cabbage aphid, *Brevicoryne*

brassicae L. (Hemiptera: Aphididae). Munis Ent. Zool. 4 (2): 424-431.

Kayani S.A., Masood A., Achakzai A.K.K., Anbreen S. 2007. Distribution of secondary metabolites in plants of Quetta-Balochistan. Pak. J. Bot. 39 (4): 1173-1179.

Külheim C., Padovan A., Hefer C., Krause S.T., Köllner T.G., Myburg A.A., Degenhardt J., Foley W.J. 2015. The eucalyptus terpene synthase gene family. BMC Genomics. DOI 10.1186/s12864-015-1598-x, doi: 10.1186/s12864-015-1598-x

Laid M., Hegazy M.E.F., Ahmed A.A., Kalla A., Belkacemi D., Ohta S. 2008. Sesquiterpene lactones from Algerian *Artemisia herba-alba.* Phytochem. Lett. 1: 85–88, doi: 10.1016/j.phytol.2008.04.002

Laznik Ž., Cunja V., Kač M., Trdan S. 2010. Efficacy of three natural substances against apple aphid (*Aphis pomi* De Geer, Aphididae, Homoptera) under laboratory conditions. Acta Agric. Slov. 97 (1): 19-23

Leicach S.R., Garau A.M., Guarnaschelli A.B., Yaber Grassa M.A., Sztarkera N.D., Datoa A. 2010. Changes in *Eucalyptus camaldulensis* essential oil composition as response to drought preconditioning. J. Plant Interact. 5 (3): 205-210, doi: 10.1080/17429145.2010.483744

Mediouni Ben Jemâa J., Haouel S., Bouaziz M., Khouja M.L. 2012. Seasonal variations in chemical composition and fumigant activity of five Eucalyptus essential oils against three moth pests of stored dates in Tunisia. J. Stored Prod. Res. 48: 61-67, 10.1016/j.jspr.2011.10.001

Miresmailli S., Bradbury R., Isman M.B. 2006. Comparative toxicity of *Rosmarinus officinalis* L. essential oil and blends of its major constituents against *Tetranychus urticae* Koch (Acari: Tetranychidae) on two different host plants. Pest Manag. Sci. 62:366–371, doi: 10.1002/ps.1157

N'guessan K., Kadja B., Zirihi G.N., Traoré D., Aké-assi L. 2009. Screening phytochimique de quelques plantes médicinales ivoiriennes utilisées en pays Krobou (Agboville, Côte-d'Ivoire). Sci. Nat. 6 (1): 1 – 15, doi: 10.4314/scinat.v6i1.48575

Ofuya T.I., Okuku I.E. 1994. Insecticidal effect of some plant extracts on the cowpea aphid *Aphis craccivora* Koch (Homoptera: Aphididae). Anz. Schädl.kd. Pflanzenschutz Umweltschutz, 67: 127-129, doi: 10.1007/BF01909033

Paolini J., El Ouariachi E., Bouyanzer A., Hammouti B., Desjobert J.M., Costa J., Muselli A. 2010. Chemical variability of *Artemisia herba-alba* Asso essential oils from East Morocco. Chem. Pap. 64 (5): 550–556, doi: 10.2478/s11696-010-0051-5

Rahim G., Qureshi R., Gulfraz M., Arshad M., Rahim S. 2012. Preliminary phytochemical screening and ethnomedicinal uses of *Teucrium stocksianum* from Malakand Division. J. Med. Plants Res. 6 (5): 704-707

Rojht H., Košir I.J., Trdan S. 2012. Chemical analysis of three herbal extracts and observation of their activity against adults of *Acanthoscelides obtectus* and *Leptinotarsa decemlineata* using a video tracking system. J.Plant Dis.Protect.119 (2) : 59–67

Rojht H., Meško A., Vidrih M., Trdan S. 2009. Insecticidal activity of four different substances against larvae and adults of sycamore lace bug (*Corythucha ciliata* [Say], Heteroptera, Tingidae). Acta Agric. Slov. 93 (1) : 31 – 36, doi: 10.2478/v10014-009-0004-2

Salari E., Ahmadi, K., Dehyaghobi, R.Z., Purhematy, A., Takalloozadeh, H.M. 2012. Toxic and repellent effect of harmal (*Peganum harmala* L.) acetonic extract on several aphids and *Tribolium castaneum* (Herbst). Chil. J. Agr. Res. 72 (1): 147-151, doi: 10.4067/S0718-58392012000100023

Salari E., Ahmadi K., Zamani R. 2010. Study on the effects of acetonic extract of *Otostegia Persica* (Labiatae) on three aphid species and one stored product pest. Adv. Environ. Biol. 4 (3)**:** 346-349

Soliman M.M.M. 2007. Phytochemical and toxicological studies of *Artemisia* L. (Compositae) essential oil against some insect pests. Arch. Phyt. Plant Prot. 40 (2): 128-138, doi: 10.1080/03235400500355808

Tomescu C.V., Brudea V., Rišca M. 2009. Preliminary research on the efficiency of some vegetal metabolites in fighting the mealy plum aphid (*Hyalopterus pruni* Geoffroi – *Ord. Homoptera*). Analele universitatii" Stefan cel mare" suceava sectiunea silvicultura serie noua nr.1

Verma R. S., Rahman L., Mishra S., Verma R.K., Singh A., Chauhan A., Yadav A.K., 2012. Volatile terpenoidcomposition of *Rosmarinus officinalis*, "Cim-Hariyali": variability in North India during annual growth. J. Chil. Chem. Soc. 57 (2): 1066-1068, doi: 10.4067/S0717-97072012000200001

Zhang Y., Adelakun T. A., Qu L., Li X., Li J., Han L., Wang T. 2014. New terpenoid glycosides obtained from *Rosmarinus officinalis* L. aerial parts. Fitoterapia, 99 : 78–85, doi : 10.1016/j.fitote.2014.09.004

COBISS Code 1.01

DOI: 10.14720/aas.2015.105.2.10
Agrovoc descriptors: setaria, grasses, physiological response, osmotic stress, salt tolerance, sowing depth, germination

Agris category code: f60, f62

Effect of pre-chilling and environmental factors on breaking seed dormancy and germination of three foxtail species

Vajihe AMINI[1], Faezeh ZAEFARIAN[1]*, Mohammad REZVANI[2]

Received July 07, 2015; accepted August 25, 2015.
Delo je prispelo 07. julija 2015, sprejeto 25. avgusta 2015.

ABSTRACT

The effect of wet and dry pre-chilling duration, pH, osmotic stress, salt stress and planting depth on seed germination and seedling emergence of three foxtail species (*Setaria glauca*, *S. verticillata* and *S. viridis*) was investigated in a series of laboratory and greenhouse experiments. Both wet and dry pre-chilling for 45 days promoted seed germination of *S. glauca* compared with the control. Pre-chilling was not significantly effective in seed dormancy breaking of *S. viridis* and *S. verticillata*. The maximum germination of foxtails (*S. verticillata, S. viridis*) was obtained when seeds were treated with pH 7 buffer solution. Increasing of osmotic and salt stress decreased seed germination of foxtails. *Setaria verticillata* seed germination was more tolerant than those of *S. glauca* and *S. viridis* to high water stress condition. *Setaria glauca* and *S. verticillata* seed germination were more tolerant to high salinity stress than *S. viridis.* Seedling emergence decreased with increasing the burial depth and no germination observed at 8 cm soil depth.

Key words: osmotic and salt stress, seed depth burial. germination, *S. glauca*, *S. verticillata* and *S. viridis*

IZVLEČEK

UČINKI HLADNEGA PREDTRETIRANJA IN OKOLJSKIH DEJAVNIKOV NA PREKINITEV DORMANCE IN KALITEV TREH VRST MUHVIČEV

Učinki trajanja mokrega in suhega hladnega predtretiranja, pH, ozmotskega in solnega stresa ter globine setve na kalitev in vznik so bili preučevani pri treh vrstah muhviča (sivozeleni muhvič- *Setaria pumila* (Poir.) Roem. & Schult.(= *S. glauca* auct.)., vretenčasti muhvič - *S. verticillata* (L.) P. Beauv. in zeleni muhvič - *S. viridis* (L.) P. Beauv.) v rastlinjaku in laboratoriju. Mokro in suho hladno predtretiranje v trajanju 45 dni je pospešilo kalitev semen sivozelenega muhviča v primerjavi s kontrolo. Isto obravnavanje ni imelo značilnega vpliva na prekinitev dormance semen pri zelenem in vretenčastem muhviču. Največja kalitev muhvičev (*S. verticillata, S. viridis*) je bila dosežena pri semenih, tretiranih s pufersko raztopino s pH 7. Povečanje ozmotskega in solnega stresa je zmanjšalo kalitev vseh vrst muhvičev Semena vretenčastega muhviča so bila bolj odporna na večji sušni stress kot semena zelenega in sivozelenega muhviča. Semena sivozelenega in vretenčastega muhviča so bila bolj odporna na velik slanostni stress kot semena zelenega muhviča. Vznik vseh treh vrst muhvičev se je zmanjševal s povečevanjem globine setve, na globini 8 cm kalitve ni bilo.

Ključne besede: ozmotski in solni stress, globina setve, kalitev, *Setaria pumila*, *S. verticillata* and *S. viridis*

[1] MSc. Student, Department of Agronomy, Faculty of Crop Sciences, Sari Agricultural Sciences and Natural Resources University, Sari, Iran

[2] Department of Agronomy, Faculty of Crop Sciences, Sari Agricultural Sciences and Natural Resources University, Sari, Iran Corresponding author: fa_zaefarian@yahoo.com

[3] Department of Agronomy, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran

1 INTRODUCTION

Foxtail species (*Setaria pumila* (Poir.) Roem. & Schult. = *Setaria glauca* auct., *S. verticillata* (L.) P. Beauv. and *S. viridis* (L.) P. Beauv.); are member of Poaceae family and native to the Eurasia. Foxtails are the worst weeds that are interfering with world agriculture (Holm *et al*., 1977) and are considered as problematic in many crops such as corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), soybean (*Glycine max* L.), potato (*Solanum tuberosum* L.) and sugarcane (*Saccharum officinarum* L.). Due to high competitive ability and reproductive potential, these species are expected to be a serious threat to summer crops and orchards in north of Iran.

Seed germination is one of the critical phases in plant life cycle (Shoab *et al.*, 2012) which is affected by dormancy and environmental factors such as light, soil pH, osmotic potential, salt stress, temperature and burial depth. The foxtails seeds are characterized by dormancy and a long period of survival in soil. *Setaria glauca*, *S. viridis* and *S. verticillata* are able to survive in soil for 13 to 39 years (Dekker, 2003).

Dormancy can be defined as an inhibition mechanism of seed germination of intact viable seed to optimize the distribution of germination over time (Bewley and Black, 1983; Hilhorst, 1995) and is one of the survival mechanisms of invasive annual weeds (Baskin *et al.* 2004). Seed dormancy is categorized as physical, physiologic, morphologic, morpho-physiologic and combined dormancy (Baskin and Baskin, 2004). For breaking dormancy and enhancing of seed germination, moist chilling or cold stratification has been widely used in different plants (Schopmeyer, 1974; AOSA, 1992; ISTA, 1999; Wang and Berjak, 2000).

Investigation of germination requirements show how a species germination process is adapted to habitat conditions and regulated by environmental factors (Van Assche *et al*., 2002). Thus, finding of some information about factors effective in seed dormancy breaking and optimal condition of germination and seedling growth are necessary for management of weeds. The aim of the present study is promotion of seed germination of foxtail species by means of moist and dry chilling and also investigation of effect of environmental factors such as soil pH, water and salt stress on seed germination and burial depth on seedling emergence.

2 MATERIALS AND METHODS

2.1 Plant materials and experimental conditions

Laboratory and greenhouse experiments were carried out at Department of Agronomy, Sari University of Agricultural Sciences and Natural Resources, Sari, Iran during 2013.

The mature seeds of *Setaria glauca*, *S. verticillata* and *S. viridis* were collected from more than 200 plants in 2012, Qaemshahr, Iran. Seeds were cleaned and stored in paper bags in darkness at room temperature (23±2 °C) until start the experiments.

Prior start laboratory experiments, seeds were surface-sterilized by soaking in 1 % sodium hypochlorite (NaOCl) for 1 min and subsequently rinsing with distilled water. Twenty five seeds were placed in Petri dishes (8 cm diameter) lined by two sheets of filter paper (Whatman No. 1). The filter paper was moistened with 5 ml of distilled water or treatment solutions. Petri dishes were sealed with Parafilm to reduce the water loss and placed in germinator with 16/8 h (day/night) photoperiod and 25/15 °C (day/night) fluctuating temperature with a light intensity of 300 µmol m^{-2} s^{-1}. Germinated seeds with at least 2 mm long seminal root were counted after a period of four weeks. The percentage of germinated seeds was calculated using following equation: number of germinated seeds/number of total seeds × 100.

2.2 Pre-treatment by cold stratification

To verify the effectiveness of the pre-chilling treatments, moisturized seeds with distilled water

and dry seeds were placed in refrigerator at temperature of 4 °C for 15, 30 and 45 days. After the pre-chilling period; seed germination was tested as in the plant materials and experimental conditions section described.

The best pre-treatment of wet or dry pre-chilling that promoted the maximum germination was considered as pre-treatment for further experiments (pH, osmotic stress, salt stress and seed burial depth).

2.3 Effect of pH on seed germination

The effect of pH on seed germination was determined by using different buffer solutions of pH 3, 5, 7, 9 and 11 prepared according to the method described by Chachalis and Reddy (2000). Seed germination tested by method described in the plant materials and experimental conditions section.

2.4 Effect of osmotic stress on seed germination

To evaluate the effect of osmotic stress, different levels of osmotic potential including 0, -0.1, -0.25, -0.5, -1 and -1.5 MPa were prepared by dissolving of 0, 99.4, 157.1, 222.2, 314.2, 384.8 g polyethylene glycol 6000 (Merck, Germany) in 1 L distilled water, respectively. Five ml of polyethylene glycol 6000 solution was added to the Petri dishes. Seed germination was tested according to the plant materials and experimental conditions section.

2.5 Effect of salt stress on seed germination

The effects of salt stress on seed germination were evaluated by soaking seeds in solution of 0, 10, 20, 40, 80, 160 and 320 mM sodium chloride (NaCl) (Merck, Germany). Germination was tested according to the plant materials and experimental conditions section.

2.6 Effect of seed burial depth on seedling emergence

Fifty seeds of foxtails were planted in soil in 16-cm-diameter plastic pots (30 cm height) at the depths of 0, 2, 5 and 8 cm in greenhouse. Soil used for experiment was a clay soil (clay, 61.4 %; silt, 7.9 %; sand, 30.7 %) with 7.49 pH and 2.58 % organic carbon. The temperature of the greenhouse was set at 28/20 °C (day/night) with a natural photoperiod. Pots were irrigated as needed to maintain soil moisture in field capacity. Seedling emergence was recorded as coleoptiles appeared. Seedling emergence counted every 7 days for 28 days.

2.7 Statistical analysis

All experiments were conducted in a complete randomized design with four replicates. Analysis of variance was performed on transformed (Arcsine transformation) data. Significant difference of means were identified by protected LSD test ($p = 0.05$) and standard error bars. The SAS program (SAS Institute Inc., Cary, NC, USA) was used for the statistical analyses.

3 RESULTS AND DISCUSSION

3.1 Effect of wet and dry pre-chilling pre-treatment on seed dormancy breaking of foxtails

Germination of foxtails seeds was significantly affected by wet and dry pre-chilling durations. Initial germination (control treatment) of *S. glauca*, *S. verticillata* and *S. viridis* was 41.54 %, 47.89 % and 53.78 %, respectively (Table 1). Both wet and dry pre-chilling for 45 days increased seed germination of *S. glauca* about 32.35 % and 48.61 % compared with the control, respectively (Table 1). In contrast, seed germination of *S. verticillata* and *S. viridis* did not significantly increase by both wet and dry pre-chilling in comparison with control (Table 1). Therefore, for continuing the experiments just *S. glauca* seeds were dry pre-chilled for 45 days prior pH, osmotic potential, salt stress and seed burial depth experiments. Positive effects of wet and dry pre-chilling on seed germination of different weed species was previously reported by Wartidiningsih *et al*. (1994) and Baskin *et al*. (1992). For Poaceae family it is well known that the duration of stratification from 3 days to 6 weeks improve seed germination (Williams 1983, Smith-Jochum and Albrecht 1988, Matus-Cádiz *et al*. 2001, Matus-Cádiz and Hucl 2003). The cold pre-chilling may change the hormonal balance of seed and increase

germination through enhancement of gibberellic acid and cytokinin activity and/or the decline of abscisic acid (Copeland and McDonald, 2001). The wet pre-chilling provides enough moisture to activate the hydrolytic enzymes that make seeds ready to germinate once they were moved to the warm temperature.

Table 1: Effect of wet and dry pre-chilling duration on germination of foxtail seeds

Germination (%)					
Wet pre-chilling			Dry pre-chilling		
S. glauca	*S. verticillata*	*S. viridis*	*S. glauca*	*S. verticillata*	*S. viridis*
41.54±0.81^{c}	47.89±0.99^{a}	53.78±0.91^{a}	41.54±0.81^{b}	47.89±0.99^{a}	53.78±0.91^{a}
52.60±1.03 ^{b}	48.45±0.47^{a}	60.35±1.59^{a}	46.16±1.42^{b}	33.08±1.12^{b}	49.70±1.75^{ab}
56.83±0.64^{ab}	39.22±0.48^{b}	34.43±1.50^{b}	52.92 ±3.27^{b}	29.83±1.22^{b}	41.94±2.15^{b}
61.40±0.65^{a}	34.28±1.38^{b}	57.00±1.50^{a}	80.83±2.68 ^{a}	19.53±1.70^{c}	59.22±2.19^{b}

Notes: Mean±Se; Values in each column followed by different superscripted letters indicate significant differences ($p< 0.05$).

3.2 Effect of pH on seed germination of foxtails

Seed germination of foxtails was significantly influenced by pH solution. Approximately, 40 % of seeds of *S. glauca* germinated at pH 3, 5 and 7 (Fig. 1a). The maximum seed germination of *S. verticillata* and *S. viridis* occurred at pH 7 (Fig. 1b and 1c). The pH 9 and 11 had an inhibitory effect on both *S. glauca* and *S. verticillata* seed germination (Fig. 1a, 1b) However, about 25 % of *S. viridis* seeds germinated at pH 9 (Fig. 1c). Inhibition of germination at pH 9 and 11 shows that soil pH is a limiting factor for seed germination. Wilson (1979) and Singh and Achhireddy (1984) indicated that a narrow range of pH from 6 to 7 was needed for germination of canadian thistle (*Cirsium arvense* L.) and strangler vine (*Morrenia odorata* Lindl.). Chachalis and Reddy (2000) showed that trumpet creeper (*Campsis radicans* L.) germination was limited by extreme pH range (pH 4 or 10). In contrast, Chachalis et al. (2008), Fani Yazdi et al. (2013) and Rezvani and Fani Yazdi (2013) reported that seed germination of venice mallow (*Hibiscus trionum* L. HIBTR), sheep sorrel (*Rumex acetosella* L.) and black nightshade (*Solanum nigrum* L.) occurred over a wide range of pH, that shows pH should not be a limiting factor for germination of some plants.

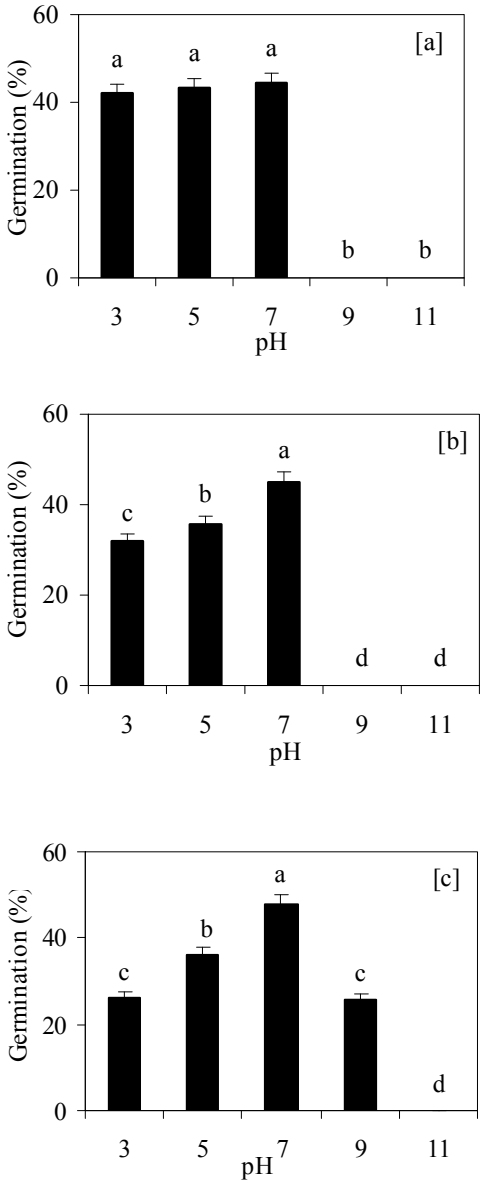


Figure 1: Effect of pH on germination of foxtails seed. *Setaria glauca* (a), *S. verticillata* (b) and *S. viridis* (c). The columns with the same letter are not significantly different. Vertical bars are standard error of the means.

3.3 Effect of osmotic stress on seed germination of foxtails

Osmotic stress significantly affected seed germination of *Setaria glauca, S. verticillata* and *S. viridis*. With increasing osmotic stress a decline in seed germination of foxtails was recorded (Fig. 2a, 2b and 2c). At osmotic stress -0.5 MPa, *S. verticillata* seed germination was higher than those of *S. glauca* and *S. viridis* (Fig. 2a, 2b and 2c). Seed germination of *S. verticillata* was more tolerant to high water stress than *S. glauca* and *S. viridis.* About 40.35 % of *S. verticillata* seeds germinated at -0.5 MPa (Fig. 2b). Seed germination of *S. glauca* and *S. viridis* was 33.05 % and 29.09 % at -0.5 MPa osmotic potential, respectively (Fig. 2a and 2c). Germination of foxtail seeds was inhibited when seeds were exposed to osmotic potential lower than -0.5 MPa. These results show that foxtails seeds are sensitive to high osmotic stress and germination is favored by a moist environment. Therefore, low moisture of soil from mid spring up to summer could be a limiting factor of emergence of foxtails in the north of Iran. Manthey and

Nalewaja (1987) showed that germination of *S. glauca* and *S. viridis* was reduced by water stress and *S. viridis* germinated more rapidly than *S. glauca*.

Germination of horseweed (*Conyza canadensis* (L.) Cronquist) decreased as osmotic potential increased from 0 (distilled water) to -0.8 MPa (Nandula *et al.*, 2006). Our results are in agreement with the work of Shaw *et al.* (1991) on redvine (*Bunnichia* sp.) and Reddy and Singh (1992) on hairy beggarticks (*Bidens pilosa* L.). Low osmotic potential was found to inhibit germination of trumpet creeper (Chachalis and Reddy, 2000), texasweed (*Caperonia palustris* (L.) A. St. Hil.) (Koger *et al*., 2004) and cadillo (*Urena lobata*) (Wang *et al*., 2009). Rezvani and Fani Yazdi (2013) and Fani Yazdi *et al.* (2013) reported that low water stress significantly reduced the germination of both black nightshade and sheep sorrel.

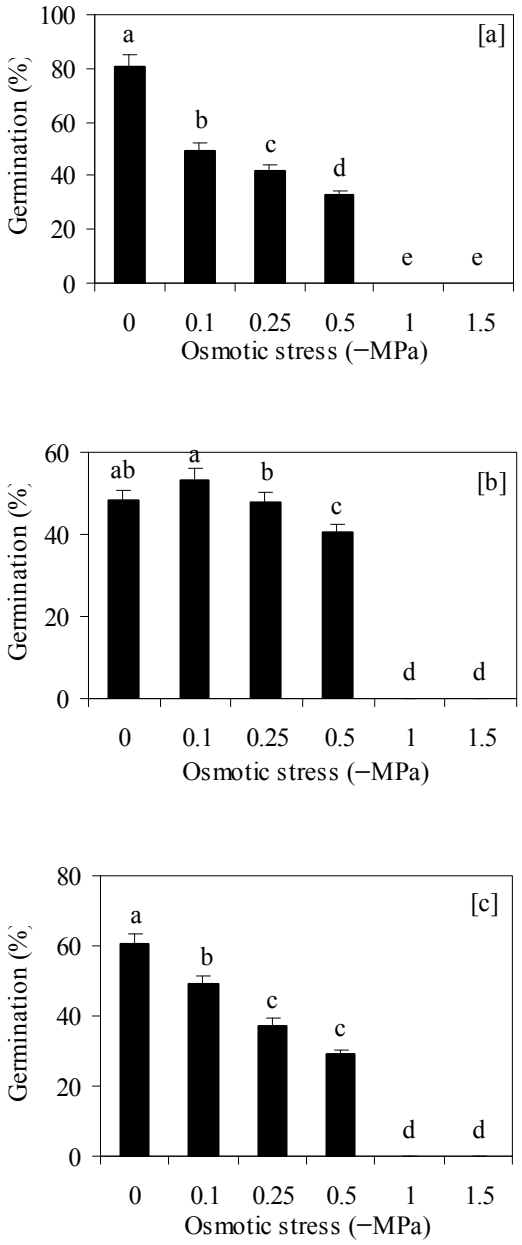


Figure 2: Effect of osmotic stress on germination of foxtail seeds. *Setaria glauca* (a), *S. verticillata* (b) and *S. viridis* (c). The columns with the same letter are not significantly different. Vertical bars are standard error of the means

3.4 Effect of salt stress on seed germination of foxtails

The germination of *S. glauca* seeds was not significantly differed among treatments in the range of 10 to 80 mM NaCl concentration. When seeds were treated with 320 mM NaCl, seed germination was inhibited (Fig. 3a). Similarly, seed germination of *S. verticillata* was not significantly differed in comparison with the control as salt stress increased from 10 to 80 mM NaCl. No germination occurred as seeds were treated with 320 mM NaCl (Fig. 3b). The germination of *S. viridis* decreased significantly as salt concentrations increased and no seed germination was observed at 160 mM NaCl (Fig. 3c). These data show that foxtails seeds were fairly tolerant to salt stress. Zia and Khan (2004), Koger *et al*. (2004), Nandula *et al*. (2006) and Lu *et al*. (2006) also reported negative effect of salt stress on seed germination of different plants. Rao *et al*. (2008) showed that germination of American sloughgrass was inhibited at 300 mM NaCl concentration.

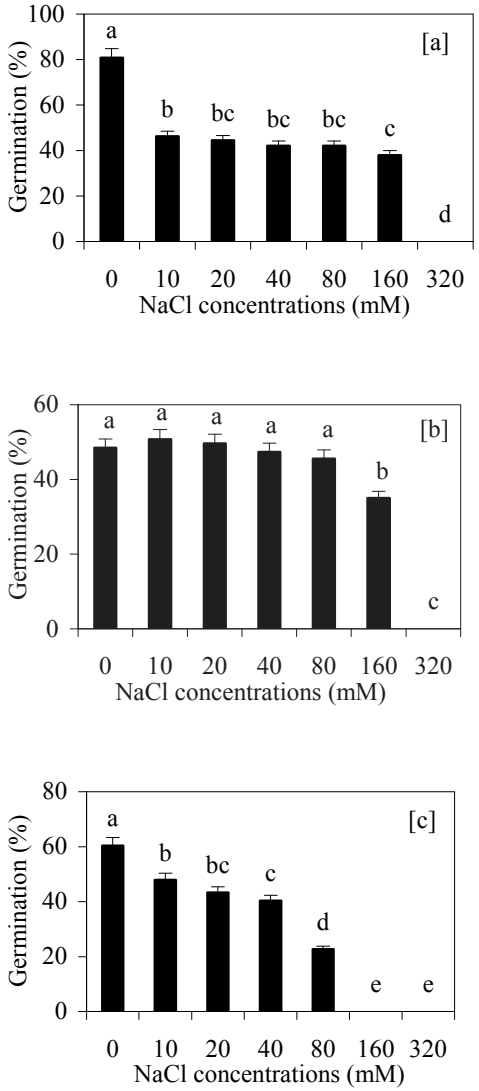


Figure 3: Effect of NaCl concentration on germination of foxtail seeds. *Setaria glauca* (a), *S. verticillata* (b) and *S. viridis* (c). The columns with the same letter are not significantly different. Vertical bars are standard error of the means

3.5 Effect of seed burial depth on seedling emergence of foxtails

Seed burial depth markedly influenced on seedling emergence of *Setaria glauca, S. verticillata* and *S. viridis*. The maximum seedling emergence of foxtails was found where seeds were sown on the soil surface. The lowest seedling emergence was obtained where planting depth was 8 cm (Fig. 4a, 4b, 4c). Decreasing in seedling emergence due to increasing burial depth could be linked primarily to seed energy reserves (Mennan and Ngouajio, 2006). Seeds of bigger size have higher energy reserve than those of smaller seed size; therefore can emerge from higher depth. Chauhan *et al.* (2006) indicated that light and seed size generally limit seedling emergence from deep in the soil. Also, low emergence from bigger depths because of the lack of oxygen diffusion and the presence of CO_2 deriving from soil biological activity has been reported by Benvenuti and Macchia (1995).

Decreased emergence by increasing in planting depth has been reported in several weed species, including hairy beggarticks (Reddy and Singh, 1992), horse purslane (*Trianthema portulacastrum* L.) (Balyan and Bhan, 1986), stranglervine (Singh and Achhireddy, 1984), and horseweed (Nandula *et al.*, 2006).

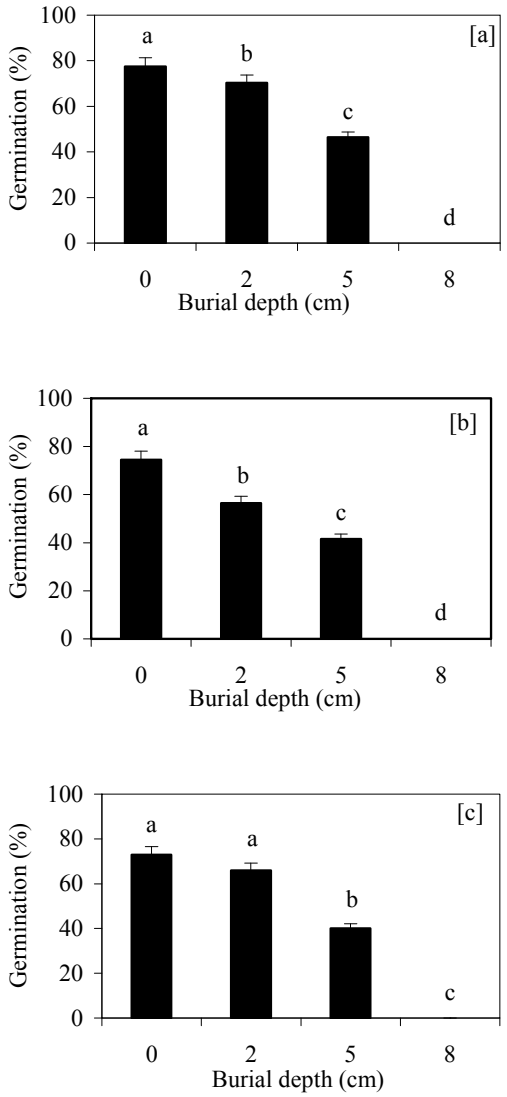


Figure 4: Effect of burial depth on germination of foxtail seeds. *Setaria glauca* (a), *S. verticillata* (b) and *S. viridis* (c). The columns with the same letter are not significantly different. Vertical bars are standard error of the means

4 CONCLUSIONS

Seed dormancy of foxtails species showed different responses to pre-chilling treatments. However, dry pre-chilling was only significantly effective in seed dormancy breaking of *S. glauca*. Moreover, pH, salt and osmotic stress and seed planting depth significantly altered seed germination of foxtails. The results of the experiments could be effective in understanding the distribution of foxtails in agricultural ecosystems in the future. High seedling emergence of these species on the soil surface could be suggesting light requirements. Therefore, spreading of foxtails would be higher in fields with no-tillage or minimum-tillage practices. Our results suggest that increasing of tillage depth could be an effective weed management strategy to reduce seedling emergence of foxtails.

5 REFERENCES

AOSA (Association of Official Seed Analysts). 1992. Rules for testing seeds. Journal of Seed Technology, 6: 1-125

Balyan, R.S., Bhan, V.M., 1986. Germination of horse purslane (*Trianthema portulacastrum*) in relation to temperature, storage conditions, and seedling depths. Weed Science, 34: 513–5

Baskin, C.C., Baskin, J.M., Hoffman, G.R., 1992. Seed dormancy in the prairie forbs *Echinacea angustifolia* var. angustifolia (Asteaceae): after-ripening pattern during cold stratification. International Journal of Plant Science, 153: 239-243, DOI: 10.1086/297027

Baskin, C.C., Milberg, P., Andersson, L., Baskin, J.M., 2004. Germination ecology of the annual weeds *Capsella bursa-pastoris* and *Descurainia sophia* originating from high northern latitudes. Weed Research, 44: 60-68, DOI: 10.1046/j.1365-3180.2003.00373.x

Baskin, J.M., Baskin, C.C., 2004. A classification system for seed dormancy. Seed Science Research, 14: 1-16, DOI: 10.1079/SSR2003150

Benvenuti, S., Macchia, M., 1995. Hypoxia effect on buried weed seed germination. Weed Research, 35: 343-351, 10.1111/j.1365-3180.1995.tb01629.x

Bewley, J.D., Black, M., 1983. Physiology and Biochemistry of Seeds In Relation to Germination. Development, germination, and growth. Vol. 1. pp. 81-100. Berlin, Heidelberg, New York, Springer Verlag.

Chachalis, D., Reddy, K.N., 2000. Factors affecting *Campsis radicans* seed germination and seedling emergence. Weed Science, 48: 212-216, DOI: 10.1614/0043-1745(2000)048[0212:FACRSG]2.0.CO;2

Chachalis, D., Korres, N., Khah, E.M., 2008. Factors affecting seed germination and emergence of venice mallow (*Hibiscus trionum*). Weed Science, 56: 509-515, DOI: 10.1614/WS-07-144.1

Chauhan, B. S., Gill, G., Preston, C., 2006. Seedling recruitment pattern and depth of recruitment of 10 weed species in minimum tillage and no-till seeding systems. Weed Science, 54: 658-668, DOI: 10.1614/WS-05-135R.1

Copeland, L.O., McDonald, M., 2001. Principles of seed science and Technology. Kluwer Academic Publisher, Norwell, MA, USA, DOI: 10.1007/978-1-4615-1619-4

Dekker, J., 2003. The Foxtail (*Setaria*) species-group. Weed Science, 51: 611-656, DOI: 10.1614/P2002-IR

Fani Yazdi, S.A., Rezvani, M., Rashed Mohassel, M.H., Ghanizadeh, H., 2013. Factors affecting seed germination and seedling emergence of sheep sorrel (*Rumex acetosella*). Romanian Agricultural Research, 30: 373-380

Hilhorst, H.W.M., 1995. A critical update on seed dormancy. I. Primary dormancy. Seed Science Research, 5: 61-73, DOI: 10.1017/S0960258500002634

Holm, L.G., Plucknett, D. L., Pancho, J.V., Herberger, J. P., 1977. The world's worst weeds-distribution and biology. The East-West Food Institute, Honolulu, HI.

ISTA (International Seed Testing Association). 1999. International rules for seed testing, Seed Science and Technology.

Koger, C.H., Reddy, K.S., Poston, D.H., 2004. Factors affecting seed germination, seedling emergence and survival of Texasweed (*Caperonia palustris*). Weed Science, 52: 989-995, DOI: 10.1614/WS-03-139R2

Manthey, D.R., Nalewaja, J.D., 1987. Germination of two foxtail (Setaria) species. Weed Technology, 1: 302-304

Matus-Cádiz, M., Hucl, P., Munasinghe, G., 2001. Seed dormancy and germination in three annual canarygrass (*Phalaris canariensis* L.) cultivars relative to spring wheat (*Triticum aestivum* L.). Seed Science Technology, 29: 523-531

Matus-Cádiz, M., Hucl, P., 2003. Comparison of pre-treatments for inducing germination in highly dormant wheat genotypes. Canadian Journal Plant Science, 83: 729-735, DOI: 10.4141/P03-008

Mennan, H., Ngouajio, M., 2006. Seasonal cycles in germination and seedling emergence of summer and winter populations of catchweed bedstraw (*Galium aparine*) and wild mustard (*Brassica Kaber*). Weed Science, 54: 114-120, DOI: 10.1614/WS-05-107R1.1

Nandula, V.K., Eubank, T.W., Poston, D.H., Koger, C.H., Reddy, K.N., 2006. Factors affecting germination of horseweed (*Conyza Canadensis*). Weed Science, 54: 898-902, DOI: 10.1614/WS-06-006R2.1

Rao, N., Dong, L., Jun, L., Zhang, H., 2008. Influence of environmental factors on seed germination and seedling emergence of American slough grass (*Beckmannia syzigachne*). Weed Science, 56: 529-533, DOI: 10.1614/WS-07-158.1

Reddy, K.N., Singh, M., 1992. Germination and emergence of hairy beggarticks (*Bidens pilosa*). Weed Science, 40: 195-199

Rezvani, M., Fani Yazdi, S.A., 2013. Factors affecting seed germination of black nightshade (*Solanum nigrum*). Acta Botanica Hungarica, 55(3–4): 397-408, DOI: 10.1556/ABot.55.2013.3-4.15

Schopmeyer, C.S., 1974 Tech. Coordinator. Seeds of woody plants in the United States. USDA, Forest Service, Agriculture Handbook No. 450. Washington, DC: US Government Printing Office.

Shaw, D.R., Mack, R.E., Smith, C.A., 1991. Redvine (*Brunnichia avata*) germination and emergence. Weed Science, 39: 33-36

Shoab, M., Tanveer A., Khaliq A., Haider Ali H. 2012. Effect of Seed Size and Ecological Factors on Germination of *Emex spinosa*. World Applied Science Journal, 17: 964-969

Singh, M., Achhireddy, N.R., 1984. Germination and ecology of milkweedvine (*Morrenia odorata*). Weed Science, 32: 781-785

Smith-Jochum, C., Albrecht, M.L., 1988. Transplanting or seeding in raised beds aids field establishment of some *Echinacea* species. Horticultural Science, 23: 1004-1005

Van Assche, J., Van Nerum, D., Darius, P., 2002. The comparative germination ecology of nine *Rumex* species. Plant Ecology, 159: 131-142, DOI: 10.1023/A:1015553905110

Wang, B.S.P., Berjak, P., 2000. Beneficial effects of moist chilling on the seeds of black spruce (*Picea mariana* (Mill.) B. S. P.). Annals of Botany, 86: 29-36, DOI: 10.1006/anbo.2000.1150

Wang, J., Ferrell, J., MacDonald, G., Sellers, B., 2009. Factors affecting seed germination of Cadillo (*Urena lobata*). Weed Science, 57: 31-35, DOI: 10.1614/WS-08-092.1

Wartidiningsih, N., Geneve, R.L., Kester, S.T., 1994. Osmotic priming or chilling stratification improves seed germination of purple coneflower. Horticultural Science, 29: 1445-1448

Williams E.D. 1983. Effects of temperature, light, nitrate and pre-chilling on seed germination of grassland plants. Annals Applied Biology 103: 161-172, DOI: 10.1111/j.1744-7348.1983.tb02752.x

Wilson, R.G., 1979. Germination and seedling development of Canada thistle (*Cirsium arvense*). Weed Science, 27: 146-151

Zia, S., Khan, M.A., 2004. Effect of light, salinity and temperature on seed germination of *Limonium stocksii*. Canadian Journal of Botany, 82: 151-157, DOI: 10.1139/b03-118

COBISS Code 1.01

DOI: 10.14720/aas.2015.105.2.11
Agrovoc descriptors: agrometeorology, *Dactylis glomerata, grasses,* herbage crops, crop yield, drought, growth, simulation models, meteorological factors

Agris category code: p40, u30, f01

Simulation of herbage yield and growth components of Cock's foot (*Dactylis glomerata* L.) in Jablje using the calibrated LINGRA-N model

Tjaša POGAČAR[1], Lučka KAJFEŽ-BOGATAJ[2]

Received June 03, 2015; accepted August 20, 2015.
Delo je prispelo 03. junija 2015, sprejeto 20. avgusta 2015.

ABSTRACT

In the study the previously calibrated LINGRA-N model was used for a long term simulation (1964–2013) of the herbage dry matter yield (*GRASS*) and growth analysis of Cock's foot (*Dactylis glomerata* L.) in Jablje. Changes in the yearly *GRASS* variability are reflected in the appearance of outliers in the second half of the study period. The biggest reductions in *GRASS* are seen in the years 1992, 1993 and 2003. These are the driest years according to meteorological variables (high maximum and minimum air temperatures, low precipitation) and also according to the simulations, with the lowest reduction factor for crop growth due to drought. The potential yield (*YIELD*) is not linearly dependent on meteorological variables. Some growth components were compared on a daily basis in a dry year (1993) and an average year (1994). In 1993, for instance, 53 % of photosynthetically active radiation was intercepted, against 75 % in 1994. Seasonal development of the actual soil moisture content was linked to the development of the leaf area index and consequently to the mass of green leaves, to the roots mass, to the mass of dead leaves and to *GRASS*. The results highlight the need for further research, on field and with simulations. As regards the latter, we have to keep in mind that they inevitably involve various uncertainties.

Key words: simulation, LINGRA-N, cock's foot, herbage yield, drought, growth analysis

IZVLEČEK

SIMULACIJA PRIDELKA ZELINJA IN KOMPONET RASTI NAVADNE PASJE TRAVE (*Dactylis glomerata* L.) V JABLJAH Z UMERJENIM MODELOM LINGRA-N

Predhodno umerjen model LINGRA-N smo uporabili za simulacijo pridelka suhega zelinja (*GRASS*) in komponent rasti navadne pasje trave (*Dactylis glomerata* L.) v 50-letnem obdobju (1964–2013) v Jabljah. Izkazalo se je, da so se v drugi polovici obravnavanega obdobja pri simulacijah *GRASS* na letni ravni začeli pojavljati osamelci. *GRASS* je bil najmanjši v letih 1992, 1993 in 2003. To so tudi najbolj suha leta, tako na podlagi meteoroloških spremenljivk kot tudi na podlagi simuliranega faktorja zmanjšanja rasti zaradi suše. Potencialni pridelek (*YIELD*) ni linearno odvisen od meteoroloških spremenljivk. Določene komponente rasti smo na dnevni skali primerjali v sušnem letu 1993 in povprečnem 1994. V letu 1993 je bilo na primer prestreženega fotosintetsko aktivnega sevanja 53 %, v letu 1994 pa 75 %. Razvoj stanja vode v tleh tekom leta smo povezali z razvojem indeksa listne površine ter posledično z razvojem mase zelenih listov, mase korenin, mase odmrlih listov in *GRASS*. Rezultati opozarjajo na pomembnost nadaljnjih raziskav, tako poljskih poskusov kot tudi modelskih simulacij. Pri slednjih se moramo zavedati, da nosijo s seboj negotovosti iz različnih virov.

Ključne besede: modeliranje, LINGRA-N, navadna pasja trava, pridelek travne ruše, suša, analiza rasti

[1] univ. dipl. meteorol., University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, SI-1000 Ljubljana, tjasa.pogacar@bf.uni-lj.si

[2] prof. dr., University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, SI-1000 Ljubljana, lucka.kajfez.bogataj@bf.uni-lj.si

This article is based on research undertaken for a doctoral dissertation by Tjaša Pogačar. Supervisor: Prof. Dr. Lučka Kajfež-Bogataj.

Članek je nastal na osnovi raziskav v okviru doktorske disertacije Tjaše Pogačar. Mentorica: prof. dr. Lučka Kajfež-Bogataj.

1 INTRODUCTION

Annual grass production varies widely, even under standard management conditions (Laidlaw, 2009). The considerable year-to-year and seasonal variation in grassland production is of major importance, as production systems must allow for the risk of unfavourable weather conditions (Trnka et al., 2006). The dependence of grassland herbage dry matter (DM) production on weather factors and their interaction with soil conditions, sward composition and management have been shown in many analyses (Trnka et al., 2006; Barrett et al., 2005; Čop, 1992).

Even individual variables important in the description of grassland growth like leaf area index (*LAI*) are strongly weather dependent. For example, when there are sufficient mineral nutrients in the soil, the development of the canopy (with $LAI < 4$) of a perennial ryegrass crop during regrowth after winter or after a cut in spring time, essentially depends on the temperature (Lambert et al., 1999).

Drought is one of the most important weather phenomena, having a major impact on grass sward growth and herbage yield. In contrast to majority field crops, grasses which constitute major part of seminatural grasslands are perennial plants and grow for several years. According to Tehnološka priporočila ... (2008), the consequences of severe droughts affect grassland sward productivity over the next years through the changes in the botanical composition of the sward, which is adapting to new growth conditions. This effect is long term and it is not obvious in monocultures, which are sown every few years. Another problem is that when rain returns after a period of drought precipitation may not be in excess of evapotranspiration so soil moisture content may not increase significantly (Laidlaw, 2009). So grassland sward makes use of periods when enough water is available and the abundant spring growth is often followed by summer hibernation. Laidlaw (2009) states that early summer droughts may not have a long term impact on yield.

In Slovenia, periods of drought are becoming increasingly problematic for forage production in summer months, especially on lighter soils (Dolničar, 2013). For example, in 2006 74 % of agricultural area damaged by drought was under permanent grasslands and pastures (Sušnik, 2006). According to climate change scenarios for Slovenia by the middle of the century (Prihodnje ... , 2014) we can expect continuous problems with drought stress due to higher air temperatures and, at least in the southern part of the country, lower summer precipitation rates.

Appropriate knowledge and understanding of the impact of climate variability on agricultural production is therefore essential for devising an adaptation strategy (Ceglar and Kajfež-Bogataj, 2012). From this point of view, crop modelling is very important for studies of the impacts of weather and climate on production. In this paper the work with the calibrated LINGRA-N model (Wolf, 2012), is described. The aim was to use the model for a long term simulation (50-year period) of the herbage dry matter (DM) yield of a grass monoculture, which brings the opportunity to observe the year-to-year variability and yield declines in years of drought. Furthermore, the growth analysis was undertaken with the intention of better understanding the interactions between growth components. This has an important role in grassland management science, as growth analyses of grass crop are rare in Slovenia, on the field or in the lab. Even if there is one, the experiment cannot be maintained for such a long period of time. Additionally, some variables of water balance were studied – their influence on the yield, its year-to-year variability or their development during average and dry years. The comparison was made with year-to-year variability of meteorological data for the central Slovenia (meteorological station Brnik).

2 METHODS AND DATA

The simulations were made with the LINGRA-N model, which was previously calibrated with herbage DM yield data for cock's foot (*Dactylis glomerata* L.) in Jablje from the experiment (KIS, 2014) that was performed in the periods 1998–2003 and 2008–2013. The average measured herbage DM yield for both periods together was 9525 $kg_{DM}\,ha^{-1}$ with the standard deviation of 1742 $kg_{DM}\,ha^{-1}$ (Figure 1). The performance of LINGRA-N was good, with *RMSE%* = 12 % and with the index of agreement (Willmott, 1982) *d* = 0.84 (Pogačar et al., 2015).

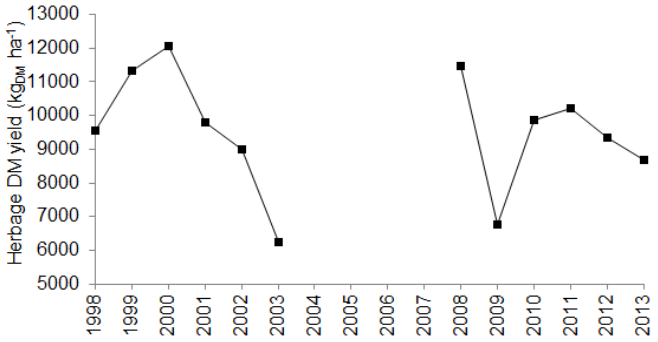


Figure 1: Average measured yearly herbage DM yield of cock's foot in Jablje for the periods 1998–2003 and 2008–2013 (data: KIS, 2014)
Slika 1: Povprečni izmerjen letni pridelek suhega zelinja navadne pasje trave v Jabljah za obdobji 1998–2003 in 2008–2013 (podatki: KIS, 2014)

2.1 Input data

The 50-year period of the simulation was set to 1964–2013 due to the availability of the meteorological data. For Jablje, the most representative meteorological station is Airport Ljubljana (Brnik). However, the distance of 12 km between the two brings some uncertainty to the modelling results, especially in the case of summer local convective events. The input for LINGRA-N includes daily data on minimum and maximum air temperatures (°C), precipitation (mm), mean wind speed ($m\,s^{-1}$), global radiation ($kJ\,m^{-2}$) and early morning vapour pressure (kPa), all obtained from the Slovenian Environment Agency (ARSO, 2014).

Air temperatures were lower at the beginning of the 50-year period (Figure 2, left) and so was global radiation (Figure 2, right). For the whole period, the average of average minimum daily air temperatures for the vegetation period (April-September) (*TminVP*) is 9.3°C, the average of average summer (June-August) minimum daily air temperatures (*TminS*) is 12.1°C, the average of average maximum daily air temperatures for the vegetation period (*TmaxVP*) is 22°C, and the average of average summer maximum daily air temperatures (*TmaxS*) is 25°C. In the second half of the period *TmaxS* dropped below this average in just seven years. *TmaxS* was extremely high in the years 2003, 2013, 2012, 1992 and 1983. It is clear that not only air temperatures but also their year-to-year variability are increasing. Something very similar holds true for the other presented air temperatures. However, the year-to-year variability of *TminVP* and *TminS* was higher in the first half of the period, due to a possibly non-climatic jump around the year 1978. Global radiation is increasing even more notably. Very high values were all reached after the year 2000: in 2011, 2003, 2009, 2000, 2007, 2012 and 2013.

The 50-year average of precipitation during the vegetation period (*RRvp*) is 734 mm, of which on average 396 mm fell in the summer time (*RRs*) (Figure 2, right). The decrease in precipitation is not obvious, but the variability increased in the second half of the 50-year period in both cases. There have lately been more years with low *RRvp* and especially with low *RRs*. *RRvp* was less than 500 mm in the years 1992, 2003, 1983 and 1993, while *RRs* was less than 250 mm in the years 1983, 1992, 2001, 2003, 2013 and 1993.

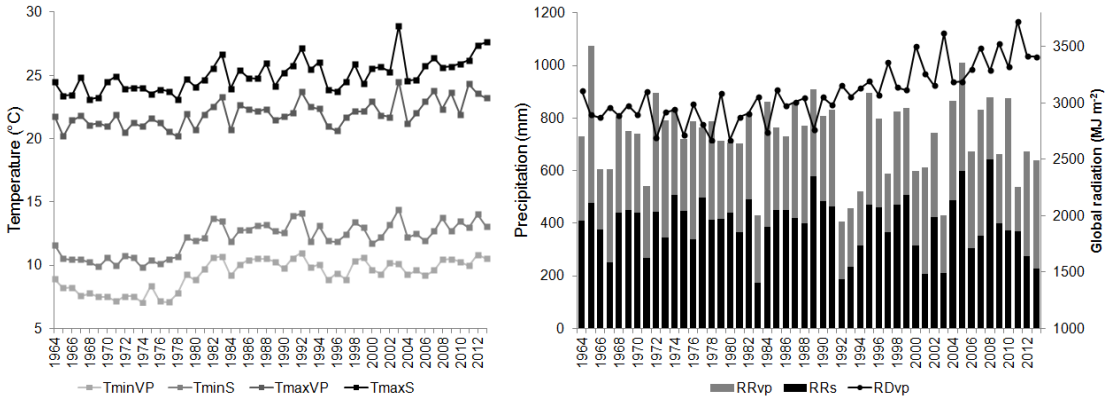


Figure 2: Left: Average minimum daily air temperature for the vegetation period (*TminVP*), average summer minimum daily air temperature (*TminS*), average maximum daily air temperature for the vegetation period (*TmaxVP*) and average summer maximum daily air temperature (*TmaxS*) in the period 1964–2013. Right: Precipitation during the vegetation period (*RRvp*), summer precipitation (*RRs*), and global radiation sum for the vegetation period (*RDvp*) in the period 1964–2013

Slika 2: Levo: Povprečna minimalna dnevna temperatura zraka za vegetacijsko obdobje (*TminVP*), povprečna poletna minimalna dnevna temperatura zraka (*TminS*), povprečna maksimalna dnevna temperatura zraka za vegetacijsko obdobje (*TmaxVP*) in povprečna poletna maksimalna dnevna temperatura zraka (*TmaxS*) v obdobju 1964–2013. Desno: Količina padavin v vegetacijskem obdobju (*RRvp*), poletna količina padavin (*RRs*) in vsota globalnega obsevanja v vegetacijskem obdobju (*RDvp*) v obdobju 1964–2013

The used soil type in Jablje is pseudogley-gley, deep and moderate, the texture is silty clay. The description can be found in Tajnšek (2003). Soil moisture content at saturation is 0.5 cm^3 cm^{-3}, soil moisture content at field capacity is 0.36 cm^3 cm^{-3} and soil moisture content at wilting point is 0.14 cm^3 cm^{-3}. The initial soil water content is set to field capacity (Pogačar et al., 2015). The rooted zone is changing with the growth of roots, every year from 30 to 40 cm. Four mowings are assumed and are set on fixed dates: 12 May, 1 July, 30 August and 17 October. The grass sward is fertilized on 1 April (60 kg_N ha^{-1}) and on the first day after the first (50 kg_N ha^{-1}) and the second (46 kg_N ha^{-1}) mowing.

Furthermore, calibrated crop and soil parameters are required as input. There are 27 of them, the most influential (Pogačar et al., 2015) are the thresholds for reductions of radiation use efficiency due to low minimum temperature (*TMNFTB = -3°C*) or high soil temperature (*TMPFTB = 25°C*), the leaf area index after mowing (*CLAI = 0.8* m^2 m^{-2}), the maximum light use efficiency (*RUETB = 2.6* g_{DM} MJ^{-1}_{PAR}), the fraction of precipitation lost by surface runoff (*RUNFR = 0.08*), the initial number of tillers (*TILLI = 7000* m^{-2}), the mineral soil nitrogen (N) available at the start of the growth period (*NMINS = 400* kg_N ha^{-1}), the fraction of total biomass to roots under stressed conditions (*FRT = 0.2*) and the recovery fractions of fertiliser N applications (*NRFTAB = 0.7*).

2.2 Overview of output variables in the LINGRA-N model

From each simulation run two output files are obtained. One gives the daily results (as model time step is 1 day) for each simulated year (Table 1). The other contains yearly cumulative or average (depending on the characteristics of the variable) values for most of the variables (exceptions are marked grey in Table 1).

Table 1: Output variables of LINGRA-N simulated for each day (DM: dry matter, N: nitrogen). Variables for which model does not calculate yearly cumulative or average values are marked grey

Preglednica 1: Izhodne spremenljivke modela LINGRA-N, simulirane za vsak dan (DM: suha snov, N: dušik). S sivo so označene spremenljivke, za katere model ne izračuna letnih povprečij oz. vsot

Variable	Unit	Description
Water balance variables		
DRAIN	mm	cumulative drainage
ESOIL	mm	cumulative soil evaporation
IRR	mm	cumulative irrigation
RAIN	mm	cumulative precipitation
RUNOF	mm	cumulative runoff
SMACT	$cm^3\ cm^{-3}$	actual soil moisture content in rooted zone
WAVT	mm	available water in rooted zone
WTOT	mm	water in rooted zone
TRANS	mm	cumulative crop transpiration
Variables based on nitrogen		
NLIV	$kg_N\ ha^{-1}$	amount of N in living crop organs
NLOSS	$kg_N\ ha^{-1}$	N loss in dead crop organs and cut grass
NMIN	$kg_N\ ha^{-1}$	amount of organic N potentially available by mineralization from the soil
NMINT	$kg_N\ ha^{-1}$	mineral N directly available from soil and fertiliser
NNI	/	nitrogen nutrition index (range 0-1)
NUPT	$kg_N\ ha^{-1}$	N uptake by crop from soil
Crop variables		
DVS	-	development stage
LAI	$m^2\ m^{-2}$	leaf area index
PAR	$MJ\ m^{-2}d^{-1}$	daily amount of photosynthetically active radiation
PARAB	$MJ\ m^{-2}d^{-1}$	daily amount of PAR as intercepted by the crop canopy
TILLER	m^{-2}	number of tillers
TRANRF	/	reduction factor for crop growth due to drought/wetness (range 0-1)
WLVD	$kg_{DM}\ ha^{-1}$	mass of dead leaves in the field
WLVG	$kg_{DM}\ ha^{-1}$	mass of green leaves in the field
WRE	$kg_{DM}\ ha^{-1}$	mass of reserves (storage carbohydrates)
WRT	$kg_{DM}\ ha^{-1}$	roots mass
TSUML	°C	temperature sum from emergence
TADRW	$kg_{DM}\ ha^{-1}$	mass of green and dead leaves in the field plus herbage DM yield
GRASS	$kg_{DM}\ ha^{-1}$	herbage DM yield
YIELD	$kg_{DM}\ ha^{-1}$	mass of harvestable leaves in the field plus herbage DM yield

In the second output file there are also yearly values of nitrogen use efficiency (*NUE*, $kg_{DM}\ kg^{-1}N$), radiation use efficiency (*RUE*, $g_{DM}\ MJ^{-1}_{PAR}$) and water use efficiency (*WUE*, $g_{DM}\ kg^{-1}_{water}$).

In this paper some of the simulated variables are studied. In the first place the herbage DM yield of grassland (*GRASS*) and the potential yield (*YIELD*), in connection with input weather variables and the reduction factor for crop growth due to drought (*TRANRF*) on a yearly basis. The dependence of *RUE* on *TRANRF* is shown. To further describe the water status, the yearly development of the actual soil moisture content in the rooted zone (*SMACT*) is examined during a dry year (1993) and an average year (1994), in which *GRASS* is very close to the average *GRASS* for the whole period. Also, the daily amount of photosynthetically active radiation (*PAR*) and the daily amount of *PAR* as intercepted by the grass crop canopy (*PARAB*) are compared with each other in the two years. This kind of a comparison is also made for the variables of the daily growth of the grass crop like the mass of green leaves (*WLVG*), the roots mass (*WRT*) and the mass of dead leaves (*WLVD*). Furthermore, the leaf area index (*LAI*) progress during dry and average years is presented.

To better understand the simulation of those variables there is a short description based on Wolf (2012) of how they are calculated in the LINGRA-N model (sections 2.3 to 2.5).

2.3 Growth variables

Growth variable *PARAB* ($MJ\ m^{-2}d^{-1}$) is calculated as the daily amount of incoming *PAR* ($MJ\ m^{-2}d^{-1}$) times the fractional light interception:

$$PARAB = PAR(1 - e^{-K_{DIF} \cdot LAI}) \quad (1)$$

where K_{DIF} is the extinction function for visible incoming radiation with the calibrated value of 0.6.

The daily assimilate production of the crop (*GTWSO*$_1$, $kg_{DM}\ ha^{-1}d^{-1}$) is dependent on *PARAB*, *RUE*, correction factors for temperature, high radiation levels, and atmospheric CO_2, and reduction factors for water and N stress (via *TRANRF* and nitrogen nutrition index *NNI*). The sum of *GTWSO*$_1$ and the available amount of reserves (*WRE*, $kg_{DM}\ ha^{-1}$) is labeled as *GTWSO*$_2$ ($kg_{DM}\ ha^{-1}d^{-1}$).

The sink limited increase in leaf area (*GLAISI*, $ha\ ha^{-1}d^{-1}$) is calculated from the number of tillers and the leaf elongation rate. The sink limited increase in total biomass (*GTWSI*, $kg_{DM}\ ha^{-1}d^{-1}$) is calculated as

$$GTWSI = \frac{GLAISI}{SLA \cdot (1 - FRT)} \quad (2)$$

where *SLA* (with the calibrated value of 0.0025 $ha\ kg^{-1}_{DM}$) is a specific leaf area and *1-FRT* (with the calibrated value of 0.8) is the above ground allocation fraction.

The actual grass growth may switch between sink and source limited growth limitation. If *GTWSO*$_2$ > *GTWSI*, the growth rate (*GTW*, $kg_{DM}\ ha^{-1}d^{-1}$) is equal to *GTWSI* and the additional amount of assimilates results in an increase in reserves. If $GTWSO_2 \leq GTWSI$ then *GTW* is equal to *GTWSO*$_2$. The increase in leaf mass (*GLV*, $kg_{DM}\ ha^{-1}d^{-1}$) is calculated from the total growth rate *GTW* and the partitioning factor (*1-FRT*), to determine the mass of green leaves (*WLVG*, $kg_{DM}\ ha^{-1}$). With the *FRT* factor the roots mass is obtained (*WRT*, $kg_{DM}\ ha^{-1}$). The daily increase in *LAI* (*GLAI*, d^{-1}) is simulated as

$$GLAI = GLV \cdot SLA, \quad (3)$$

with *SLA* as in (2).

Furthermore, the relative death rates of the leaves (*RDR*, d^{-1}) due to N shortage (with *NNI* < *1*; *RDR*$_n$, d^{-1}) and due to ageing as dependent on the mean daily temperature (*RDR*$_{tb}$, d^{-1}), due to shading (with high *LAI* values; *RDR*$_{sh}$, d^{-1}) or due to drought (as dependent on *TRANRF*; *RDR*$_{dr}$, d^{-1}) are determined:

$$RDR = RDR_n + \max(RDR_{tb}, RDR_{sh}, RDR_{dr}). \quad (4)$$

Next, the death rate of leaves (*DLV*, $kg_{DM}\ ha^{-1}d^{-1}$) is calculated from *RDR*, followed by the calculation of the mass of the dead leaves (*WLVD*, $kg_{DM}\ ha^{-1}$). The decrease in *LAI* (*DLAI*, d^{-1}) is calculated practically in the same way as the leaf death rate. Only to allow regrowth after, for example, a period of severe drought stress, *LAI* ($m^2\ m^{-2}$) remains during the growth period always at least on the value of predefined *CLAI*. The change in the leaf area (*RLAI*, d^{-1}) is equal to *GLAI* minus *DLAI*

2.4 Herbage DM yield, potential yield and crop efficiency

For the calculation of the herbage DM yield (*GRASS*, $kg_{DM}\ ha^{-1}$), the harvestable leaf mass (*HRVBL*, $kg_{DM}\ ha^{-1}$) has to be determined first. It is equal to the green leaf mass in the field (*WLVG*) minus the leaf mass that remains in the field after mowing:

$$HRVBL = WLVG - \frac{CLAI}{SLA} \quad (5)$$

where *CLAI* is the leaf area index after mowing (0.8 $m^2\ m^{-2}$) and *SLA* is as in (2). *GRASS* increases at every mowing by the value of *HRVBL* on the mowing day. Potential yield (*YIELD*, $kg_{DM}\ ha^{-1}$) is determined by the equation

$$YIELD = GRASS + HRVBL. \quad (6)$$

For the crop efficiency simulations another variable *TADRW* ($kg_{DM}\ ha^{-1}$) is determined as

$$TADRW = GRASS + WLVG + WLVD. \quad (7)$$

It presents the mass of green and dead leaves in the field together with the herbage DM yield. Radiation use efficiency (*RUE*, $g_{DM}\ MJ^{-1}{}_{PAR}$) is derived at the end of the growth period from *TADRW* divided by the total intercepted solar radiation during the growth period. The calculation of water use efficiency (*WUE*, $g_{DM}\ kg^{-1}{}_{water}$) is similar: at the end of the growth period *TADRW* is divided by the total water amount used by evapotranspiration during the growth period.

2.5 Water balance

LINGRA-N calculates evapotranspiration and water balance in the same way as the WOFOST model (Supit and Van der Goot, 2003). The processes directly affecting the root zone soil moisture content are percolation, surface runoff, infiltration, crop transpiration and soil evaporation. The actual soil moisture content (*SMACT*, $cm^3\ cm^{-3}$) can be established according to Driessen (1986 op. cit. Supit and Van der Goot, 2003):

$$SMACT = \frac{IN_{up} + (IN_{low} - T_a)}{RD} \Delta t \quad (8)$$

where the rate of net influx through the upper root zone boundary (IN_{up}, $cm\ d^{-1}$) is

$$IN_{up} = P + I_e - E_s - SR \quad (9)$$

and the rate of net influx through the lower root zone boundary (IN_{low}, $cm\ d^{-1}$) is

$$IN_{low} = -PERC \quad (10)$$

and T_a ($cm\ d^{-1}$) is the calculated actual transpiration rate of crop, *RD* (cm) the calculated actual rooting depth, Δt the determined time step (1 d), *P* ($cm\ d^{-1}$) input daily precipitations, I_e ($cm\ d^{-1}$) from input recalculated effective daily irrigation (*not used – it is not a common practice to irrigate grass swards)*, E_s ($cm\ d^{-1}$) the calculated soil evaporation rate, *SR* ($cm\ d^{-1}$) the calculated rate of surface runoff and *PERC* ($cm\ d^{-1}$) the calculated percolation rate.

The method, introduced by Penman (1956, 1948 op. cit. Supit and Van der Goot, 2003) and adapted according to Choisnel et al. (1992 op. cit. Supit and Van der Goot, 2003), is used for daily totals of canopy transpiration and soil evaporation and is described in Supit and Van der Goot (2003). The reduction of the grass growth rate and the transpiration rate due to drought stress is calculated via:

$$TRANRF = T_a / T_p = \frac{SMACT - SMW}{SMCR - SMW} \quad (11)$$

where T_a and *SMACT* are defined as in (8), T_p ($cm\ d^{-1}$) is the potential transpiration rate of crop, *SMW* ($cm^3\ cm^{-3}$) soil moisture content at wilting point and *SMCR* ($cm^3\ cm^{-3}$) critical soil moisture content. *SMCR* is defined as the quantity of stored soil moisture below which water uptake is impaired and the plant closes its stomata. *TRANRF* affects *RUE* and the growth rate of the crop, the leaf death rate and the distribution of assimilates to the roots.

3 RESULTS AND DISCUSSION

As previously mentioned, there is great year-to-year variation of grassland herbage DM yields. Coefficient of variation for experimental herbage DM yield data in Jablje is 18 %. For instance, measured annual grassland herbage DM yields in Austria tend to vary within ±10-20 %, but during some years (e.g. 2003) these deviations can be much greater (Schaumberger et al., 2007). In the period 1995–2004, the average coefficient of variation for experimental grassland herbage DM

yields in France was about 16 % (Smit et al., 2008).

The simulated *GRASS* (Figure 3) has about the same variability throughout the 50-year period, however, in the second half outliers start to appear, which can be alarming in terms of the negative effect of climate change.

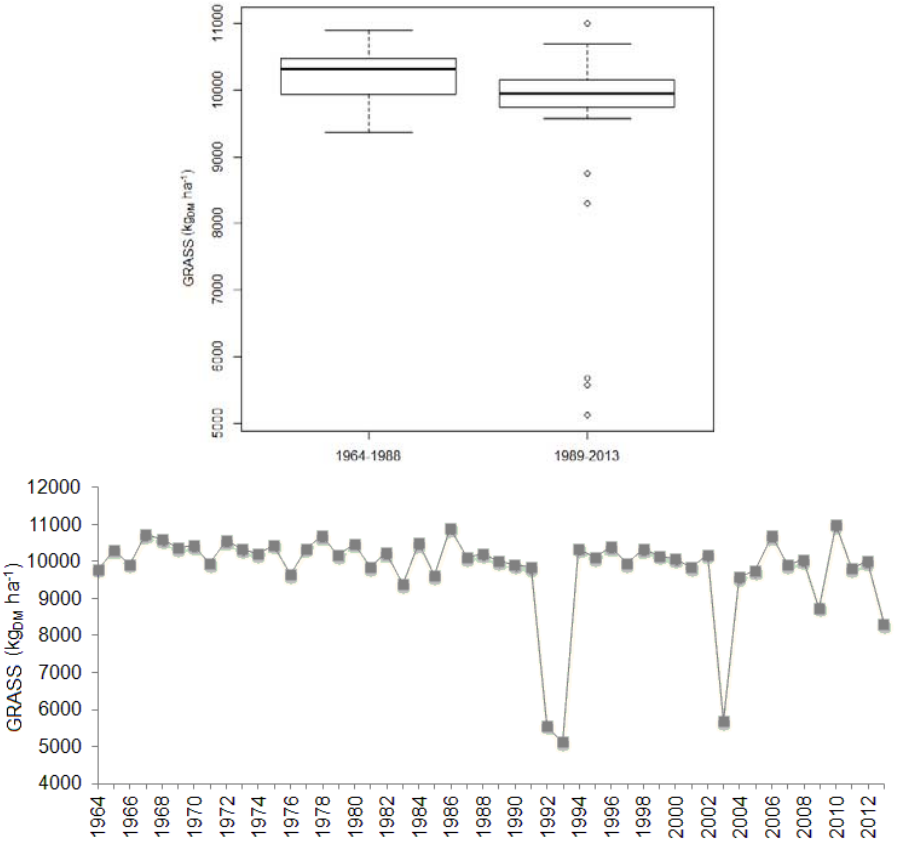


Figure 3: Upper: Boxplots of simulated yearly herbage DM yield (*GRASS*) of cock's foot in Jablje for the first (1964–1988) and the second (1989–2013) half of the 50-year period. Lower: Simulated yearly herbage DM yield (*GRASS*) of cock's foot in Jablje for the whole period 1964–2013

Slika 3: Zgoraj: Okvirja z ročaji za simuliran letni pridelek suhega zelinja (*GRASS*) navadne pasje trave v Jabljah v prvi (1964–1988) in drugi (1989–2013) polovici obravnavanega 50-letnega obdobja. Spodaj: Simuliran letni pridelek suhega zelinja (*GRASS*) navadne pasje trave v Jabljah za celotno obdobje 1964–2013

The biggest reductions in the simulated herbage DM yield are seen in the years 1992, 1993 and 2003 (approximately 4 t $ha^{-1}year^{-1}$). As it is seen in Figure 2, these are also years with very low precipitation in the summer and in the vegetation period. Only 47 % of average summer precipitation for the period 1964–2013 was measured in 1992, 53 % in 2003 and 59 % in 1993. For the vegetation period proportions were a little higher, 55, 59 and 62 %, respectively. Also, in the years 1992 and 2003 extremely high minimum and maximum daily air temperature averages were recorded for both the summer and the vegetation period. As regards global radiation, it was extremely high in the vegetation period of 2003. Altogether, it is clear that the *GRASS* reductions were due to drought conditions.

Sušnik and Pogačar (2010) studied indicators like the number of dry days and soil moisture deficit to define drought years for grass sward in six locations across Slovenia for the period 1973–2009, and compared them to drought reports published in Agrometeorological bulletins, which can be found in the archive of the Slovenian Environment Agency. In years 1992, 1993 and

2003 the most intense and the longest droughts were detected in all locations, which correspond to the simulation results.

Furthermore, the observed connections led to the testing of *YIELD* dependence on weather variables. *YIELD* was used in this case instead of *GRASS* to avoid a direct influence of the mowing dates on the final result. Among all input weather variables, calculated as the average or sum for the summer and for the vegetation period, there is none linearly related to *YIELD*. However, it can be again seen (Figure 4) that very low *YIELD* is connected to very high (maximum) air temperatures and very low precipitation. Smit *et al.* (2008) claim the grass sward production in Europe to be strongly correlated with the annual precipitation and less with the annual temperature sum or the length of the growth period. On the other hand, the 20-year experiment on permanent grassland in Ljubljana also showed only very small positive correlation between the annual precipitation and the herbage DM yield (Lekšan, 1995).

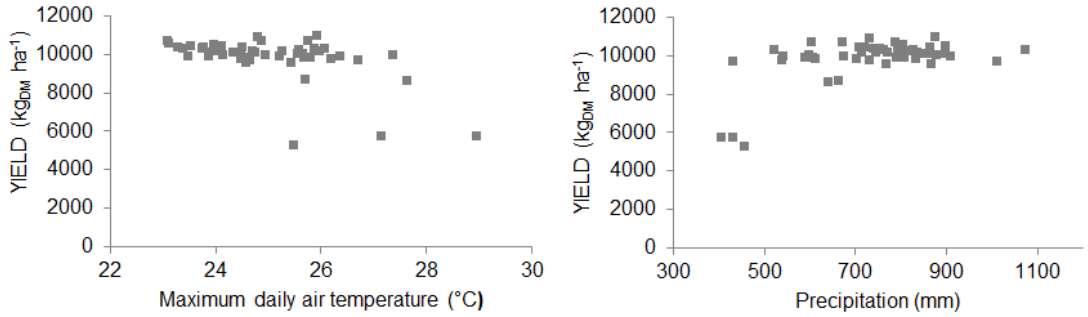


Figure 4: Scatterplots of the potential yield (*YIELD*) versus the summer average of maximum daily air temperature (left) and *YIELD* versus the vegetation period sum of precipitation (right) for cock's foot in Jablje for the whole period 1964–2013

Slika 4: Razsevna diagrama, ki prikazujeta potencialni pridelek (*YIELD*) v odvisnosti od poletnega povprečja maksimalne dnevne temperature zraka (levo) in v odvisnosti od količine padavin v vegetacijskem obdobju (desno) za navadno pasjo travo v Jabljah za celotno obdobje 1964–2013

The given years with the lowest *GRASS* were also the years with the lowest *TRANRF* (Figure 5). As *TRANRF* is the model's measure of drought conditions, these were detected as the driest years in the simulation. Also, in the years 1971, 1983, 1994, 2001, 2006, 2007, 2011, 2012 and 2013 *TRANRF* fell under 0.95, denoting dry years.

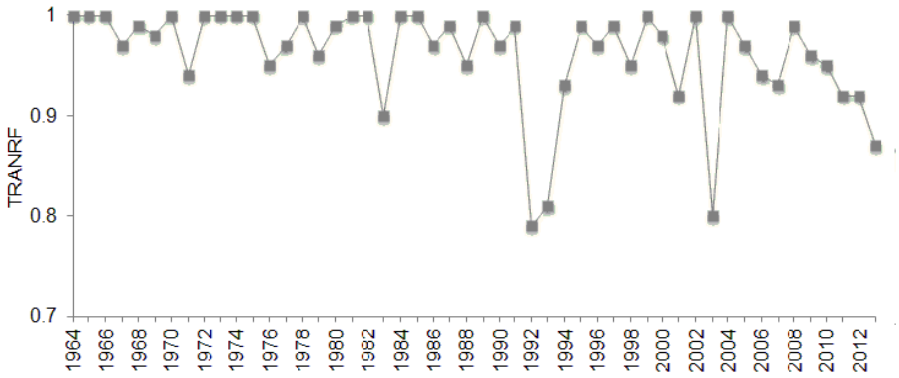


Figure 5: Simulated reduction factor for crop growth due to drought (*TRANRF*) for cock's foot in Jablje for the whole period 1964–2013

Slika 5: Simuliran faktor zmanjšanja rasti zaradi suše (*TRANRF*) navadne pasje trave v Jabljah za celotno obdobje 1964–2013

Smit *et al.* (2008) also claim that are herbage DM yields especially affected by droughts. Also similar as in our case, in Ireland, herbage DM yield reductions of 1.4 to 4.0 t $ha^{-1}year^{-1}$ have been estimated to be lost for intensively managed grassland in the driest regions due to limiting soil moisture availability (Brereton and Keane, 1982 op. cit. Laidlaw, 2009).

Drought stress has a major influence on *RUE* (Bonesmo and Belanger, 2002). This can be seen in Jablje as the course of *RUE* is very similar to the course of *TRANRF* (Figure 6). For *RUE* versus *TRANRF* (not presented) the coefficient of determination is $r^2 = 0.84$, which means that 84% of *RUE* variability can be explained with the changing *TRANRF*.

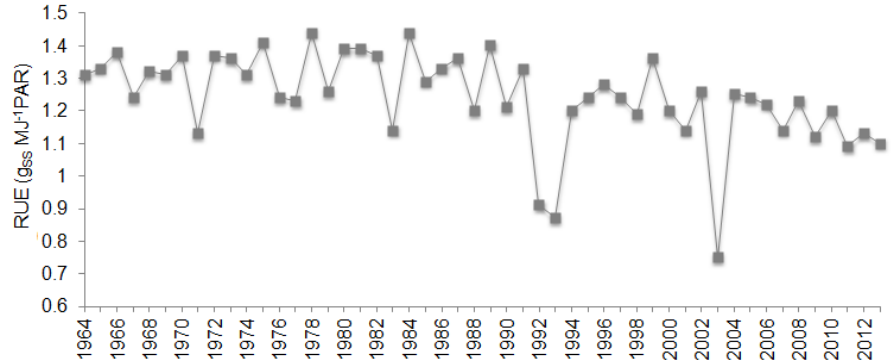


Figure 6: Simulated radiation use efficiency (*RUE*) of cock's foot in Jablje for the whole period 1964–2013
Slika 6: Simulirana učinkovitost izrabe sončnega obsevanja (*RUE*) navadne pasje trave v Jabljah za celotno obdobje 1964–2013

Water status can be also monitored as actual soil moisture content on a daily scale with variable *SMACT*. Figure 7 (upper right) shows a pattern of *SMACT* in the dry year of 1993 and in the average year of 1994. In 1993 *SMACT* stayed on a very low level from the beginning of May to the end of the August, while in 1994 it only fell to this level twice in the whole year. This is reflected very strongly in other variables. *YIELD* (Figure 7, upper left) was not increasing at all in the dry period of 1993, the same happened in 2003. In contrast, for example in the years 1994 and 2010 *YIELD* was increasing almost steadily throughout the vegetation period, only a little more slowly in the summer time. Naturally, *YIELD* depends on *LAI* (Figure 7, lower left), which remained under 2 $m^2 m^{-2}$ during the dry period of 1993. In 1994 *LAI* was below this value just at the beginning and at the end of the year, and on mowing days (four extreme falls of *LAI* can be seen). Otherwise it rose as high as 5 to 9 $m^2 m^{-2}$.

Cumulative *PARAB* in Jablje was 1100 MJ_{PAR} $m^{-2}year^{-1}$ in the dry year of 1993, which is 53 % of *PAR*, and 1650 MJ_{PAR} $m^{-2}year^{-1}$ in 1994, which is 75 % of *PAR* (Figure 7, lower right). For example, in the research of Wolf (2006), who made simulations of rye grass growth with LINGRA for five years for optimal water and nutrient supply, *YIELD* appears to increase from Wageningen (The Netherlands) to Bologna (Italy) to Sevilla (Spain). He claims this was caused by the length of the growing season and by cumulative *PARAB*, which increased for the three locations from 1200–1600 MJ_{PAR} $m^{-2}year^{-1}$ to 1700–2000 MJ_{PAR} $m^{-2}year^{-1}$ and 2700–2800 MJ_{PAR} $m^{-2}year^{-1}$, respectively.

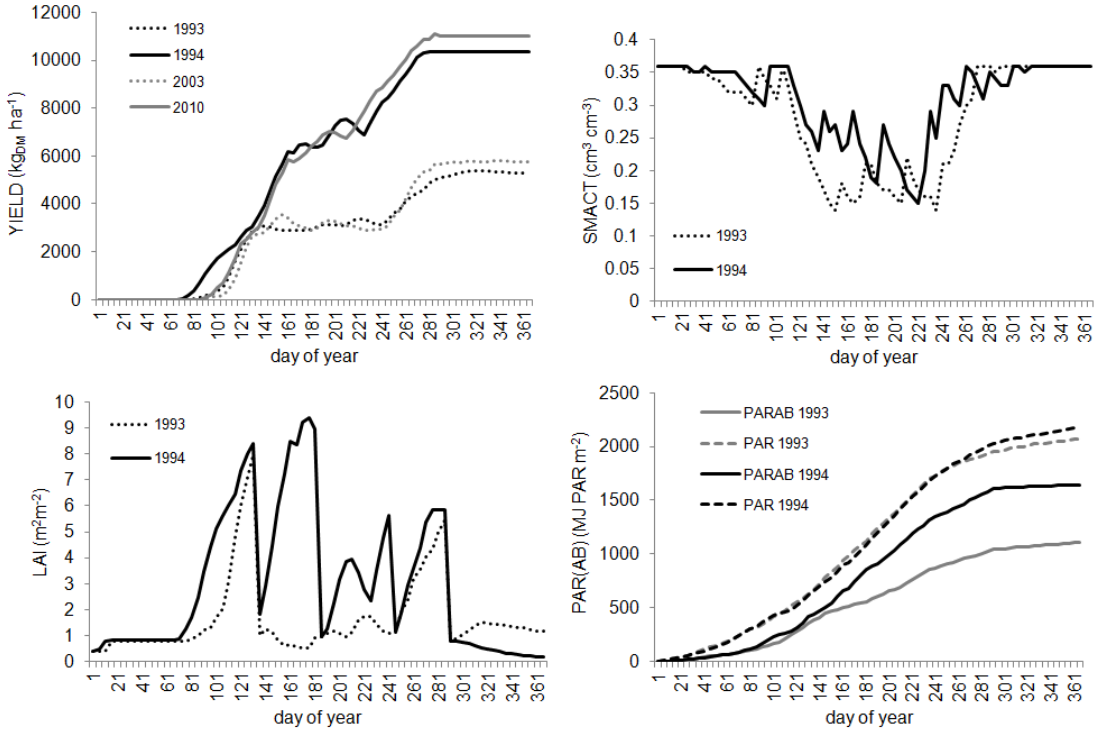


Figure 7: Simulated potential yield (*YIELD*, upper left), soil moisture content (*SMACT*, upper right), leaf area index (*LAI*, lower left), cumulative amount of photosynthetically active radiation (*PAR*) and cumulative amount of *PAR* as intercepted by the crop canopy (*PARAB*) (lower right) of cock's foot in Jablje in the dry year of 1993 and the average year of 1994 (*YIELD* also in 2003 and 2010)

Slika 7: Simuliran potencialni pridelek (*YIELD*, zgoraj levo), vsebnost vode v tleh (*SMACT*, zgoraj desno), indeks listne površine (*LAI*, spodaj levo) ter kumulativno fotosintetsko aktivno sevanje (*PAR*) in prestreženo fotosintetsko aktivno sevanje (*PARAB*) (spodaj desno) za navadno pasjo travo v Jabljah za suho leto 1993 in povprečno leto 1994 (*YIELD* tudi za leti 2003 in 2010)

According to Wolf (2006), the increase in *PARAB* results in a higher *YIELD* and in a much higher *WLVD*, because the higher biomass production results on average in a higher *LAI* and thus in more leaf senescence due to self-shading. The same can be said for the simulations in Jablje (Figure 7, Figure 8).

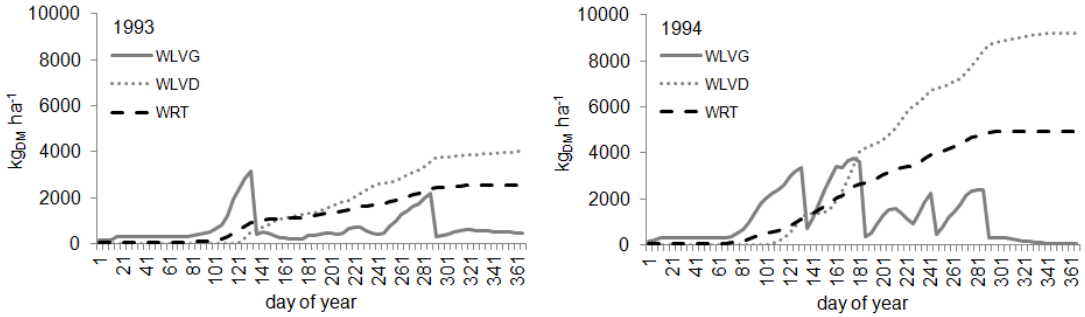


Figure 8: Simulated mass of green leaves (*WLVG*), mass of dead leaves (*WLVD*) and roots mass (*WRT*) of cock's foot in Jablje in the dry year 1993 (left) and in the average year of 1994 (right)

Slika 8: Simulirana masa zelenih listov (*WLVG*), masa odmrlih listov (*WLVD*) in masa korenin (*WRT*) navadne pasje trave v Jabljah v suhem letu 1993 (levo) in v povprečnem letu 1994 (desno)

As expected from the definition, the shape of the *WLVG* curve is the same as the shape of the *LAI* curve, due to constant *SLA*. In 1993, during the long summer drought *WLVG* was almost 0 all the time, so two intermediate mowings cannot be seen (Figure 8, left). On the other hand, four mowings are clearly seen in four extreme decreases of *WLVG* in 1994 (Figure 8, right). Because of the drought, roots also grew very slowly in 1993 and at the end of the growth period reached only half of the *WRT* that was reached at the end of 1994. What is more, the mass of dead leaves (*WLVD*) in 1993 was only 44 % of the *WLVD* in 1994, due to low available green biomass.

4 CONCLUSIONS

Fundamentally, this research shows the value of applying the calibrated LINGRA-N model for a 50-year (1964–2013) herbage yield simulation and growth analysis. It provides insights in the interactions between several weather and crop variables or their seasonal development and herbage yield variability. It is important to have an opportunity to better understand the growth components, and simulations can reveal their dynamics and impacts on herbage yield, based on weather and soil conditions. Overall, crop models are a very useful and important tool for this kind of research.

As regards the simulated herbage DM yield (*GRASS*), recent changes in its variability are reflected in the appearance of outliers in the second half of the study period. The biggest reductions in *GRASS* were detected in the years 1992, 1993 and 2003. These years were also recognised as years with very low precipitation and very high minimum and maximum daily air temperature averages in the summer and in the vegetation period, so we were able to conclude that the *GRASS* reductions were due to drought conditions. The given years with the lowest *GRASS* were also the years with the lowest reduction factor for crop growth due to drought (*TRANRF*). As the latter is the model's measure of drought conditions, these were detected as the driest years in the simulation, too.

Radiation use efficiency variability was strongly dependent on *TRANRF*. Drought had a major impact on the cumulative amount of *PAR* as intercepted by the crop canopy (*PARAB*), which reached just 53 % of *PAR* in the dry year of 1993. Seasonal development of the actual soil moisture content (*SMACT*) was linked to the development of the leaf area index and consequently to the mass of green leaves, to the roots mass, to the mass of dead leaves and to *GRASS*.

However, some of the obtained results remain indicative without confirmation of the simulated values through field measurements. Angulo *et al.* (2013) also recommend that future work should focus on obtaining more comprehensive, high quality data allowing application of improved methods for model calibration. For modelling it would be of great importance to plan grassland field experiments multiple years in advance that would, in addition to measurements of herbage yield, include measurements of variables or parameters like leaf area index, specific leaf area, leaf appearance rate, tiller density or mass of green leaves. Measurements of soil moisture content would also be useful. Further important factors include the vicinity of the meteorological station, the availability of soil data and, possibly, swards to be one to two years old. Naturally, this would be a major project with a great need of financial support.

The results highlight the need for further research, on field and with simulations. As regards the latter, we have to keep in mind that they inevitably involve various uncertainties. These uncertainties originate from input (meteorological, soil, management) data, from calibrated (for a specific period) model parameters, from model structure and concept. In order to identify potential problems caused by seasonal weather variability, which is increasing due to climate change, and to objectively assess its impact on the grassland production, it is necessary to perform various different simulations. For Slovenia, it would be of greater importance to make such simulations for permanent grasslands, but as for now the calibration has not been successful (Pogačar et al., 2015) we need to first obtain results for various grass monocultures and various locations, and try to proceed from there.

5 REFERENCES

ARSO. 2014. Slovenian Environment Agency (http://www.arso.gov.si/): Climatological data - database output

Angulo C., Rötter R., Lock R., Enders A., Fronzek S., Ewert F. 2013. Implication of crop model calibration strategies for assessing regional impacts of climate change in Europe. Agricultural and Forest Meteorology, 170: 32-46, doi: 10.1016/j.agrformet.2012.11.017

Barrett P.D., Laidlaw A.S., Mayne C.S. 2005. GrazeGro: a European herbage growth model to predict pasture production in perennial ryegrass swards for decision support. European Journal of Agronomy, 23: 37-56, doi: 10.1016/j.eja.2004.09.006

Bonesmo H., Belanger G. 2002. Timothy yield and nutritive value by the CATIMO model; I. Growth and nitrogen. Agronomy Journal, 94: 337-345, doi: 10.2134/agronj2002.0337

Ceglar A., Kajfež-Bogataj L. 2012. Simulation of maize yield in current and changed climatic conditions: addressing modelling uncertainties and the importance of bias correction in climate model simulations. European Journal of Agronomy, 37, 1: 83-95, doi: 10.1016/j.eja.2011.11.005

Čop J. 1992. Sezonska razporeditev rasti zelinja štirih vrst trav avtohtonega izvora v predalpskem območju Slovenije. Master thesis. Ljubljana, University of Ljubljana, Biotechnical Faculty, Department for Agronomy: 82 p.

Dolničar J. 2013. Poznavanje travne ruše za gospodarno pridelovanje krme. Glas dežele, junij 2013. http://www.glasdezele.si/articles/2013/poznavanje-travne-ru%C5%A1e-za-gospodarno-pridelovanje-krme (16. 10. 2013)

KIS. 2014. Agricultural Institute of Slovenia (http://www.kis.si/): herbage yield data of grass monocultures - database output

Laidlaw A.S. 2009. The effect of soil moisture content on leaf extension rate and yield of perennial ryegrass. Irish journal of agricultural and food research, 48: 1-20

Lambert R., Peeters A., Toussaint B. 1999. LAI evolution of a perennial ryegrass crop estimated from the sum of temperatures in spring time. Agricultural and Forest Meteorology, 97: 1-8, doi: 10.1016/S0168-1923(99)00059-3

Lekšan D. 1995. Vpliv vremena na pridelek različno gnojenega travnika. Graduation thesis. Ljubljana, University of Ljubljana, Biotechnical Faculty, Department for Agronomy: 34 p.

Pogačar T., Ipavec D., Verbič J., Kajfež-Bogataj L. 2015. Calibration of the LINGRA-N model to simulate herbage yield of grass monocultures and permanent grassland in Slovenia. Acta agriculturae Slovenica, 105, 1: 111-123, doi: 10.14720/aas.2015.105.1.12

Prihodnje spremembe podnebja v Sloveniji. 2014. Ljubljana, Ministry of the environment and spatial planning, Slovenian environment agency: 3 p. http://meteo.arso.gov.si/uploads/probase/www/climate/PSS/scenariji/podnebni_scenariji.pdf (21. 5. 2015)

Schaumberger A., Trnka M., Eitzinger J., Formayer H., Bartelme N. 2007. Agrometeorological monitoring of Austrian grasslands using GIS based modeling. Geophysical Research Abstracts, 9, poster: 3 p. http://meetings.copernicus.org/www.cosis.net/abstracts/EGU2007/10449/EGU2007-J-10449.pdf (20. 1. 2014)

Smit H.J., Metzger M.J., Ewert F. 2008. Spatial distribution of grassland productivity and land use in Europe. Agricultural Systems, 98: 208-219, doi: 10.1016/j.agsy.2008.07.004

Supit I., Van der Goot E. (eds.). 2003. Updated system description of the WOFOST crop growth simulation model as implemented in the CGMS applied by the European Commission. Joint Research Centre of the European Commission, Luxembourg: http://www.treemail.nl/download/treebook7/index.htm

Sušnik A. 2006. Vodni primanjkljaj v Sloveniji in možni vplivi podnebnih sprememb. Master thesis. Ljubljana, University of Ljubljana, Biotechnical Faculty, Department for Agronomy: 147 p.

Sušnik A., Pogačar T. 2010. Modeliranje vodne bilance travinja kot orodje pri analizi suše v obdobju 1973–2009. In: Novi izzivi v poljedelstvu 2010. Rogaška Slatina, 2.-3. 12. 2010. Kocjan Ačko D., Čeh B. (eds.). Ljubljana, Slovensko agronomsko društvo: 299-306

Tajnšek A. 2003. Deset let trajnih poskusov IOSDV v Sloveniji, Jable in Rakičan 1993–2003. In: Namen in cilj trajnih poljskih poskusov IOSDV Jable in IOSDV Rakičan. Žalec, 12. 12. 2003. Tajnšek A., Čeh Brežnik B., Kocjan Ačko D. (eds.). Proceedings of the conference, Slovensko agronomsko društvo: 7-24

Tehnološka priporočila za zmanjšanje občutljivosti kmetijske pridelave na sušo: poljedelstvo, travništvo, zelenjadarstvo in hmeljarstvo. 2008. Ljubljana, Ministry of Agriculture, Forestry and Food: 44 p. http://www.arsktrp.gov.si/fileadmin/arsktrp.gov.si/pageuploads/Aktualno/Aktualno/2013/Tehnoloska_priporocila_za_zmanjsanje_obcutljivosti_na_suso.pdf (18. 11. 2013)

Trnka M., Eitzinger J., Gruszczynsk G., Buchgraber K., Resch R., Schaumberger A. 2006. A simple statistical model for predicting herbage production from permanent grassland. Grass and forage science, 61: 253-271, doi: 10.1111/j.1365-2494.2006.00530.x

Willmott C.J. 1982. Some comments on the evaluation of model performance. Bulletin of the American Meteorological Society, 63: 1309-1313, doi: 10.1175/1520-0477(1982)063<1309:SCOTEO>2.0.CO;2

Wolf J. 2006. Grassland data from PASK study and testing of LINGRA in CGMS. ASEMARS Project report no. 2. Wageningen, Alterra: 38 p. http://models.pps.wur.nl/sites/models.pps.wur.nl/files/LINGRA-PASK_report_Wolf-2006.pdf (8. 1. 2010)

Wolf J. 2012. LINGRA-N: Simple generic model for simulation of grass growth under potential, water limited and nitrogen limited conditions. User guide for LINGRA-N. Wageningen, Wageningen University: 65 p. http://www.wageningenur.nl/en/Publication-details.htm?publicationId=publication-way-343434373232 (28. 3. 2013)

COBISS Code 1.01

DOI: 10.14720/aas.2015.105.2.12
Agrovoc descriptors: soybeans, glycine max, weed control, *Abutilon theophrasti,* spacing, population dynamics, seed production, biological comptition

Agris category code: h01, h60, f03

Study of relationship between soybean (*Glycine max* (L.) Merr.)planting spatial arrangements and velvetleaf (*Abutilon theophrasti* L.) population dynamic

Hamid SALEHIAN[1*], Maryam NAJAFIAN[1]

Received July 20, 2015; accepted September 02, 2015.
Delo je prispelo 20. julija 2015, sprejeto 02. septembra 2015.

ABSTRACT

The velvetleaf is an important annual weed in the Mazandaran province, Iran. Seeds are the only way of propagation and renewal of this weed. More knowledge was gained regarding soil seed bank and its seed production to improve the management of the velvetleaf weed in the future. There is a minimal information concerning the impact of the soybean planting pattern on the dynamics of the velvetleaf population. For this reason two different fields have been studied with the cooperation of the Agriculture Discipline of the Islamic Azad University, in Qaemshahr, Iran, during 2009 and 2012. In this study the effect of two types of soybean row spacing were used, 50 cm-wide and 36 cm - narrow, and three emergences of the velvetleaf weed population (periods 0-10, 10-20 and 20-30 days after soybean sowing were implemented) were studied. Seed production, leaf area and dry matter increased in each plant population of the velvetleaf weed in the 50-cm soybean rows. Mortality rates were decreased in velvetleaf's seedling population in the wider spaced rows. By observation it was seen an increased production of seeds in the first batch of seedlings. It appears that we must remove the first weed emergence flushes within three to four weeks after the soybean emergence to prevent reduced yields in the soybean crop and further increase of the velvetleaf seed bank.

Key words: row spacing, cohort, velvetleaf, soybean

IZVLEČEK

PREUČEVANJE RAZMERJA MED PROSTORSKO UREDITVIJO SETVE SOJE (*Glycine max* (L.) Merr.) IN POPULACIJSKO DINAMIKO BRŽUNASTEGA OSLEZOVCA (*Abutilon theophrasti* L.)

Bržunasti oslezovec je pomemben enoletni plevel v provinci Mazandaran, Iran. Njegovo razmnoževanje je izključno s semeni. V raziskavi so bila pridobljena nova spoznanja o semenski banki in produkciji semena za izboljšanje uravnavanja bržunastega oslezovca v bodoče. Malo je informacij, ki se nanašajo na vpliv načina setve soje na dinamiko polpulacij bržunastega oslezovca. V ta namen sta bili v sodelovanju z Agriculture Discipline of the Islamic Azad University, Qaemshahr, Iran, v letih 2009 in 2012 preučevani dve polji, na katerih sta bila preučevana načina setve soje v vrstah s širšim razmikom, 50 cm, in v vrstah z ožjim razmikom, 36 cm, na vznik populacij bržunastega oslezovca v treh različnih obdobjih po setvi soje (0-10, 10-20 in 20-30 dni). Produkcija semena, listna površina in vsebnost suhe snovi so se povečali v vsaki naslednji populaciji bržunastega oslezovca pri setvi soje v vrstah s širšim, 50 cm razmikom. Mortaliteta kalic bržunastega oslezovca se je pri tem načinu setve soje iz populacije v populacijo zmajševala. Pri tem smo opazili povečano produkcijo semena rastlin bržunstega oslezovca, ki so vzkalile v prvem obdobju po setvi soje. Iz tega lahko zaključimo, da je potrebno zatiranje plevelov v prvem obdobju po setvi soje, to je v treh do štirih tednih po vzniku soje, če hočemo preprečiti zmanjšanje pridelka soje in nadaljne povečevanje semena bržunastega oslezovca v semenski banki. Neupoštevanje širine razmika med vrstami pri setvi soje kaže na pomen tega dejavnika pri uravnavanju populacij te plevelne vrste.

Ključne besede: razporeditev vrst setve, kohorte, bržunasti oslezovec, soja

[1] Department of Weed Science. Faculty of Agriculture and Natural Resources, Islamic Azad University, Qaemshahr Branch, Iran; Email : hamisalehian@gmail.com as correspondence

1 INTRODUCTION

Several new methods and technologies were used to reduce the population of the weed to adequate levels. However for preserving the existing environment and other natural resources it is essential the use of various methods and techniques such as integrated weed management control (IWM). The purpose of this method in this part is to reduce our reliance on chemical weed control which can be accomplish through development and utilization of integrated weed management (IWM) as well as integrating other preventive measures (Knezevic and Horak, 1998). Integrated weed management (IWM) emphasizes the health of the product, and a crucial understanding of this method and its accurate application (Swanton et al., 2008). This approach has demonstrated the relevance and importance of weed mortality and decline of the weeds fitness (Williams et al., 1998). It is a combination of various methods by cultural, chemical and mechanical means (Swanton et al., 2008) as well as agronomical operations which also play a vital role (Pylon et al., 1997; Hock et al., 2005). Articles on the timing and placement of fertilizer, seeding rate and reducing crop row spacing have been studied and reported by Walker and Buchanan, (1982). Planting the soybean with a smaller distance between the plant rows leads to acceleration in canopy closure thus increasing its relative competitiveness and weed suppression particularly in the final weed cohorts (Knezevic et al., 2003b; Mickelson and Renner, 1997; Mulugeta and Boerboom, 2000; Nice et al., 2001; Yelverton and Coble, 1991; Yong et al., 2001). Knezevic et al (2003 a, b) demonstrated in his study that with decrease in row spacing of the soybean the toleration of the crop was increased to constrain the weed competition at the onset of the season. The critical weed free period was delayed and in general severity of weed damage declined. Other studies have shown that by increasing the plant dry mass, the seed production per bush were increased (Martens and Jansen, 2002; Thompson et al., 1991; Samson and Werk, 1986). Therefore, it can be purposed that the crop planting pattern affects the production ability of velvetleaf seed. It is insufficient research regarding the influence of the planting pattern on the velvetleaf seed production.

Effective, logical and long term weed management is based on the constant reduction of weeds' population and seed bank density. One of the most important factors in this area is to minimize seed production per plant. This result has been done by reduction of weed density or by mean of diminishing the seed production.

Process of emergence is a critical event in the life cycle of the velvetleaf weed (Forcella et al., 2000). Period of weed emergence and dynamics of weed emergence flushes play a critical role on loss of the crop yield (O'Donovan et al., 1985; Swanton et al., 2008). The growth of the velvetleaf seedlings which emerge at the beginning of the season is often compared with the later ones. The seedlings developed in the early part of the season demonstrate more competitiveness, produce a higher biomass and number of seeds, and furthermore have added impact on the crops' yield ability (Massinga et al., 2001; Norsworthy et al., 2007; Steckel and Sprague, 2004).

The low competitiveness of the seedlings which emerge later in the season is mainly due to the interspecies competition and their mortality rate under the canopy. This was noted to occur most likely in the dense row spacing and it was as a consequence of shading by crop (Buehring et al., 2002; Norsworthy et al., 2007).

For the future success and development of integrated weed management (IWM), further biological studies of the various weeds inhibiting the soybean culture is necessary. Velvetleaf is a serious and troublesome weed of the soybean in the north of Iran and a global dilemma (Shafigh et al., 2006; Rezvani et al., 2008; Sadeghi et al., 2003; Warwick and Blank, 1988; Hock et al., 2005; Zeinali and Ehteshami 2003). The majority of the velvetleaf seedlings sprouted at the beginning of the season and some emerged mid-season (Egley and Williams, 1991). Velvetleaf seedlings are able to complete their life cycle in the crop canopy (Mitch, 1991) and can produce as much as 17000 seeds per plant (Warwick and Black, 1988).

The goal of this study is to see the interactions between the various velvetleaf cohorts during (0-

10, 10-20, and 20-30 days after crop planting) in the disparate soybean planting patterns (36 and 50-cm row spacing).

2 MATERIAL AND METHODS

This experiment has been done in the scientific-research fields of the Islamic Azad University in Qaemshahr, Iran; formatted and randomized in complete block design, in three replications in the two season crops of 2009 and 2012. Treatments for this study were different planting patterns in the soybean culture. Soybean seeds were planted in two different row spacing in order to achieve a 45 plants per m^{-2} on 15 April 2009 and 18 April 2012. At the first plant-group, the distance between rows was 36 cm (narrow spaces) and the seedlings intervals were 6 cm in the rows and in the second plant-group the distances between rows were 50 cm (wide spaces) and 4 cm intervals between the seedlings in the row, respectively.

The soybean seeds ('JK' variety) were disinfected with Benomyl fungicide (2000 ppm), and then planted at a depth of 2 cm. A segment of the university's research-field was selected which was known to be positive for contamination of the velvetleaf seed. Experimental units were 7 m long and 3.2 m up to 4.5 m wide with 10 rows.

The field was tillaged twice (the autumn in previous year and prior to planting after the next year). According to the soil tests there were no need to apply potassium (K_2O), however, phosphorus (P_2O_5) and nitrogen (urea) fertilizers were used at 80 kg h^{-1} and 5 kg h^{-1}, respectively.

By Auger, five soil samples were taken from each plot, prior to sowing the soybean seeds, the diameter and depth of each sample was 6 cm and 10 cm, respectively. The purpose of these samples were to confirm the presence and removal of the velvetleaf seeds (*Abutilon theophrasti* Medik), all samples were taken to the university laboratory. Soil samples were put into plastic bowls which contained potassium hexametaphosphate (5 %), then were mixed with distilled water disintegrating soil structures, then the solution of soil solid particles was filtered through a sieves of 1 mm diameter, at last following confirmation and identification of the velvetleaf seeds, the seeds were collected. Subsequent to counting the seeds in the augered sectioned areas, the seeds' density per unit soil surface was estimated.

For recording and sampling during the growth season two quadrats, 1m × 1m, were selected in the center of each plot. For determination of the effects of the velvetleaf seedlings emergence stages their appearance were divided into three cohorts: zero to 10, 10 to 20 and 20 up 30 days after soybean sowing. Calculations for the number of seed per unit soil volume and surface, rate of recruitment was measured. The seedling mortality was also calculated in the form of percentage. The other undesirable weeds were controlled by hand-hoeing.

Developmental stages were based on the number of fully expanded leaves per plant (Fehr and Cavines, 1977). Velvetleaf bushes were clipped at the soil surface, divided into 40 - cm segments, then leaves, stems and capsules were separated from each other at physiological maturity of the soybean. The leaf area (LA) of the velvetleaf was measured with a leaf area meter (LTD AM 200). Different plant components were then dried at 80 C° in the oven (Memmert DIM 40050) and weighed. Seeds that had scattered on the ground before or at the final harvest were not collected, and seeds losses were not estimated. Analysis of variance was performed using PROC MIXED (SAS, 1999) to test data normality and significance ($P < 0.05$) of growing scenario, soybean row spacing and velvetleaf relative emergence.

3 RESULTS AND DISCUSSION

The average number of velvetleaf seed in the soil was 943 and 991 seeds m^{-2} at the 2009 and 2012, respectively. This amount was in agreement with other studies (Lindquist et al., 1995; Munger et al., 1987). This result could have risen from the

intense contamination of soil with this weed or was caused by a lack of continual crop planting.

Initially, the rate of the velvetleaf seedlings emergence cannot be influenced by a soybean planting pattern. Differences in the velvetleaf seedlings emergence in two different rows spacing's can be related to their content on the weed seed bank (Table 1). The mean of the velvetleaf seed density during two years of study in the soil seed bank in wide and narrows rows were 1215 and 720 seed per m^{-2}.Therefore higher number of velvetleaf seedlings in wider rows soybean planting appears to be a logical outcome. The lack of necessity to light for velvetleaf seed germination may explain this result (Bello et al., 1995).

Table 1: Velvetleaf seed rate in seed bank, emergence seedlings density and recruitment percentage in various soybean row spacing for 2009 and 2012[1]

Year	Seed bank (sb) (seed/m^2) Row spacing (cm)		Seedling density (sd) (p/m^2) Row spacing (cm)		Recruitment sd/sb×100 Row spacing (cm)	
	36	50	36	50	36	50
2009	708	1179	10	22	1.4	1.8
2012	732	1251	12	25	1.6	2.0

1- Within a row the same letter indicates that the values did not differ significantly by LSD test, according to P=0.05

Velvetleaf population recruitment was insignificant regarding planting pattern (averaging 26 %, Table 1). In comparison with other experiments our study gave results with smaller values of this parameter (Puricelli et al., 2002). Lindquist et al (1995) also found this consistency in his study. This information can be used to predict the approximate amount of emergence percentage from the weed seed bank. Of course, in the majority of weed species more differences are recorded between the content in weed seed bank and emerging seedlings (Forcella et al., 1997). These variations maybe caused by the sizes of the sampling areas or the time of samplings (Derksen and Watson, 1998).

The rate of the velvetleaf seedlings mortality was lower in the wide-rows (Table 2). Despite slower growth of velvetleaf seedlings in the narrow rows, they were able to survive relative to their condition (higher in the second cohort). Puricelli et al (2002), in his study regarding the interference between *Anoda cristatae* L. and the soybean, reported similar results. However, Scursoni et al (1999) observed mortality of *Avena fatua* L. plants in the densest stands of barley, due to the acceleration in the canopy closure. Nonetheless, in this study, further delay in the emergence of the velvetleaf population relative to the soybean caused the decrease in their survival. High survival of early cohorts of velvetleaf seedlings in both row spacing of soybean indicated important role on information of their soil seeds bank.

Observation of the velvetleaf seedling population in the last cohorts, during the soybean harvest it has shown that they did not survive. Therefore it was noted that the control measures must be predominantly focused on the removal of the early cohorts. Therefore the results were demonstrated that the loss of the soybean yield was a result of the competition with the first cohorts of velvetleaf seedlings (Cowan et al., 1998; Dielman et al., 1996; Steckel and Sprague, 2004). Researches have showed that velvetleaf plants which emerged 35 days after soybean planting did not decrease crop yield significantly (Zimdahl, 1988). This indicates the importance of time of velvetleaf seedlings emergence for their survival (Hock et al, 2005), the same was proved also for *Digitaria sanguinalis* (L.). (Gallart et al, 2010; Oreja and dela Funte. 2005) and some other weed species (Lindquist et al., 1995).

Table 2: Emerged seedlings number, mortality and survival percentage of velvetleaf in cohorts and planting patterns for 2009 and 2012

Year	Cohorts[1]	Emerged seedling number (p/m^2)		Mortality (%)		Survival (%)	
		row spacing		row spacing		row spacing	
		36 cm	50 cm	36 cm	50 cm	36 cm	50 cm
2009	1	5 b**1**	14 c	43 c	40 c	57 c	60 c
	2	3 a	5 b	60 b	66 b	40 b	34 b
	3	2 a	3 a	100 a	100 a	0 a	0 a
2012	1	7 c	18 c	40 c	37 c	60 c	63 c
	2	4 b	9 b	51 b	60 b	49 b	40 b
	3	2 a	4 a	98 a	100 a	2 a	0 a

1-It means emergence date of velvetleaf at 10 th intervals after soybean planting (for details refer to the text in table 3)
2-Within a top column, the same letter indicates that the values did not differ significantly by LSD test, according to *P*=0.05

Dry matter accumulation in the velvetleaf population was affected by the planting pattern (Table 3). The velvetleaf populations benefited more in their biomass in the wide soybean row spacing. Seedlings that emerged in the first cohort in 50-cm row generated almost four times higher plants with respect to the second cohort. Similarly the velvetleaf populations in the narrow soybean row spacing as with the first cohort in comparison with the second cohort had 200 % more dry matter. Overall the seedlings that were emerged earlier produced additional biomass. These outcomes were confirmed by the findings of Massinga et al. 2001, Puricelli et al. 2002 and Hock et al., 2005.

Leaf areas of velvetleaf plants accumulated in the third and fourth layer 77 % and 71 % for the 50-cm-row and 36-cm-row soybean respectively, an average in both years. The highest leaf accumulation area in the upper strata's of the velvetleaf canopies indicates higher efficiency in competition for intercepting the light in comparison with the soybean. Other experiments have reported similar reactions during mixed culture between soybean with velvetleaf (Heindl and Burn, 1983; Hock et al., 2005) and the redroot pigweed (Legere and Schreiber, 1989). Hock et al (2005) reported that velvetleaf population had low leaf area in the lower layers when it was located with soybean in mixed cultures.

Table 3: Velvetleaf seed production and plant dry mass in cohorts at different planting patterns different for 2009 and 2012 season

Year	Row spacing	Emergence date of velvetleaf		Leaf stage of soybean	Seed number per plant	Plant dry mass (g)
2009	36	May 15	May 25	V_E	1098 (143)	18.4
		May 26	Jun 4	V_1	509 (143)	9.6
		Jun 5	Jun 14	V_2	0	0
	50	May 15	May 25	V_E	2372 (143)	39.96
		May 26	Jun 4	V_1	683 (143)	10.0
		Jun 5	Jun 14	V_2	0	0
2012	36	May 19	May 29	V_E	716 (26)	17.9
		May 30	Jun 9	V_1	264 (29)	6.6
		Jun 10	Jun 19	V_2	0	0
	50	May 19	May 29	V_E	1881 (169)	37.6
		May 30	Jun 9	V_1	527 (47)	7.9
		Jun 10	Jun 19	V_2	90 (8)	0

1- Standard error based on least square means (P=0.05)

The velvetleaf leaf area was influenced by the soybean-planting pattern and its' relative time of emergence ($P < 0.05$) (Figure 1). Leaf area of velvetleaf plants within the soybean showed more reduction in 36-cm-row planting at the second cohort. In the narrow-row spacing on average in both years the leaf area of velvetleaf population in the first and second cohort were reduced up to 24 % and 30 %, respectively. With a further-decline of the leaf area in 36-cm-row; expressing further competition for the light, more so in the second cohort.

The time of emergence plays an important part in the velvetleaf seedlings survival, in so far as those emerging early produced a higher seed population and increased further soil seed banks, as illustrated in Table 3. The variations are demonstrated and can be justified by the unsymmetrical competition for plants in different cohorts (Schwinning and Weiner, 1998). Thus, the bushes which were emerged early had a longer growing period and also were larger. Hock et al (2005) and Gallart et al (2010) demonstrated the importance of time emergence for seed production in velvetleaf and orchard grass weed populations. Thus the dynamics of the seed bank can be influenced by each cohort and were resulted in producing seeds with different dormancy's and reactions of their environmental conditions (Baskin and Baskin, 2001).

Seed production in the velvetleaf populations were reduced in the narrow-row spacing and due to their delayed time of emergences (Table 3). Seedlings that emerged in the first cohort in wide rows generated twice the amount of seeds on each of the plants.

These differences were smaller in the later cohorts. Furthermore, the seed production in different flushes in the wide rows had a higher variance. The competition for light accounts for the decline in seed productions (Puricelli et al., 2002; Buehring et al., 2002; Benvenuti et al., 1994). It appears that the first flushes must be removed to prevent further weed population from the seed banks, and planting pattern of the crops should be considered as well.

For both soybean planting patterns an increase in weed mass were observed, with the increase of dry matter in each plant, seed production was raised (Figure 2). For this reason the mean of seed numbers in the remaining plants at the end of the

seasons' growth for the first flush were measured. Higher seed production in each velvetleaf bush in the wide-rows can be correlated to the differences in biomass and percentage of mortality in both planting patterns. It was used a simplest linear model for calculation of the seed productions where the independent variable was the weight of each plant (Mertens and Jansen, 2002). Intercept of regression model for seed production was increased when seedling size rose, this situation was seen in the wide-rows. For this reason the number was 181 in the wide rows spacing (as it was compared to 102 in narrow-rows). Similar trends were seen during various other studies, such as the effect different row spacing's for wheat on the seed production in a three weed species study (Mertens and Jansen, 2002).

The treatments in this study demonstrated that the narrow – row spacing resulted in less seed production and bigger seedling mortality. Dry matter and seed production was estimated for each plant (as an alternative of per area unit). This method has some advantages. At first, it revealed the weed community increase or decrease. Moreover, the velvetleaf population had no even distribution in the field. Therefore we should not compare our results on the dry matter and seed density of velvetleaf with those obtained per unit area, the comparisons would have been incorrect.

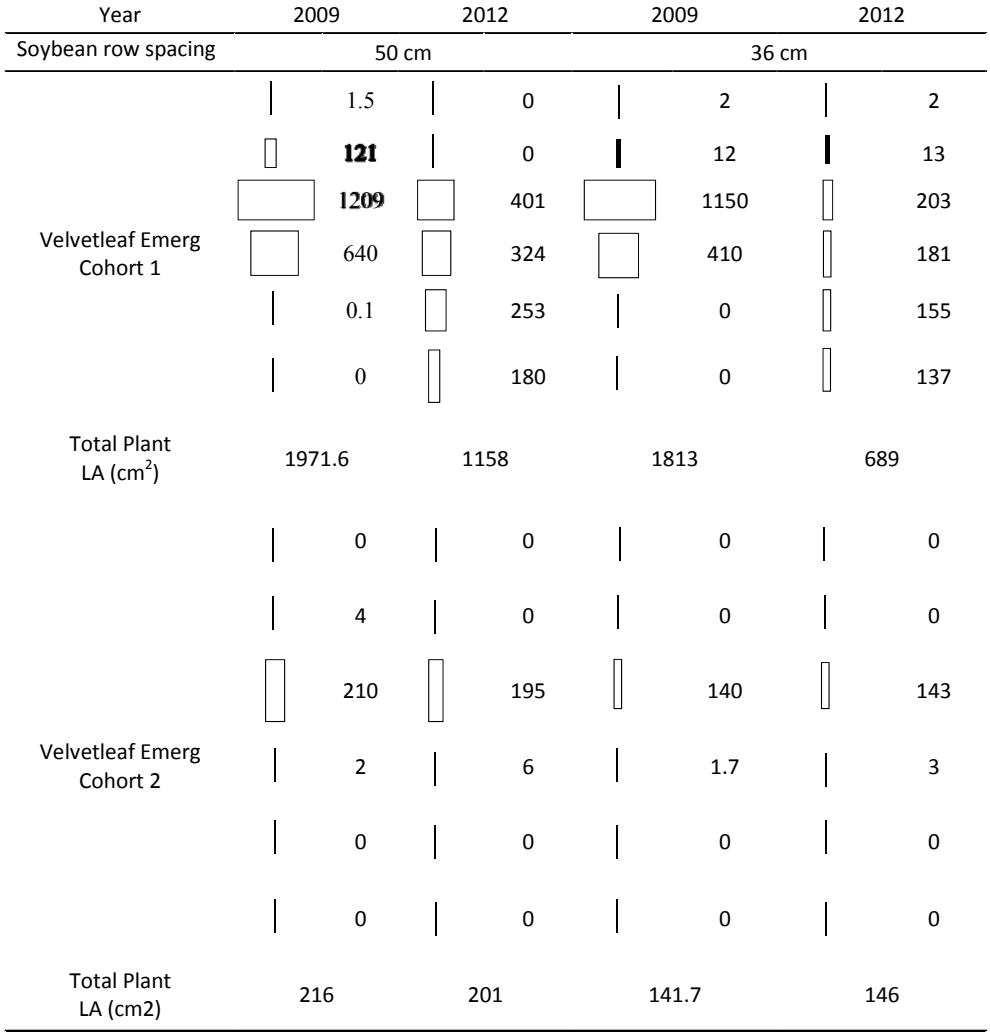


Figure 1: Velvetleaf leaf area (LA) distribution at soybean maturity for years 2009 and 2012 as influenced by crop row spacing and time of velvetleaf emergence in velvetle af-soybean plots. Each rectangle represents a 40 – cm height increment. Total LA is shown at the bottom each symbolic plant.

This study emphasizes the importance of integrated weed management through the method of weed emergence control time and using an inexpensive planting patterns. Furthermore, the data on leaf area, total of dry matter and seed production also suggested the greater need for control of early emerging rather than late-emerging velvetleaf of populations.

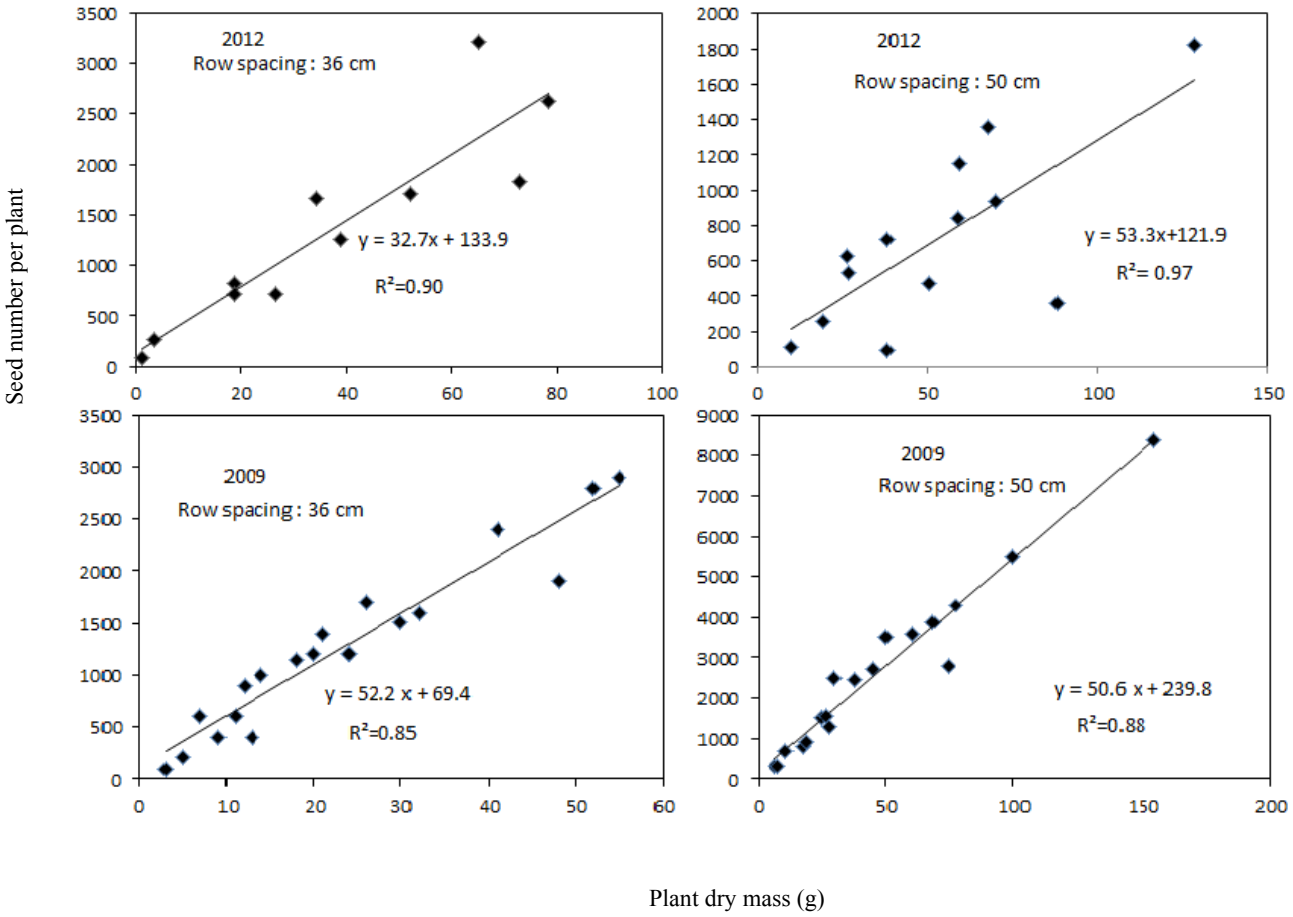


Figure 2: Relationship between the number of seeds produced by velvetleaf plant and plant dry mass in various soybean planting patterns for years 2009 and 2012

4 CONCLUSIONS

A long term integrated weed management plan should be considered for early control of the velvetleaf population rather than the late-emerging population. The data collected in this study on the leaf area, total dry matter and seed production confirm the need for better control techniques of the velvetleaf population. Furthermore, this study has demonstrated a necessity for further studies and research for successful results such as reduction and effective control of the weed seed stocks in the soil. These methods and techniques would not degrade the environment, quality of the land or agricultural crops and most of all it is very important their cost effect.

5 REFERENCES

Baskin C.C., Baskin J.M. 2001. Seeds: Ecology, Biogeography and Evolution of Dormancy and Germination. Academic Press, Sandi ego.

Bello I.A., Owen M.D.K., Valenti H.M.H. 1995. Effect of shade on Velvetleaf (*Abutilon theophrasti*) growth, seed production, and dormancy. Weed Technology, 3:452-45

Benvenuti M., Macchia S., Stefani A. 1994. Effects of shade on reproduction and some morphological characteristics of *Abutilon theophrasti* medicus, *Dutura stramonium* L. and *Sorghum halepense* L.pers.s. Weed Research, 34:283-288; DOI: 10.1111/j.1365-3180.1994.tb01996.x

Buehring N.W., Nice G.R.W., Shaw D.R. 2002. Sicklepod (*Senna obtusifolia*) control and soybean (*Glycine max*) response to soybean row spacing and population in three weed management systems. Weed Technology, 16: 131-141; DOI: 10.1614/0890-037X (2002)016

Cowan P, Weaver S.E., Swanton C.J.1998 .Interference between pigweed (*Amaranthus* spp.), and Barnyard grass (*Echinochloa crus-galli*) and Soybean (*Glycine max*). Weed Science, 46:539-533; DOI: 10.1614/WS-03-004R

Derksen D.A., Watson D.R. 1998. Weed community composition in seed banks ,seedlings, and mature plant communities in a multi-year trial in western Canada. Aspects of Applied Biology, 51:43-50

Dielman A., Hamill A.S., Fox G.C., Swanton C.J. 1996. Decision rules for post emergence control of pigweed (*Amaranthus* spp.) interference in Soybean*(Glycine max*).Weed Science, 44:126-132

Egley G.H., Williams R.D. 1991. Emergence periodicity of six summers annual weed species. Weed Science, 4: 595-600

Fehr, W.R., Caviness.C.E.1977.Stages of soybean develpment.Spec.Rep.80.Iowa Agric.Home Econ.Exp.Stn., Iowa State Uni. Ames

Forcella F.,Wilson R.G., Dekker J. 1997. Weed seed bank emergence across the Corn Belt .Weed Science, 45:67-76

Forcella F., Benech Arnold R.L., Sanchea R., Ghersa C. 2000. Modeling seedling emergence. Field Crops Research, 67: 123-1, doi: 10.1016/S0378-4290(00)00088-5

Gallart M., Mas M.T.,Verdu A.M.C. 2010. Demography of *Digitaria sanguinalis* :effect of the emergence time on survival, reproduction, and biomass. Weed Biology and Management, 10:132-140; DOI: 10.1111/j.1445-6664.2010.00375

Heindl J.C., Burn W.A.1983. Light and shade effects on abscission and C- photaassimilate partitioning among reproductive structures in soybean. Plant Physiology, 73:434-439; DOI:10.1104/pp.73.2.434

Hock S.M., Knezevic S.Z., Martin A.R., Lindquist J.L. 2005. Influence of soybean row width and velvetleaf emergence time on velvetleaf (*Abutilon theophrasti*).Weed Science, 53: 160-165; DOI: 10.1614/WS-04-122R

Knezevic S.Z., Horak M.J. 1998. Influence of emergence time and density on redroot pigweed (*Amaranthus retroflexus*).Weed Science, 46: 665-672

Knezevic S.Z., Evans S.P., Mainz M. 2003a. Row spacing influences the critical timing for weed removal in soybean (*Glycine max*). Weed Technology, 17: 666-673; DOI: 10.1614/WT02-49

Knezevic S.Z., Evans S.P., Mainz M. 2003b. Yield penalty due to delayed weed control in corn and soybean. [Crop Management Journal Online] www.plantmanagementnetwork.org/pub/cm/research/2003/delay./

Legere A., Schreiber M.M.1989.Competition and canopy architecture as affected by Soybean (*Glycine max*) row width and density of redroot pigweed (*Amaranthus retroflexus*) .Weed Science, 37:84-92

Lindquist J.L., Max Well B.D., Buhler D.D., Gun Solus J.L. 1995. Velvetleaf (*Abutilon theophrasti*): Recruitment, survival,seed production, and interference in Soybean (*Glycine max*).Weed Science, 43:226-232

Martens S.K., Jansen J.H. 2002.Weed seed production, crop planting pattern, and mechanical weeding in wheat. Weed Science, 50:748-756; DOI: 0043-1745(2002)050

Massinga R.F., Currie R.S., Horak M.J., Boyer J. 2001. Interference of Palmer Amaranth in corn. Weed Science, 49: 202-208.; DOI: 0043-1745(2001)049

Mickelson J.A., Renner K.A. 1997. Weed control using reduced rates of post emergence herbicides in narrow and wide row soybean. Journal of Production Agriculture, 12: 35-43

Mitich L.W. 1991.Velvetleaf. Weed Technology, 5: 253-255

Mulugeta D., Boerboom C.M. 2000. Critical time of weed removal in glyphosate -resistant soybean (*Glycine max)*. Weed Science, 48: 35-42, doi: 10.1614/0043-1745(2000)048[0035:CTOWRI]2.0.CO;2

Munger P.H., Chandler J.M., Cothren J.T., Hons F.M. 1987. Soybean (*Glycine max*) velvetleaf (*Abutilon theophrasti*) interspecific competition. Weed Science, 35: 647- 653

Nice G.R.W., Buehrin N.W., Shaw D.R. 2001. Sicklepod response to shading ,soybean (*Glycine max*) row

spacing and population in three management systems. Weed Technology, 15: 155-162; DOI: 0890-037X (2001)015

Norsworthy J.K., Jha P., Bridges W. 2007. Sicklepod survival and fecundity in wide- and narrow-row glyphosate-resistant soybean (*Glycine max*). Weed Science, 55: 252-259; DOI: 10.1614/WS-06-155

O'Donovan J.T., Remy E.A., O'Sullivan P.A., Dew D.A., Sharma A.K. 1985. Influence of the relative time of emergence of wild oat (*Avena fatua*) on yield loss of barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*).Weed Science, 33: 498- 503

Oreja F.H., dela Fuente E.B.2005. Population dynamics of *Digitaria sanguinalis* L. scop. In soybean in the Pampa.Proc.XVII Congreso of the Latinoamerican Weed Science Associations (ALAM).Varadero.Cuba.1-5.

Puricelli E., Oriolli G., Sabbatini M.R. 2002. Demography of *Anoda cristata* in wide-and narrow row soybean. Weed Research, 42:456-463; DOI: 10.1046/j.1365-3180.2002.00307

Pyon J.Y., Guh J.O., KU Y.C. 1997. Environment-friendly cultural and mechanical practices for weed management.Korean Journal Weed Science, 17: 124-134

Rezvani H., Latifi N., Zeinali E. 2008. Determination of critical period for velvetleaf (*Abutilon theophrasti*) control in summer seeded soybean, Williams's cultivar. Electronic Journal Crop Production, 1(2): 45-65

Sadeghi H., Baghestani M.A., Akbary G.A., Hegazy A. 2003. Evaluation of soybean (*Glycine max*) and some weed species growth indices under competition condition. Plant Pest and Disease Journal, 71: 87-106

Samson D.A., Werk K.S .1986.Size - dependent effects in the analysis of reproductive effort in plants. American Naturalist, 127:667-680, doi: 10.1086/284512

SAS Institute .2001.SAS/STAT user's guide .Version 8.Vols.1-3.Cary, NC: SAS Institute 1028 p.

Schwinning S., Weiner J. 1998. Mechanisms determining the degree of size asymmetry in competition among plants. Oecologia, 113:447-455, doi: 10.1007/s004420050397

Scursoni J., Benech Arnold R., Hirchoren H. 1999. Demography of wild oat in barley crops: effect of crop, sowing rate, and herbicide treatment. Agronomy Journal, 91: 479-485, doi: 10.2134/agronj1999.00021962009100030020x

Shafigh M., Rashed Mohassel M.H., Nassiri Mahallati M. 2006. The competitive aspect of soybean (*Glycine max*) and Velvetleaf (*Abutilon theophrasti*) in response to population density and planting date. Jour. Iranian. Field Crops Research, 4:71-81

Steckel L.E., Sprague C.L. 2004. Late-season common water hemp (*Amaranthus rudis*) interference in narrow- and wide-row soybean. Weed Technology, 18: 947-952; DOI: 10.1614/WT-03-131R

Swanton C.J., Weise S.W. 1991. Integrated weed management: the rationale and approach. Weed Technology, 5: 663-687

Swanton C.J, Mahoney K.J., Chandler K., Gulden R.H. 2008. Integrated weed management: Knowledge-Based weed management systems. Weed Science, 56: 168-172; DOI: 10.1614/WS-07-126.1

Thompson B.K., Weiner J., Warwick S.I.1991. Size-dependent reproduction output in agricultural weeds. Canadian Journal of Botany, 69:442-446, doi: 10.1139/b91-061

Walker R.H. and Buchanan G.A. 1982. Crop manipulation in integrated weed management systems. Weed Science, 30 (Suppl.1): 17-23

Warwick S.I., Black L.D. 1988. The biology of Canadian weeds. 90. *Abutilon theophrasti*. Canadian Journal of Plant Science, 68: 1069-1085, doi: 10.4141/cjps88-127

Williams M.M., Mortensen D.A., Doran J.W. 1998. Assessment of weed and crop fitness in cover crop residues for integrated weed management. Weed Technology, 46: 595-603

Yelverton F.H., Coble H.D. 1991. Narrow row spacing and canopy formation reduces weed resurgence in soybeans (*Glycine max*). Weed Technology, 5:169-174

Young B.G., Young J.M., Gonzani L.C., Hart S.E., Wax L.M., Kapusta G. 2001. Weed management in narrow and wide-row glyphosate-resistant soybean (*Glycine max*). Weed Technology, 15: 112-121; DOI: 0890-037X (2001)015

Zeinali E., Ehteshami M.R. 2003. Biology and control of important weed species .Gorgan University of Agricultural Science and Natural Resources. Gorgan, Iran .pp.412

Zimdahl. R.L.1988. The Concept and application of the critical weed-free period. CRC Press. Boca Raton, FL, USA

COBISS Code 1.01

DOI: 10.14720/aas.2015.105.2.13
Agrovoc descriptors: *Daucus carrota*, varieties, chemical compounds, chemical composition, carotenes, carotenoids, polyphenols, antioxidants

Agris category code: q04

Carrot (*Daucus carota* L. ssp. *sativus* (Hoffm.) Arcang.) as source of antioxidants

Judita BYSTRICKÁ[1], Petra KAVALCOVÁ[2], Janette MUSILOVÁ[1], Alena VOLLMANNOVÁ[3], Tomáš TÓTH[4], Marianna LENKOVÁ[4]

Received June 16, 2015; accepted September 08, 2015.
Delo je prispelo 16. junija 2015, sprejeto 08. septembra 2015.

ABSTRACT

Carrot (*Daucus carota* L. *s*sp. *sativus* (Hoffm.) Arcang.) is a significant source of vitamins (A, B, C) and beta carotene. Further it contains vitamins B, C, E, H, folic acid and pantothenic acid. Carrot is an important source of trace elements (K, Na, Ca, Mg, P, S, Mn, Fe, Cu and Zn). Consumption of carrot improves eyesight, lowers cholesterol and improves digestion. In this work we evaluated and compared content of total polyphenols, ß-caroteneand antioxidant activity in five varieties of carrot ('Jitka', Kardila', 'Katlen', 'Rubína' and 'Koloseum') Samples of carrot were collected at full maturity stages from area of Bardejov. Samples of fresh carrot were homogenized (25 g) in 50 ml 80 % ethanol and analysed after sixteen hours. The content of the total polyphenols was determined by using the Folin-Ciocalteu reagent (FCR). The content of ß-carotene was determined spectrophotometricaly at 450 nm. Antioxidant activity was measured using a compound DPPH˙ (2.2-diphenyl-1-picrylhydrazyl) at 515.6 nm using spectrophotometer. Total polyphenols content in samples ranged from 81.25 ± 13.11 mg/kg to 113.69 ± 11.57 mg/kg and content of ß-carotenes ranged from 24.58 ± 2.38 mg/kg to 124.28 ± 3.54 mg/kg. We also evaluated and compared the antioxidant activity in selected varieties of carrot, which varied from 6.88 ± 0.92 % to 9.83 ± 0.62 %. Statistically significant the highest value of total polyphenols was recorded in variety of Koloseum (113.69 ± 11.57 mg/kg). This variety is also characterized by the highest content of ß-carotene (124.28 ± 3.54 mg/kg)as well as the highest value of antioxidant activity (9.83 ± 0.62 %).

Key words: carrot, cultivar, ß-carotenes, polyphenols, antioxidant activity

IZVLEČEK

KORENJE (*Daucus carota* L. ssp. *sativus* (Hoffm.) Arcang.) KOT VIR ANTIOKSIDANTOV

Korenje (*Daucus carota* L. ssp. *sativus* (Hoffm.) Arcang.) je pomemben vir vitaminov (A, B, C) in beta karotena. Dodatno vsebuje vitamine B, C, E, H, folno in pantotentsko kislino. Korenje je tudi pomemben vir elementov v sledeh kot so K, Na, Ca, Mg, P, S, Mn, Fe, Cu in Zn. Uživanje korenja izboljšuje vid, zmanjšuje količino holesterola in izboljšuje prebavo. V tej raziskavi smo ovrednotili in primerjali vsebnost celokupnih polifenolov, beta karotena in antioksidacijsko aktivnost v petih sortah korenja ('Jitka', 'Kardila', 'Katlen', Rubína' and 'Koloseum'). Vzorci korenja so bili nabrani ob tehnološki zrelosti na območju Bardejova. Vzorci svežega korenja so bili homogenizirani (25 g) v 50 ml 80 % etanola in analizirani po 16 urah. Vsebnost celokupnih polifenolov je bila določena z uporabo Folin-Ciocalteu reagenta (FCR). Vsebnost beta karotena je bila določena spektrofotometrično pri 450 nm. Tudi antioksidacijaka aktivnost je bila izmerjana spektrometrično z uporabo DPPH˙ (2.2-difeenil-1-pikcrilhidrazil) pri 515.6 nm. Vsebnost celukopnih polifenolov v vzorcih je bila med 81.25 ± 13.11 mg/kg in 113.69 ± 11.57 mg/kg, vsebnost ß-karotena pa med 24.58 ± 2.38 mg/kg in 124.28 ± 3.54 mg/kg. Ovrednotili in primerjali smo tudi antioksidacijsko aktivnost v izbranih sortah korenja, ki je bila med 6.88 ± 0.92 % in 9.83 ± 0.62 %. Največjo, statistično značilno vsebnost polifenolov smo izmerili pri sorti Koloseum (113.69 ± 11.57 mg/kg). Ta sorta je bila značilna tudi po največji vsebnosti ß- karotena (124.28 ± 3.54 mg/kg) kot tudi po največji antioksidacijski aktivnosti (9.83 ± 0.62 %).

Ključne besede: korenje, sorta, ß-karoten, polifenoli, antioksidacijska aktivnost

[1] Assoc. Prof. Ing., Ph.D, Dept. of Chemistry, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra; Slovak Republic

[2] Ing., Dept. of Chemistry, Dept. of Chemistry, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra; Slovak Republic

[3] Prof., RNDr., Ph.D, Dept. of Chemistry, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra; Slovak Republic

[4] Assoc. Prof. RNDr. Ing., Ph.D, Dept. of Chemistry, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra; Slovak Republic

1 INTRODUCTION

Carrots in Slovak Republic are among most popular root vegetables. It is the most important crop of Apiaceae family. Member of this family have small, mostly white, 5-parted flowers arranged in umbrella-like inflorescence called umbel (Essig, 2013).Carrots were first used for medical purposes and gradually used as food (Carlos and Dias, 2014). This vegetable is an important source of bioactive compounds with beneficial effect for the consumer health.Carrots are consumed in different ways, they can be eaten raw or cooked.

Fruits and vegetables are an important part of our diet. They provide, not only the major dietary fiber component of food, but also a range of micronutrients, including minerals, vitamins and antioxidant compounds, such as carotenoids and polyphenols (Augspole et al., 2014). Increased consumption of fruits and vegetables containing high levels of phytochemicals has been recommended to prevent chronic diseases related to oxidative stress in the human body (Liu 2003; Rao and Rao, 2007; Pandey and Rizvi, 2009. Fruits and vegetables are valuable sources of health-promoting substances active in neutralization of reactive oxygen species (Augustynowicz et al., 2014). Among them carrot belongs to horticultural crops of high recognition and importance due to its nutritional value and high concentration of bioactive constituents (Leja et al., 2013).

Carrot is one of the most important vegetables in the world; its bioactive constituents may be beneficial to a vast number of consumers. It is rich in pro-healthy antioxidants both of lipophilic (carotenoids) and hydrophilic (phenolic compounds) characters (Hager and Howard, 2006; Sharma et al., 2012; Leja et al., 2013). Carrots are a good source of carbohydrates and minerals like Ca, P, Fe and Mg (Sharma et al., 2012).

This root vegetable contains valuable phytochemicals. The presence of phytochemicals, in addition to vitamins and provitamins, in fruits and vegetables has been recently considered of crucial nutritional importance in the prevention of chronic diseases, such as cancer, cardiovascular disease, and diabetes (Nambia et al., 2010; Jamuna et al., 2011; Myojin et al., 2008). The complex mixture of phytochemicals in fruits and vegetables provides a better protective effect on health than single phytochemicals. Carrot could release approximately half of their phytochemical contents in the colon (Chu et al., 2002).

Carrots are noted for their rich antioxidants, especially β-carotene. In recent years, worldwide consumption of carrots has been steadily increasing because of their nutritional benefits. Carrots have potentially beneficial health effects, anti-carcinogenic, antioxidant, and immune-boosting properties, as well as the pro-vitamin activity of some carotenoids (Fiedor and Burda, 2014; Tanaka et al., 2012). The most important micronutrient is β-carotene, which is a lipid-soluble carotenoid. Its typical chemical structure, consisting of a polyene chain with 11 conjugated double bonds and β-ring at each end of the chain (Augspole et al., 2014).

Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called “free radicals.” Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function (Gupta et al., 2012; Pandey et al., 2012; Prasad and Rajkumar, 2014). Antioxidants are our first line of defence against free radical damage, and are critical for maintaining optimum health and wellbeing. Antioxidants can scavenge free radicals and protect the human body from oxidative stress, which is the main cause of some cancers and heart diseases (Sun et al., 2003).

Vitamin C, vitamin E, and beta carotene are among the most widely studied dietary antioxidants. Vitamin C is important water-soluble antioxidant in extracellular fluids. It is capable of neutralizing free radicals in the aqueous phase. Vitamin E, a major lipid-soluble antioxidant, is the most effective chain-breaking antioxidant within the cell membrane where it protects membrane fatty acids from lipid peroxidation. Vitamin C regenerates vitamin E. Beta carotene and other carotenoids are also believed to provide antioxidant protection to lipid-rich tissues (Shukla et al., 2014; Kumari et al., 2014).

Polyphenols can be characterized as products of plants secondary metabolism of. The phenolic compounds contain aromatic ring with one or more substituent –OH groups. Polyphenols are formed by many and very diverse group of substances, simple phenolic and polymerized phenolic compounds. Therefore they are often called polyphenols (Balasundram, 2006).

Phenolic compounds can act as antioxidants by interfering with oxidation processes through chainbreaking reaction activities (primary oxidation) or through scavenging of free radicals (secondary oxidation) (Ndhlala et al., 2010; Augspole et al., 2014). Antioxidants are capable of stabilizing, or deactivating, free radicals before they attack cells (Kumari et al., 2014; Agarwal, 2012).

The presented work is a part of a broader topics dealing with polyphenolic compounds and carotenes with antioxidant effects in selected varieties of carrot. The main purpose of this study was to determine the influence of cultivar on the content of the total polyphenols, carotenes as well as antioxidant activity in carrot.

2 MATERIALS AND METHODS

2.1 Climate conditions of location

This study was carried out in area of Bardejov, area without negative influences and i mission sources. It is located in the north-eastern Slovakia of region Šariš, with 49.1357, 20.4335 coordinates. The attitude of the village is in the middle of 276 m a.s.l. Average annual air temperature is 7.4 °C, and annual rainfall is 700 mm.

2.2 Plant samples

Five carrot (*Daucus carota* L. ssp. *sativus* (Hoffm.) Arcang.) cultivars ('Jitka', 'Kardila', 'Katlen', 'Rubína' and 'Koloseum') were obtained from a local producer in are Bardejov, Slovak Republic. All cultivars were cultivated conventionally under the same condition. Only NPK fertilization has been used for the achievement of favourable soil macroelements content. The soils on which the carrots were grown, can be characterized as acidic to neutral (pH/KCl = 5.51 – 6.60), with medium to high content of humus (% Hum. = 2.98 to 3.76), very high phosphorus (P = 257.50 – 310.15 mg/kg), potassium (K = 321.19 – 387.6 mg/kg) and magnesium content (Mg = 221.30 – 276.53 mg/kg). Samples of five cultivars of carrots were collected at full maturity stages. From the same places, from the arable layer (0 – 20 cm), soil samples were also taken with pedological probe GeoSampler fy. Fisher.

2.3 Characteristics of varieties

Jitka is medium-late varieties of carrot. It is well storable and suitable for industrial processing.

Kardila is a late variety, suitable for winter storage. Koloseum is late variety of carrot, well storable and suitable for eating.

The variety has high dry matter content and long shelf life.

Katlen is late variety, very profitable for the storage and industrial processing.

Rubína is late, traditional variety of carrot.

2.4 Sample preparation

Samples of selected varieties of carrot were homogenized (25 g) in 50 mL 80 % ethanol for sixteen hours.Samples were kept under laboratory room temperature in dark bottles and dark light conditions until pre-analytical operations. These extracts were used for analyze. The experiment was based on four replications.

2.5 Determination of total polyphenols

Total polyphenols were determined by the method of Lachman et al. (2003) and expressed as mg of gallic acid equivalent per kg fresh mater. Gallic acid is usually used as a standard unit for phenolics content determination because a wide spectrum of phenolic compounds. The total polyphenol content was estimated using Folin-Ciocalteau assay. The Folin-Ciocalteau phenol reagent was added to a volumetric flask containing 100 ml of extract. The content was mixed and 5 ml of a sodium carbonate solution (20 %) was added after 3 min. The volume was adjusted to 50 ml by adding of distilled water.

After 2 hours, the samples were centrifuged for 10 min. and the absorbance was measured at 765 nm of wave length against blank. The concentration of polyphenols was calculated from a standard curve plotted with known concentration of gallic acid.

2.6 Determination of carotens

ß-carotene after releasing by ethanolic hydroxide and after extraction into petrolether could be determined by spectrophotometry at wavelength 450 nm. Content of ß-carotene in carrot was assessed by method of calibration curve with measuring of absorbance of standard solutions of potassium dichromate. Carrot (1 g) was put in flask and added 20 ml of ethanolic solution NaOH, then 20 ml of HCl (1:1) was added. The flask content was quantitatively put on filter and washed by acetone till its non-soluble part was colourless. Filtrate was put into separating funnel (500 mL), added 40 ml petrolether and filled to ¾ water content. The procedure is 2 – 3 times repeated till the water ethanolic phase is colourless.

2.7 Determination of antioxidant activity

Antioxidant activity was measured by the Brand-Williams et al. (1995) method-using a compound $DPPH^{\cdot}$ (2.2-diphenyl-1-pikrylhydrazyl). 2.2-diphenyl-1-pikrylhydrazyl ($DPPH^{\cdot}$) was pipetted to cuvette (3.9 ml) then the value of absorbance which corresponded to the initial concentration of $DPPH^{\cdot}$ solution in time Ao was written. Then 0.1 ml of the followed solution was added and then the dependence A = f(t) was immediately started to measure. The absorbance of 1, 5 and 10 minutes at 515.6 nm in the spectrophotometer Shimadzu UV/VIS – 1240 was mixed and measured. The percentage of inhibition reflects how antioxidant compound are able to remove $DPPH^{\cdot}$ radical at the given time.

Inhibition (%) = (Ao - At / Ao) x 100

2.8 Statistical analysis

Results were statistically evaluated by the Analysis of Variance (ANOVA – Multiple Range Tests, Method: 95.0 percent LSD) using statistical software STATGRAPHICS (Centurion XVI.I, USA).

3 RESULTS AND DISCUSSION

In this work the content of total polyphenols in carrot was watched and evaluated. The results are shown in Table 1.

Table 1: Average content of total polyphenols (mg/kg) in selected varieties of carrots

vegetable	variety	TPC (mg/kg)
	Jitka	81.25 ± 13.11^{a}
	Kardila	88.71 ± 7.47^{ab}
	Katlen	97.10 ± 11.38^{ab}
	Rubína	102.18 ± 6.68^{bc}
	Koloseum	113.69 ± 11.57^{d}
$HD_{0,05}$	15.8749	
$HD_{0,01}$	21.9469	

LSD Test on the significance: α: < 0.05

Total polyphenols content in samples ranges from 81.25 ± 13.11mg/kg to 113.69 ± 11.57 mg/kg. Statistically significant the highest value of total polyphenols was recorded in variety of Koloseum (113.69 ± 11.57 mg/kg). The lowest content of total polyphenols was recorded in variety of Jitka (81.25 ± 13.11 mg/kg). Based on the measured values of total polyphenols varieties of carrot can be classified as follows: Koloseum (113.69 mg/kg) > Rubína (102.18 mg/kg) > Katlen (97.10 mg/kg) > Kardila (88.71 mg/kg) > Jitka (81.25 mg/kg) Algarra et al. (2014) reported that the content of polyphenols in carrot was 94 mg/kg. Bembem a Sadana (2014) determined higher content of polyphenols in carrot, in comparison with our results. Their value was 320 mg/kg. The highest levels of polyphenols in carrots recorded Leahu et al. (2013), namely 652 ± 0.85 mg/kg. Jamada et al. (2011) referred that the content of total polyphenols was in interval from 455 to 697 mg/kg. Polyphenols are the most widespread and most numerous group of plant secondary metabolites and are an integral part of the diet of all living organisms. Natural polyphenolic compounds are ranked among the most abundant substance exhibiting antioxidant activity in our diet.

Another indicator that has been evaluated and compared was the content of ß-carotenes in varieties of carrot. Carrot is considered one of the most important source of carotenoids, especially ß-carotene.

The results of the determinations of ß-carotenes in the samples of carrot are shown in Table 2.

Table 2: Average content of ß-carotenes (mg/kg) in selected varieties of carrots

vegetable	**variety**	**ß-carotenes (mg/kg)**
	Jitka	24.58 ± 2.38^{a}
	Kardila	47.42 ± 3.97^{b}
	Katlen	44.19 ± 3.01^{b}
	Rubína	29.19 ± 3.76^{a}
	Koloseum	124.28 ± 3.54^{c}
$HD_{0,05}$	5.10442	
$HD_{0,01}$	7.05682	

LSD Test on the significance: α: <0.05

On the basis of gained results we can conclude, that statistically significant the highest value of ß-carotenes was recorded in variety Koloseum (124.28 ± 3.54 mg/kg). The lowest content of ß-carotenes was detected in variety Jitka (24.58 ± 2.38 mg/kg). In variety Koloseum the average content of ß-carotenes was 5.05 times higher than in the variety Jitka.

Karnjanawipagul et al. (2010) reported that the content of ß-carotenes in carrot samples was in the range from 72.3 – 145.9 mg/kg. Ullah et al. (2011) indicated 112.1 mg/kg ß-carotenes in carrot. Rebecca et al. (2014) published a higher value of ß-carotenes in carrot. Their value represented 183 mg/kg ß-carotenes in carrot.

Carotenoids with polyphenols are a phytochemicals that are responsible for the antioxidant activity of carrots. They protect human body against cardiovascular disease, arteriosclerosis and cancer (Ciccone et al., 2013; Relevy et al., 2015).

In the present work it was detected, that antioxidant activity in samples of carrot ranges from 6.88 ± 0.92 % (in variety of Jitka) to 9.83 ± 0.62 % (in variety of Koloseum) (Table 3).

Table 3: Average values of antioxidant activity (% inibition) in carrot

vegetable	variety	AOA (% inhibition)
	Jitka	6.88 ± 0.92^{a}
	Kardila	9.42 ± 0.68^{c}
	Katlen	8.75 ± 0.78^{bc}
	Rubína	7.54 ± 0.94^{ab}
	Koloseum	9.83 ± 0.62^{c}
$HD_{0,05}$	1.20821	
$HD_{0,01}$	1.67034	

LSD Test on the significance: α: <0.05

In the variety Koloseum the average value of antioxidant activity is 1.4- times higher than that of the variety Jitka (6.88 %) and 1.3- times higher than in the variety Rubína (7.54 %). Our obtained results are in accordance with findings Algarra et al. (2014), who also determined the values of antioxidant activity in carrot in the interval from 1.4 % to 17.6 %. Bembem et al. (2014) also determined the value of antioxidant activity in carrot (11.2 %).

In this paper also relations among content of polyphenols, ß-carotenes and antioxidant activity were evaluated (Figure 1 – 6). Our work was in coherence with the findings of Číž et al. (2010), Hu (2012) who indicated correlations between content of polyphenols in the onion, carrot, potato, cabagge and antioxidant activity.

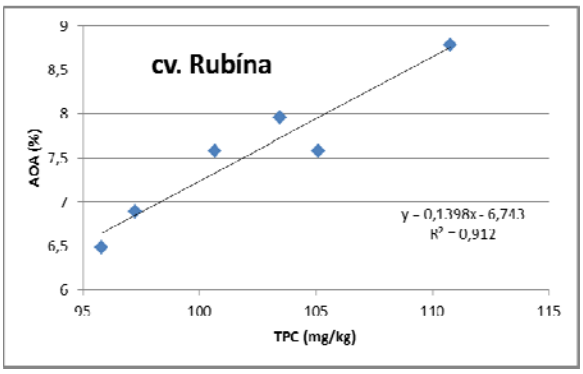


Figure 1: Relathionship between TPC and AOA in carrot 'Rubína'

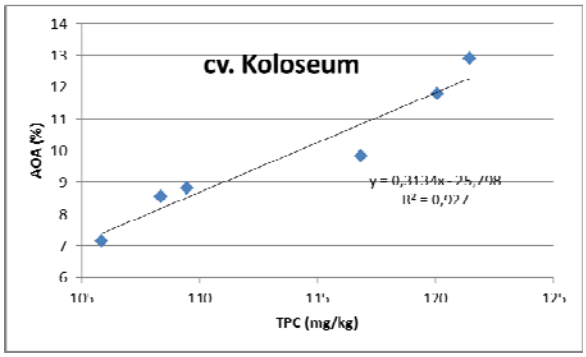


Figure 2: Relathionshipbetween TPC and AOA in carrot 'Koloseum'

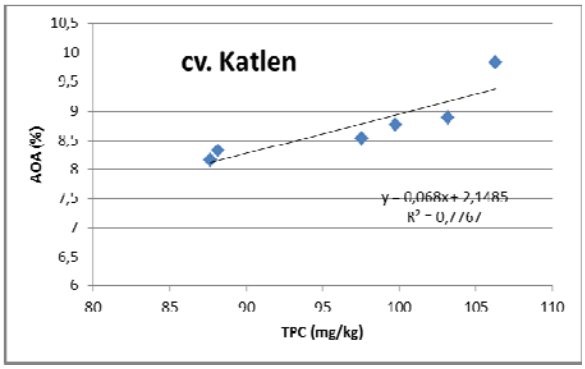


Figure 3: Relathionship between TPC and AOA in carrot 'Katlen'

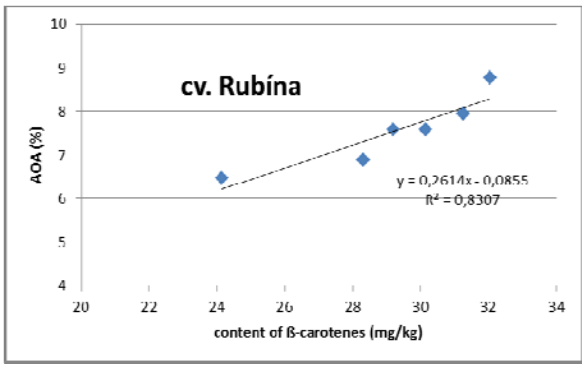


Figure 4: Relathionship between content of ß-carotenes and AOA in carrot 'Rubína'

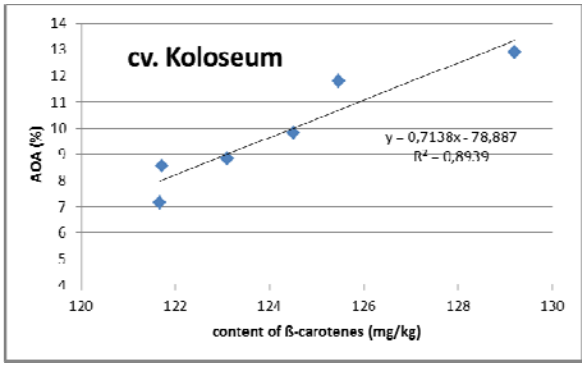


Figure 5: Relathionship between content of ß-carotenes and AOA in carrot 'Koloseum'

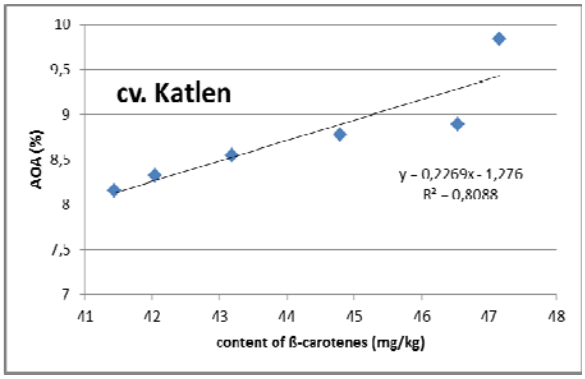


Figure 6: Relathionship between content of ß-carotenes and AOA in carrot 'Katlen'

4 CONCLUSION

Vegetable generally is a source of substances of high biological and nutritional value. Carrot is important in human nutrition and also in animal nutrition. Carrot is very popular vegetable for its important vitamins (group B, provitamin A, vitamin C), sugars and minerals in particular Ca, F, Se and Mg. It is also a rich source of chemoprotective compounds that protect the body against many diseases of civilization. The content of biologically active substances (polyphenols) in carrot root depends on various factors such as: area in which the carrot is grown (agrochemical characteristic of soil), climatic conditions in the region during the growing season, cultivation technology but also the variety. The obtained results suggest that the carrot is a rich source of carotenes. We determined the highest content in 'Koloseum' 113.69±11.57 mg/kg and also there was determined the highest value of antioxidant activity 9.83±0.62.

5 ACKNOWLEDGEMENT

This work was supported by scientific grant VEGA 1/0290/14, VEGA 1/0456/12. This work was co-funded by European Community under project no 26220220180: Building Research Centre „AgroBioTech".

6 REFERENCES

Agarwal S. 2012. Lycozen-gt: a super anti-oxidant multivitamin, multimineral formulation with goodness of lycopene, green tea & grape seed extract for excellent protection. International Journal of Research in Pharmacology and Pharmacotherapeutics, 1: 103-120

Algarra M., Fernandes A., Mateus N., Freitas V., Joaquim C.G., Silva E., Casad CH. 2014. Anthocyanin profile and antioxidant capacity of black carrots (Daucus carota L. ssp. sativus var. atrorubens Alef.) from Cuevas Bajas, Spain. Journal of Food Composition and Analysis, 33: 71-76, doi: 10.1016/j.jfca.2013.11.005

Augspole I., Rackejeva T., Kruma Z., Dimins F. 2014. Shredded carrots quality providing by treatment with Hydrogen peroxide. 9th Baltic Conference on "Food for Consumer Well - Being" FOODBALT 2014, 150-154

Augustynowicz J., Dlugosz-Grochowska O.G., Kostecka-Gugata A.M., Leja M., Kruszek M.K., Swiderski A. 2014. Callitriche cophocarpa – a new rich source of active phenolic compounds. Central European Journal of Chemistry, 12: 519-527, doi: 10.2478/s11532-013-0404-3

Balasundram N. 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurence, and potential uses. Food Chemistry, 99: 191-203, doi: 10.1016/j.foodchem.2005.07.042

Bembem K., Sadana B. 2014. Effect of different cooking methods on the antioxidant components of Carrot. Bioscience Discovery, 5: 112-116

Brand-Williams W., Cuvelier M.E., Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft and Technologie, 28: 25-30, doi: 10.1016/S0023-6438(95)80008-5

Carlos J., Dias S. 2014. Nutritional and Health Benefits of Carrots and Their Seed Extracts. Food and Nutrition Sciences, 5: 2147-2156, doi: 0.4236/fns.2014.522227

Chu Y., Sun J., Wu X., Liu R.H. 2002. Antioxidant and Antiproliferative Activities of common Vegetables. Journal of Agricultural and Food Chemistry, 50: 6910-6916, doi: 10.1021/jf020665f

Ciccone M.M., Cortese F., Gesualdo M., Carbonara S., Zito A., Ricci G., De Pascalis F., Scicchitano P., Riccioni G. 2013. Dietary intake of carotenoids and their antioxidant and anti-inflammatory effects in cardiovascular care. Mediators of Inflammation, Volume 2013, 11 pages.

Číž M., Čížová H., Denev P., Kratchanova M., Slavov A., Lojek A. 2010. Different methods for control and comparison of the antioxidant properties of vegetables. Food Control, 21, 518-523, doi: 10.1016/j.foodcont.2009.07.017

Essing F.B. 2013. What's in a Family? The Apiaceae. Florida Gardening, 18: 36-37

Fiedor J., Burda K. 2014. Potential Role of Carotenoids as Antioxidants in Human Health and Disease. Nutriens, 6: 466-488, doi: 10.3390/nu6020466

Gupta V., Kunari S., Kumar A. 2012. Mechanism of oxygen free radical generation and Endogenous Antioxidants. The Journal of Phytopharmacology, 1: 89-100

Hager T.J., Howard L.R. 2006. Processing Effects on Carrot Phytonutriens. Horticultural Science, 41: 74-79

Hu Ch. 2012. Factors affecting phytochemical composition and antioxidant activity of Ontario vegetable crops. (A thesis presented to the University of Guelphl). Guelphl, Ontario, Canada. P. 194

Jamada M., Yamauchi J., Hosoyamada Y. 2011. The properties of the components of carrot leaf, and their effect on serum lipids in cholesterol-fed rats. Journal for the Integrated Study of Dietary Habits, 22: 148-152, doi: 10.2740/jisdh.22.148

Jamuna K.S., Ramesh C.K., Srinivasa T.R., Raghu K.L. 2011. In vitro antioxidant studies in some common fruits. International Journal of Pharmacy and Pharmaceutical Sciences, 3: 60-63

Karnjanawipagul P., Nittayanuntawech W., Rojsanga, P., Suntornsuk, L. 2010. Analysis of ß-carotene in carrot by spectrophotometry. Journal of Pharmaceutical Science, 37: 8-16

Kumari S., Maguddajao A.V.Z., Prashar S, Kumar C.J.S. 2014. The antioxidant revolution - to protect against diseases & to maintain optimum health and wellbeing. International Journal of Pharmacology Research, 4: 47-51

Lachman J., Proněk D., Hejtmánková A., Pivec V., Faitová K. 2003. Total polyphenol and main flavonoid antioxidants in different onion (*Allium cepa* L.) varieties. Scientia Horticulturae, 30: 142-147

Leahu A., Damian C., Carpiuc N., Oroian M., Avramiuc M. 2013. Change in colour and physicochemical quality of carrot juice mixed with other fruits. Journal of Agroalimentary processes and technologies, 19: 241-246.

Leja M., Kaminská I., Kramer M., Maksylewicz-Kaul A., Kammerer D., Carle R., Baranski R. 2013. The Content of Phenolic Compounds and Radical Scavenging Activity Varies with Carrot Origin and Root Color. Plant Foods Human Nutrition, 68: 163-170, doi: 10.1007/s11130-013-0351-3

Liu R.H. 2003. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. American Society for Clinical Nutrition, 78: 517-520

Myojin C., Enami N., Nagata A., Yamaguchi T., Takamura H., Matora T. 2008. Changes in the Radical-Scavenging Activity of Bitter Gourd (Momordica charantia L.) during Freezing and Frozen Storage with or without Blanching. Journal of Food Science, 73: 546-550, doi: 10.1111/j.1750-3841.2008.00886.x

Nambia V.S., Daniel M., Guin P. 2010. Characterization of Polyphenols from Coriander leaves (coriandrum sativum), red amaranthus (a. paniculatus) and green amaranthus (a. frumentaceus) using paper chromatography: and their health implications. Journal of Herbal Medicine and Toxicology, 4: 173-177

Ndhlala A.R., Moyo M., Van Staden J. 2010. Natural Antioxidants: Fascinating or Mythical Biomolecules? Molecules, 15: 6905-6930, doi: 10.3390/molecules15106905

Pandey R.B., Rizvi S.I. 2009. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Medicine and Cellular Lengevity, 2: 270-278, doi: 10.4161/oxim.2.5.9498

Pandey S., Kumar P, Verna S. 2012. Comparing of antioxidant and DPPH induced free radical scavenging activity of Sesbania grandiflora and Acacia nilotica plants. The Journal of Phytopharmacology, 1: 33-42

Prasad M.P., Rajkumar R. 2014. In vitro antioxidant assay of citrus species using DPPH method. Indian Journal of Advances Plant Research, 1: 01-03

Rao A.V., Rao L.G. 2007. Carotenoids and human health. Pharmacological research, 55: 207-213, doi: 10.1016/j.phrs.2007.01.012

Rebecca L.J., Sharmila S., Das M.P., Seshiah C. 2014. Extraction and purification of carotenoids from vegetables. Journal of Chemical and Pharmaceutical Research, 6: 594-598

Relevy N.Z., Rühl R., Harari A., Grosskopf I., Barshack I., Ben-Amotz A., Nir U., Gottlieb H., Kamari Y., Harats D., Shaish A. 2015. 9-cis ß-carotene Inhibits Atherosclerosis Development in Female LDLR-/-Mice. Functional Foods in Health and Disease, 5: 67-79

Sharma K.D., Karki S., Thakur N.S., Attri S. 2012. Chemical composition, functional properties and processing of carrot. Journal of Food Science and Technology, 49: 22-32, doi: 10.1007/s13197-011-0310-7

Shukla G., Sarika M., Saritha D., Kumar C.J.S. 2014. Lycotenforte capsules: a multiple nutrient antioxidant, anti-microbial, anti-inflammatory protection with anti-aging benefits. International. Journal of Innovative Drug Discovery, 4: 25-30

Silva Dias J.C. 2014. Nutritional and Health Benefits of Carrots and Their Seed Extracts. Food and Nutrition Sciences, 5: 2147-2156, doi: 10.4236/fns.2014.522227

Sun T., Powers J.R., Tang J. 2003. Evaluation of the antioxidant activity of asparagus broccolia their juices. Food Chemistry 105: 101-106, doi: 10.1016/j.foodchem.2007.03.048

Tanaka T., Shnimizu M., Moriwaki H. 2012. Cancer Chemoprevention by Carotenoids. Molecules, 17: 3202-3242, doi: 10.3390/molecules17033202

Ullah N., Khan A., Khan F.A., Khurram M., Hussan M., Khayam M.U., Amin M., Hussain J. 2011. Composition and isolation of beta carotene from different vegetables and their effect on human serum retina level. Middle-East Journal of Scientific Research, 9: 496-502

COBISS Code 1.01

DOI: 10.14720/aas.2015.105.2.14
Agrovoc descriptors: soybeans, *Glycine max*, phosphate fertilizers, biofertilizers, spacing, planting, weed control, crop management, crop yield

Agris category code: f01, f04, f08, h01

The effects of planting arrangement and phosphate biofertilizer on soybean under different weed interference periods

G. R. MOHAMMADI[1,*], S. CHATRNOUR[1], S. JALALI[1] and D. KAHRIZI[1]

Received November 11, 2014; accepted August 24, 2015.
Delo je prispelo 11. novembra 2014, sprejeto 24. avgusta 2015.

ABSTRACT

This study was conducted to evaluate the effects of planting arrangement and phosphate biofertilizer on soybean yield and yield components under different weed interference periods at the Agricultural Research Farm of Razi University, Kermanshah, west Iran. The experiment was a factorial with three factors arranged in a randomized complete block design with four replications. The first factor was planting arrangement (50 and 5 cm (P1) or 25 and 10 cm (P2) for inter-row and inter-plant spacings, respectively), the second factor was phosphate biofertilizer (no-inoculation (I0) and inoculation (I1)) and the third factor was weed treatment (full season weed-free condition (W0), weedy condition until soybean 4-trifoliate stage (W1), weedy condition until soybean flowering stage (W2) and full season weedy condition (W3)). Results revealed that the highest soybean yield occurred when weeds were controlled throughout the growing season and soybean was planted at the inter-row and inter-plant spacings of 25 and 10 cm, respectively (P2) whether phosphate biofertilizer was used or not. For both planting arrangements, full season weedy condition at the lack of the biofertilizer led to the lowest soybean yield produced. Weed biomass was not significantly affected by use of biofertilizer. The highest weed biomass was established in plots without weed control throughout the whole growing season and soybean was planted in a wider row spacing and a less uniform spatial arrangement (P1). Moreover, For W2 and W3 treatments, soybean planted in a narrower row spacing and a more uniform spatial arrangement (P2) produced a notable lower weed biomass, so that, this planting arrangement reduced weed biomass by 31.8 and 31.7 % in W2 and W3, respectively as compared to the P1 planting arrangement. It can be concluded that soybean planting in a more uniform spatial arrangement via a narrower row spacing can significantly improve soybean yield and suppress weeds. Phosphate biofertilizer had no significant effect on soybean yield when soybean was planted as the P2 and weeds were controlled throughout the growing season.

Key words: *Glycine max*, phosphate biofertilizer, planting arrangement, soybean yield, weed control

IZVLEČEK

UČINKI NAČINOV SETVE IN UPORABE FOSFORJEVIH BIO-GNOJIL NA PRIDELEK SOJE OD ČASOVNO RAZLIČNIH ZATIRANJ PLEVELOV

V raziskavi, ki je bila izvedena na Agricultural Research Farm, Razi University, Kermanshah, zahodni Iran, so bili ovrednoteni učinki prostorske razporeditve rastlin (načinov setve) in uporabe fosforjevih bio-gnojil na pridelek soje in njegove komponente pri različnih zapleveljenostih. Poskus je bil zasnovan kot naključni bločni, trifaktorski poskus s štirimi ponovitvami. Prvi preučevani dejavnik je bila razporeditev rastlin v odvisnosti od načina setve, 50 in 5 cm (P1) ali 25 in 10 cm (P2), kot razdalji setve med vrstami in znotraj vrste. Drugi dejavnik je bila uporaba fosforjevih bio-gnojil (brez inokulacije (I0) in z inokulacijo (I1)) in tretji je bilo obravnavanje s pleveli (cela sezona brez plevelov (W0), zapleveljeno do stopnje razvoja, ko ima soja 4 trojnate liste (W1), zapleveljeno do začetka cvetenja soje (W2) in zapleveljeno celo rastno sezono (W3). Rezultati so pokazali, da je bil pridelek soje največji pri zatiranju plevelov skozi celo rastno sezono in ko je bila soja posejana v vrstah s 25 cm razmikom in z 10 cm razdaljo med rastlinami v vrsti (P2), ne glede na uporabo fosforjeva bio-gnojila. Zapleveljenost celo sezono in odsotnost gnojenja z bio-gnojili je dala ne glede na način setve najmanjši pridelek. Uporaba bio-gnojil ni značilno vplivala na biomaso plevelov. Največja biomasa plevelov je bila, kadar ti niso bili zatirani celo rastno sezono in, ko je bila soja posejana v vrstah s širšim razmikom, torej z manj enakomerno prostorsko razporeditvijo (P1). Pri obravnavanjih W2 in W3, ko je bila soja posejana v vrstah z manjšim razmikom in so bile rastline bolj enakomerno razporejene (P2), so imeli pleveli opazno manjšo biomaso. Takšni razporeditvi rastlin soje (W2 in W3) sta zmanjšali biomaso plevelov za 31.8 in 31.7 %, v primerjavi z raporeditvijo pri obravnavanju P1. Zaključimo lahko, da setev soje v vrstah z ožjim razmikom značilno poveča njen pridelek in zavre rast plevelov. Uporaba fosforjevih bio-gnojil ni imela značilnega vpliva na pridelek soje, kadar je bila ta posejana v vrstah z ožjim razmikom, P2, in če so bili pleveli nadzorovani celo rastno sezono.

Ključne besede: *Glycine max*, fosforjeva bio-gnijila, razporeditev rastlin, pridelek soje, nadzor plevelov

[1] Dept. of Crop Production and Breeding, Faculty of Agriculture and Natural Resources, Razi University, Kermanshah, Iran

[*] Corresponding author: mohammadi114@yahoo.com

1 INTRODUCTION

Soybean (*Glycine max* L.) is an important two-purpose crop which is extensively grown as a source of edible oil and protein for human nutrition in Iran. In soybean, weed infestation is considered a persistent and complex constraint in many regions of the world, as it influences soybean growth and development through competition for nutrients, water and light (Vollmann et al. 2010) as well as the production of allelopathic compounds (Rice 1984; Bhowmik and Doll 1982). Weeds are a serious constraint to easy harvesting in soybean and can reduce yield and economic returns. Thus, weed control is considered a key factor for successful soybean production, and various weed management systems have been developed for that purpose (Buhler and Hartzler, 2004). Weed control in soybean can be labor intensive or involve the intensive use of herbicides in Iran. Intensive herbicide use can increase costs, pose a threat to the environment and may promote the development of herbicide resistancein weeds. The implementation of an integrated weed management (IWM) system is seen by many weed scientists as a means of achieving the goal of reducing the amount of herbicide used while still maintaining crop yield (Swanton and Weise 1991).

According to Johnson et al. (1997) there is a trend towards reducing crop row width as a means of increasing crop competition to suppress weeds. Early results in narrow-row soybean show that this method can provide adequate weed control and soybean yield (Steckel et al. 1990; Prostko and Meade 1993). Narrow rows make more efficient use of available resources and should allow quicker canopy closure and thus quicker shading of the ground thereby improving weed control (Fernandez et al. 2002). In general, crop competitive ability can also be increased by improving planting uniformity. Olsen et al (2005a; 2005b) reported that wheat produced more biomass and had less weed biomass as crop planting uniformity increased. According to Weiner et al. (2001) a more uniform planting distribution should enable crops to compete more successfully with weeds. In Iran, soybean is usually planted in a wide row spacing (50 cm). This row spacing can reduce potential crop yield and economic return due to less efficient use of available resources such as light, water and nutrients by the soybean plants and increase weed infestation.

Moreover, the competitive relationship between crop and weeds is highly dependent on many factors including the characteristics of the crop and the weeds, the environmental variables, the cultural practices (Knezevic et al. 2002) and supply and availability of nutrients (Evans et al. 2003; Di Tomaso 1995). The availability of nutrients can influence the timeliness and extent of early season competition from weeds (Weaver et al. 1992). Phosphorus is an important element which can affect the competitive interactions between a crop and weeds. It is only second to nitrogen as a mineral nutrient required for plant growth (Ogbo 2010). Most of the soils in Iran are phosphorous deficient or marginally deficient and a massive increase in the rate of application of chemical fertilizers has been adopted to ameliorate this deficiency (Cox et al. 1993). However, a large proportion of the phosphorous content of chemical fertilizers is quickly transformed to the insoluble form such as calcium phosphate, thereby making them unavailable to plants. In addition, there are global concerns that the un-balanced use of chemical fertilizers has a role in environmental degradation and climate change (Day and Quinn 1989; Daynard et al. 1971). However, nutrients applied to soils are also available for weeds and these un-wanted plants are better able to utilize added nutrients than crops (Carlson and Hill 1986; Peterson and Nalewja 1992). Therefore, in an attempt to reduce environmental risk and cost with chemical fertilizer use and increase crop nutrient use efficiency, phosphorous biofertilizers (phosphate-solubilizing microorganisms) has been considered as possible substitutes for traditional mineral P fertilizer. These microorganisms have been distinguished by their relative ability to dissolve calcium phosphate and apatite in association with plant roots. This activity was attributed to organic acid and chelating metabolites produced by these microorganisms (Deinum et al. 1996; Dong and Pierdominici 1995). However, phosphate biofertilizer have shown variation in their performance in related to their environmental condition.

This study was carried out to investigate the effects of phosphorus biofertilizer and planting arrangement on soybean under different weed pressure treatments in Kermanshah, west Iran.

2 MATERIALS AND METHODS

The study was carried out in 2009 at the Agricultural Research Farm of Razi University, Kermanshah, west Iran. The soil type was a silty clay with a pH of 7.8 and 0.8 % organic matter. The land was plowed and disked before planting. The soybean cultivar was 'Williams' (a cultivar that is commonly planted in the region). All soybean seeds were inoculated with *Bradyrhizobium japonicum* Kirchner bacterium prior to sowing. The crop was planted on 9 May 2009 at a constant density of 40 plants m^{-2}. Soybean is a summer and irrigated crop in western Iran; therefore, it is not dependent on seasonal rainfall. Irrigations were carried out at 7-9 day intervals throughout the growing season in term of crop need.

The experiment was a factorial with three factors arranged in a randomized complete block design with four replications. The first factor was planting arrangement (50 and 5 cm (P1) or 25 and 10 cm (P2) for inter-row and inter-plant spacings, respectively), the second factor was phosphate biofertilizer (no-inoculation (I0) and inoculation (I1)) and the third factor was weed treatment (full season weed-free condition (W0), weedy condition until soybean 4-trifoliate stage (W1), weedy condition until soybean flowering stage (W2) and full season weedy condition (W3)). Each plot consisted of six soybean rows of 8 m long with predetermined inter-row and inter-plant spacings. Before planting, the seeds were also inoculated with phosphate biofertilizer (Barvar 2) containing the phosphate solubilizing microorganisms *Pantoea agglomerans* Eving and Fife and *Pseudomonas putida* Trevisan. Weed removal was carried out by hand.

At maturity, soybean plants located at 4 m^2 from each plot were harvested by hand and allowed to dry to a constant mass and weighed and biological yield (total aboveground dry mass) was determined. Subsequently, they were threshed and cleaned and seed yield was calculated. Then harvest index (HI) was calculated according to the following equation:

$$HI = (\text{Seed yield} / \text{Biological yield}) \times 100$$

Additionally, 100-seed weight were determined according to the recommendations of the International Seed Testing Association (ISTA) (Draper, 1985). Before harvesting, the number of pods per plant and the number of seeds per pod were measured on 5 randomly selected plants in the centre rows of each plot, except from the rows that were used for yield measurement. Weed biomass was also measured by harvesting weeds at the ground level in three random 0.5×0.5 m quadrats in each plot at the end of the growing season for the W3 treatment and before each weed removal for the W1 and W2 treatments. Then weeds dried at 75° C to constant mass and weighed. Data analyses were carried out using SAS (SAS Institute 2003).

3 RESULTS AND DISCUSSION

Analysis of variance (Table 1) revealed that all of the traits under study including soybean seed yield (SY), the number of pods per plant (PPP), the number of seeds per pod (SPP), 100-seed weight (SW), harvest index (HI) and weed biomass (WB) were significantly affected by weed treatments (at the 0.01 level of probability). There was a significant three-way interaction (weed treatment×planting arrangement×phosphate biofertilizer) for SY, PPP and SPP. The significant two-way interactions including weed treatment×planting arrangement, weed treatment× phosphate biofertilizer and planting arrangement×phosphate biofertilizer were observed for HI. However, SW was significantly affected by the two-way interactions including weed treatment×planting arrangement, weed treatment×phosphate biofertilizer. However, WB

was influenced by a two-way interaction (weed treatment×planting arrangement) and phosphate biofertilizer alone or in combination with other factors had no significant effect on this trait.

Table 1: Analysis of variance of the traits under study

Source of Variance	Mean Square					
	Seed yield	Pod/plant	Seed/pod	100-seed weight	Harvest index	Weed biomass
Replication	309.03 ns	7.80 ns	0.04 ns	0.33 ns	1.24 ns	672420.05 ns
Weed Interference (WI)	94876.00 **	6854.90 **	0.19 **	1.80 **	314.09 **	2231939.50 **
Phosphate Biofertilizer (PB)	1407.20 *	172.50 **	0.01 ns	0.94 *	285.30 **	150.60 ns
Planting Arrangement (PA)	5682.70 **	3719.40 **	0.03 ns	1.02 *	3.77 ns	3226900.00 **
WI×PB	2124.90 **	752.40 **	0.09 **	0.91 *	42.80 **	236226.10 ns
WI×PA	113.50 ns	658.60 **	0.06 **	1.09 **	57.30 **	926873.05 *
PB×PA	989.40 ns	516.90 **	0.08 **	0.02 ns	147.80 **	74342.09 ns
WI×PB×PA	3973.90 **	204.12 **	0.06 **	0.57 ns	25.15 ns	53670.50 ns
Error	335.60	17.23	0.01	0.23	9.32	257976.06

ns, * and **: Non significant and significant at the 0.05 and 0.01 level of probability, respectively

The highest SY was obtained when weeds were controlled for all of the growing season (W0) and soybean was planted at the inter-row and inter-plant spacings of 25 and 10 cm, respectively (P2) whether phosphate biofertilizer was used or not (Table 2). For both planting arrangements, full season weedy condition (W3) and at the lack of the biofertilizer (I0) led to the lowest SY (Table 2). It seems that in weed free condition and a more uniform planting arrangement soybean yield is not significantly affected by phosphate biofertilizer due to a lower competition for this essential element. However, in the presence of weeds, phosphate biofertilizer could reduce the harmful effects of these unwanted plants. In general, soybean seed yield decreased when the weed interference period increased. Although, in most cases, the reductions were lower when soybean was planted as the P2 planting arrangement when compared with the P1 planting arrangement (Table 2). Row spacing and spatial uniformity can play important roles to manage weeds in cropping systems. Mohammadi et al. (2012) reported that corn yield was improved and weed biomass was decreased in response to decreasing row spacing. Moreover a more uniform crop spatial (as seen at the P2 planting arrangement) decreases competition within the crop population early in the growing season (Olsen and Weiner 2007) and maximizes the total shade cast by the crop by reducing self shading (Weiner et al. 2001). According to Kristensen et al. (2008) in the presence of weeds the highest yields were obtained with high spatial uniformity.

The highest PPP was occurred in the plots in which weeds were removed throughout the growing season, phosphate biofertilizer was applied and soybean was planted as the P2 planting arrangement (Table 2). This can be attributed to the lack of weed harmful effects on the crop, more crop spatial uniformity and consequently a lower competition among the soybean plants. Moreover, many researchers have reported an improve in growth and P-uptake by crops through the inoculation of phosphate solubilizing microorganisms in pot experiments (Vassilev et al. 2006; Omar 1998) and under field conditions (Valverde et al. 2006; Duponnois et al. 2005; De Freitas et al. 1997). In a study, Mittal et al. (2008) observed two-fold increase in seed number of chickpea due to the use of phosphate solubilizing microorganisms.

Table 2: Soybean plant traits as influenced by weed treatment, planting arrangement and phosphate biofertilizer

Weed treatment	Planting arrangement	Phosphate biofertilizer	Soybean plant traits		
			Seed yield (g m^{-2})	Pods/plant	Seeds/pod
W0	P1	I0	305.6 b	86.6 c	2.1 ef
		I1	319.3 b	66.3 d	2.3 cde
	P2	I0	323.3 ab	101.7 b	2.3 cde
		I1	346.9 a	117.1 a	2.4 abc
W1	P1	I0	189.5 ef	55.3 fg	2.5 ab
		I1	206.9 de	63.5 de	2.4 abc
	P2	I0	239.3 c	68.8 d	2.5 ab
		I1	200.1 de	82.5 c	2.1 ef
W2	P1	I0	161.3 gh	56.2 fg	2.1 ef
		I1	191.9 ef	43.2 i	2.2 def
	P2	I0	220.1 cd	63.0 de	2.4 abc
		I1	173.7 fg	52.4 gh	2.2 def
W3	P1	I0	134.3 ij	49.2 h	2.1 ef
		I1	141.6 hi	38.4 ij	2.1 ef
	P2	I0	114.9 j	36.7 j	2.1 ef
		I1	182.9 efg	58.5 ef	2.2 def
LSD (0.05)			26.1	5.9	0.2

Dissimilar letters at each column indicate the significant difference at the 0.05 level of probability (LSD test).
Abbreviations: W0, W1, W2 and W3: full season weed free condition, weedy condition until soybean 4-trifoliate stage, weedy condition until soybean flowering stage and full season weedy condition, respectively. P1: soybean planted at the inter-row and inter-plant spacings of 50 and 5 cm, respectively; P2: soybean planted at the inter-row and inter-plant spacings of 25 and 10 cm, respectively. I0 and I1: no inoculation and inoculation with phosphate biofertilizer, respectively.

The number of seeds per pod didn't show an obvious response to the treatments under study, although, in most cases, full season weedy condition and weedy condition until soybean flowering stage led to the lowest values of this trait (Table 2). Weed interference until 4-trifoliate stage didn't significantly influence 100-seed weight when compared with full season weed free condition, although, the longer weed interference reduced this yield component, notably (Fig. 1). However, for all of the weed treatments, the use of phosphate biofertilizer didn't significantly affect soybean 100-seed weight (Fig. 1). Moreover, for all weed treatments, 100-seed weight was higher when soybean was planted as the P2 planting arrangement (Fig. 2). Although, the positive effect of this planting arrangement on soybean seed mass was more obvious in the plots in which weeds were controlled throughout the growing season (Fig. 2).

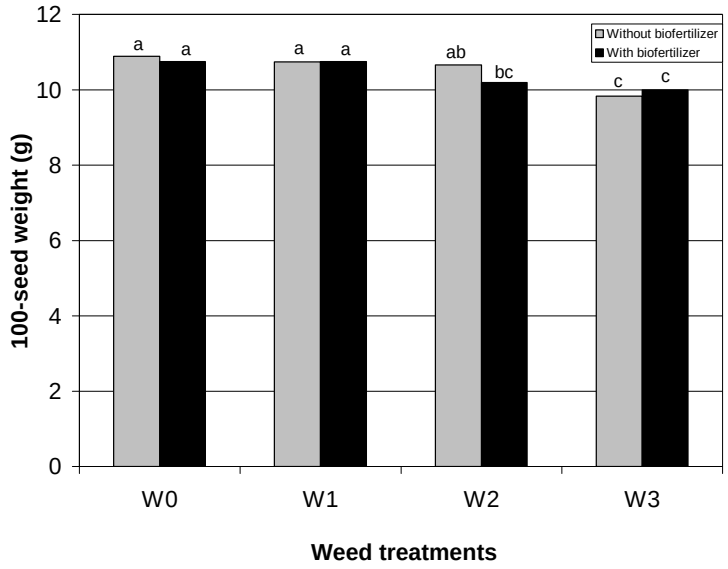


Figure 1: The effect of phosphate biofertilizer on soybean 100-seed weight under different weed treatments. Abbreviations: W0, W1, W2 and W3: full season weed free condition, weedy condition until soybean 4-trifoliate stage, weedy condition until soybean flowering stage and full season weedy condition, respectively.

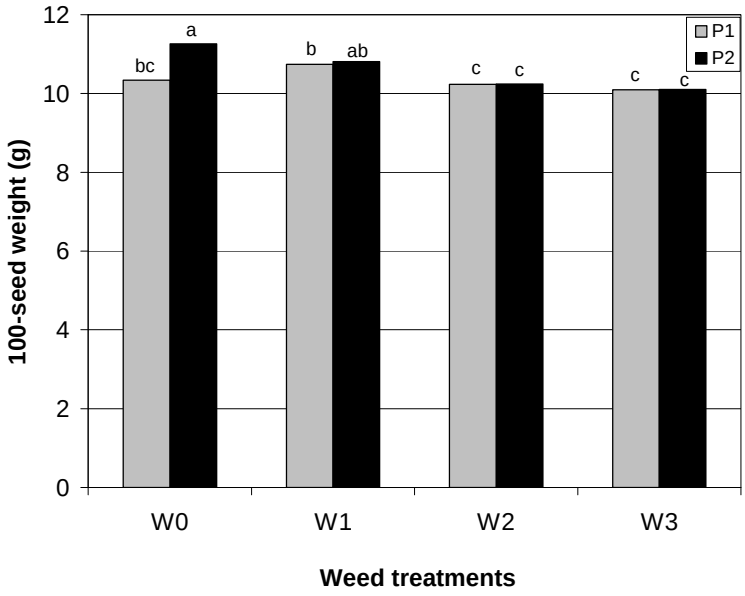


Figure 2: Soybean 100-seed weight as influenced by different planting arrangements and weed treatments. Abbreviations: W0, W1, W2 and W3: full season weed free condition, weedy condition until soybean 4-trifoliate stage, weedy condition until soybean flowering stage and full season weedy condition, respectively. P1: soybean planted at the inter-row and inter-plant spacings of 50 and 5 cm, respectively; P2: soybean planted at the inter-row and inter-plant spacings of 25 and 10 cm, respectively.

Harvest index was significantly influenced by phosphate biofertilizer×planting arrangement interaction (Table 1). The highest HI was observed in the more uniform spatial arrangement (P2) and when phosphate biofertilizer was applied (Fig. 3). Moreover, weed free condition for the entire growing season led to the highest HI when soybean was planted as the P2 planting arrangement (Fig. 4). Harvest index is the fraction of the total crop biomass allocated to the economic yield (Williams et al. 1989; Sto¨ckle et al. 1994) and a higher HI indicates a more crop efficiency to allocate the produced biomass to the seeds. It seems that, the lower inter- and intra-specific competitions and higher phosphorus available for the crop can significantly increase the biomass allocated to soybean generative organs and consequently improve HI.

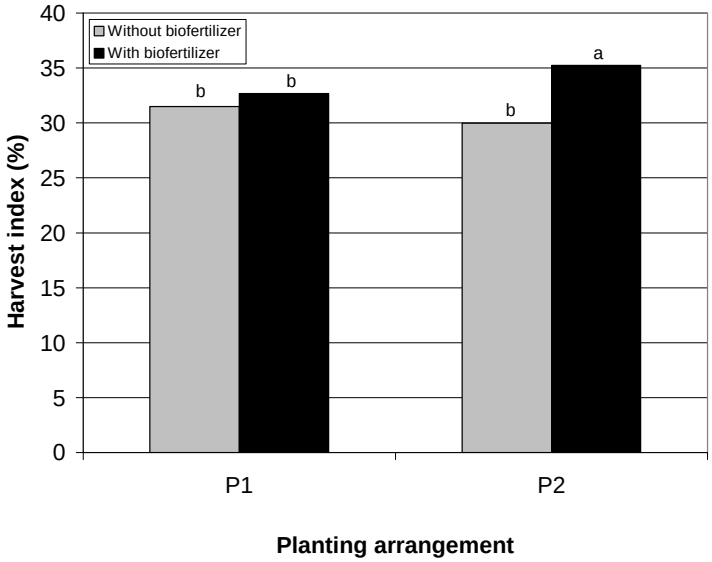


Figure 3: The effect of phosphate biofertilizer on soybean harvest index under different planting arrangement.
Abbreviations: P1: soybean planted at the inter-row and inter-plant spacings of 50 and 5 cm, respectively; P2: soybean planted at the inter-row and inter-plant spacings of 25 and 10 cm, respectively.

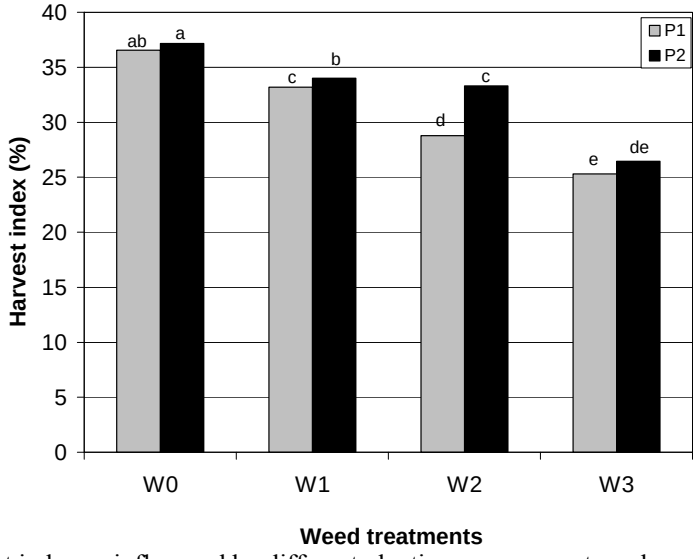


Figure 4: Soybean harvest index as influenced by different planting arrangements and weed treatments.
Abbreviations: W0, W1, W2 and W3: full season weed free condition, weedy condition until soybean 4-trifoliate stage, weedy condition until soybean flowering stage and full season weedy condition, respectively. P1: soybean planted at the inter-row and inter-plant spacings of 50 and 5 cm, respectively; P2: soybean planted at the inter-row and inter-plant spacings of 25 and 10 cm, respectively.

Weed biomass was also significantly affected by weed treatment×planting arrangement interaction (Table 1). The highest weed biomass was produced when weeds were not controlled throughout the growing season and soybean was planted in wider row spacing and a less uniform spatial arrangement (P1) (Fig. 5). For W2 and W3 treatments, soybean planted in a narrower row spacing and a more uniform spatial arrangement (P2) produced a notable lower weed biomass, so that, this planting arrangement reduced weed biomass by 31.8 and 31.7 % in W2 and W3, respectively as compared to the P1 planting arrangement. However, in W1 treatment there was no significant difference between the two planting arrangements in term of weed biomass (Fig. 5) indicating the important weed suppressing effect of a more uniform planting arrangement in the higher weed pressure conditions. In general, crop canopy expansion and soil cover vary with planting arrangement (Ottman and Welch 1989; Tetio-Kagho and Gardner 1988). According to Fernandez et al. (2002) radiation interception and use efficiencies as well as nitrogen use efficiency of crop were positively related to the increased planting uniformity and for maximum weed suppression, crop should be planted in a square or triangular lattice arrangement. In another study, Mohammadi et al. (2012) found that both crop yield and weed control can be improved by increasing the planting spatial uniformity in a corn cropping system.

There was a negative and significant correlation between soybean yield and weed biomass produced (r = -0.76). It can be concluded that increasing soybean yield in the P2 plots is mainly due to a higher weed suppressive effect of this planting arrangement. However, a lower intra-specific competition can also play an important role. Kristensen et al. (2008) reported that in the presence of weeds the highest yields were obtained with high crop density and high spatial uniformity.

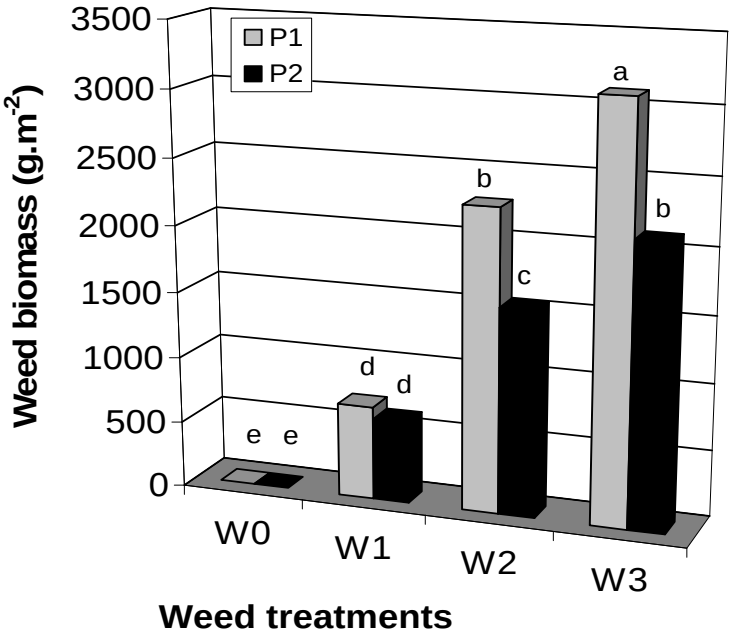


Figure 5: Weed biomass as influenced by different planting arrangements and weed treatments.
Abbreviations: W0, W1, W2 and W3: full season weed free condition, weedy condition until soybean 4-trifoliate stage, weedy condition until soybean flowering stage and full season weedy condition, respectively. P1: soybean planted at the inter-row and inter-plant spacings of 50 and 5 cm, respectively; P2: soybean planted at the inter-row and inter-plant spacings of 25 and 10 cm, respectively.

4 CONCLUSION

This study revealed that soybean planting in a more uniform spatial arrangement via a narrower row spacing can significantly improve soybean yield and reduce weed growth especially in a higher weed pressure condition. Phosphate biofertilizer had no positive effect on soybean yield when soybean was planted in a more uniform spatial arrangement (P2) and weeds were controlled for the entire growing season. However, in the presence of weeds and a decreased planting uniformity (P1), the biofertilizer could significantly improve soybean yield indicating a P-limitation in this condition probably due to the higher intra- and inter-specific competitions for this essential element.

5 REFERENCES

Bhowmik P.C. and Doll J.D. 1982. Corn and soybean response to allelopathic effects of weed and crop residues. Agron J. 74: 601-606, doi: 10.2134/agronj1982.00021962007400040005x

Buhler D.D. and Hartzler R.G. 2004. Weed biology and management. In: Boerma, H.R., Specht, J.E. (Eds.), Soybeans: Improvement, Production and Uses. 3rd ed., Series Agronomy, No. 16. American Society of Agronomy, Madison, WI, pp. 883–918

Carlson, H. L. and Hill J. E. 1986. Wild oat (*Avena fatua*) competition in spring wheat: effects of nitrogen fertilization. Weed Sci. 34: 29–33

Cox, W.J., S. Kalange, D.J.R. Cherney and Reid W.S. 1993. Growth, yield and quality of forage maize under different N management practices. Agron. J. 85: 341-347, doi: 10.2134/agronj1993.00021962008500020033x

Day, R.W. and G.P. Quinn 1989. Comparison of treatments after an analysis of variance in ecology Ecological Monographs, 59: 433-463, doi: 10.2307/1943075

Daynard, T. B., J. W. Tanner and G. Duncan 1971. Duration of the grain filling period and its relation to grain yield in corn, *Zea mays* L. Crop Sci. 11: 45–48, doi: 10.2135/cropsci1971.0011183X001100010015x

De Freitas, J.R., Banerjee, M.R., Germida, J.J. 1997. Phosphatesolubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (*Brassica napus* L.). Biology and Fertility of Soils 24: 358–364, doi: 10.1007/s003740050258

Deinum, B.R.D. Sulastri, M.H.J. Zeinab and A. Maassen. 1996. Effects of light intensity on growth, anatomy and forage quality of two tropical grasses (*Brachiaria brizantha* and *anicum maximum* var. trichoglume). Neth J Agric Sci 44: 111–124

Di Tomaso J.M. 1995. Approaches for improving crop competitiveness through the manipulation of fertilization strategies. Weed Sci 43: 491-497

Dong M. and Pierdominici M.G. 1995. Morphology and growth of stolons and rhizomes in three clonal grasses, as affected by different light supply. Vegetatio 116: 25–32

Draper S.R. 1985. International rules for seed testing. Seed Sci Technol 13: 342-343

Duponnois, R., Colombet, A., Hien, V. and Thioulouse, J. 2005. The mycorrhizal fungus *Glomus intraradices* and rock phosphate amendment influence plant growth and microbial activity in the rhizosphere of Acacia holosericea. Soil Biology and Biochemistry 37, 1460–1468, doi: 10.1016/j.soilbio.2004.09.016

Evans S.P., Knezevic S.Z., Shapiro C. and Lindquist J.L. 2003. Nitrogen level affects critical period for weed control in corn. Weed Sci 51: 408-417, doi: 10.1614/0043-1745(2003)051[0408:NAITCP]2.0.CO;2

Fernandez, O.N., O.R. Vignolio and E.C. Requesens 2002. Competition between corn (*Zea mays*) and bermudagrass (*Cynodon dactylon*) in relation to the crop plant arrangement, Agronomie, 22: 293-305, doi: 10.1051/agro:2002015

Johnson, G.A., Hoverstad, T.H. and Greenwald, R.E. 1997. Integrated weed management using narrow row crop spacing, herbicides and cultivation.Agron.J. 90, 40–46, doi: 10.2134/agronj1998.00021962009000010008x

Knezevic, S.Z., Evans, S.P., Blankenship, E.E., Van Acker, R.C. and Lindquist, J.L. 2002. Critical period for weed control: the concept and data analysis. Weed Sci 50: 773-786, doi: 10.1614/0043-1745(2002)050[0773:CPFWCT]2.0.CO;2

Kristensen L., Olsen J. and Weiner J. 2008. Crop density, sowing pattern, and nitrogen fertilization

effects on weed suppression and yield in spring wheat. Weed Sci. 56: 97-102, doi: 10.1614/WS-07-065.1

Mittal, V., O. Singh, H. Nayyar, J. Kaur and R. Tewari 2008. Stimulatory effect of phosphate-solubilizing fungal strains (*Aspergillus awamori* and *Penicillium citrinum*) on the yield of chickpea (*Cicer arietinum* L. cv. GPF2). Soil Biol. Biochem., 40: 718-727, doi: 10.1016/j.soilbio.2007.10.008

Mohammadi, G. R., M. E. Ghobadi and S. Sheikheh Poor. 2012. Phosphate biofertilizer, row spacing and plant density effects on corn (*Zea mays* L.) yield and weed growth. American Journal of Plant Sciences. 3: 425-429, doi: 10.4236/ajps.2012.34051

Ogbo, F.C. 2010. Conversion of cassava wastes for biofertilizer production using phosphate solubilizing fungi. Bioresource Technology 101: 4120–4124, doi: 10.1016/j.biortech.2009.12.057

Olsen, J. and J. Weiner. 2007. The influence of *Triticum aestivum* density, sowing pattern and nitrogen fertilization on leaf area index and its spatial variation. Basic Appl. Ecol. 8: 252–257, doi: 10.1016/j.baae.2006.03.013

Olsen, J., L. Kristensen, and J. Weiner. 2005a. Effects of density and spatial pattern of winter wheat on suppression of different weed species. Weed Sci. 53: 690–694, doi: 10.1614/WS-04-144R2.1

Olsen, J., L. Kristensen, J. Weiner, and H.-W. Griepentrog. 2005b. Increased density and spatial uniformity increases weed suppression by spring wheat (*Triticum aestivum*). Weed Res. 45: 316–321, doi: 10.1111/j.1365-3180.2005.00456.x

Omar, S.A., 1998. The role of rock phosphate solubilizing fungi and vesicular arbuscular mycorrhiza (VAM) in growth of wheat plants fertilized with rock phosphate. World Journal of Microbiology and Biotechnology 14: 211–219, doi: 10.1023/A:1008830129262

Ottman, M. J. and Welch, L. F. 1989. Planting patterns and radiation interception, plant nutrient concentration, and yield in corn. Agron J. 81: 167-174, doi: 10.2134/agronj1989.00021962008100020006x

Peterson, D.A., Nalewaja, J.D. 1992. Environment influences green foxtail competition with wheat. Weed Technol. 6: 607–610.

Prostko, E.P., Meade, J.A. 1993. Reduced rates of postemergence herbicides in conventional soybean (*Glycine max*).Weed Sci. 38: 541–545.

Rice, E.L. 1984. Allelopathy. Orlando, Florida, Academic Press (Second Edition), 422 pp.

SAS Institute, 2003. SAS/STAT. User's Guide. Version 9.1. SAS Inst., Inc., Cary, NC.

Steckel, L.E., Defelice, M.S. and Sims, B.D. 1990. Integrating reduced rates of postemergence herbicides and cultivation for broadleaf weed control in soybeans (*Glycine max*). Weed Sci. 38: 541–545

Sto¨ckle, C.O., Martin, S.A. and Campbell, G.S. 1994. CropSyst, a cropping systems simulation model: water/nitrogen budgets and crop yield. Agric. Sys. 46: 335–359, doi: 10.1016/0308-521X(94)90006-2

Swanton, C.J. and Weise, S.F. 1991. Integrated weed management: the rationale and approach. Weed Technol 5: 657-663

Tetio-Kagho F. and Gardner F.P. 1988. Responses of maize to plant population density: I. Canopy development, light relationships, and vegetative growth. Agron J. 80: 930-935, doi: 10.2134/agronj1988.00021962008000060018x

Valverde, A., Burgos, A., Fiscella, T., Rivas, R., Velazquez, E., Rodriguez, C. and Igual, J.M. 2006. Differential effects of co inoculations with Pseudomonas jessenii PS06 (a phosphate solubilizing bacterium) and Mesorhizobium ciceri c-2/2 strains on the growth and seed yield of chickpea under greenhouse and field conditions. Plant and Soil 287: 43–50, doi: 10.1007/s11104-006-9057-8

Vassilev, N., Medina, A., Azcon, R. and Vassilev, M. 2006. Microbial solubilization of rock phosphate on media containing agro-industrial wastes and effect of the resulting products on plant growth and P-uptake. Plant and Soil 287: 77–84, doi: 10.1007/s11104-006-9054-y

Vollmann, J., Wagentristl, H. and Hartl, W. 2010. The effects of simulated weed pressure on early maturity soybeans. Eur J Agron 32: 243-248, doi: 10.1016/j.eja.2010.01.001

Weaver, S.E., Kropff, M.J. and Groeneveld, R.M.W. 1992. Use of ecophysiological models for crop-weed interference: the critical period of weed interference. Weed Sci 40: 302-307

Weiner, J., H. W. Griepentrog, and L. Kristensen. 2001. Suppression of weeds by spring wheat (*Triticum aestivum*) increases with crop density and spatial uniformity. J. Appl. Ecol. 38: 784–790, doi: 10.1046/j.1365-2664.2001.00634.x

Williams, J.R., Jones, C.A., Kiniry, J.R. and Spanel, D.A. 1989. The EPIC crop growth model. Trans. ASAE, 32: 497–511, doi: 10.13031/2013.31032

COBISS Code 1.01

DOI: 10.14720/aas.2015.105.2.15
Agrovoc descriptors: *Trichogramma brassicae*, Hymenoptera, parasitoids, biological control, biological pest control, caterpillars, Slovenia

Agris category code: h01, h10

Prva najdba parazitoidne ose *Trichogramma brassicae* Bezdenko, 1968 (Hymenoptera, Trichogrammatidae) v Sloveniji

Tanja BOHINC[1], Stefan SCHMIDT[2], Juan Carlos MONJE[3], Stanislav TRDAN[4]

Received July 10, 2015; accepted September 07, 2015.
Delo je prispelo 10. julija 2015, sprejeto 07. septembra 2015.

IZVLEČEK

V prispevku predstavljamo parazitoidno oso, katero smo v Sloveniji prvič našli avgusta 2014, in sicer v jajčnem leglu kapusove sovke (*Mamestra brassicae*) na zelju. Parazitoidna osa *Trichogrammma brassicae* spada med jajčne parazitoide in je znana kot naravni sovražnik metuljev (Lepidoptera). Prvotno so omenjeno parazitoidno oso uporabljali za zatiranje koruzne vešče (*Ostrinia nubilalis*), pozneje pa je pridobila na pomenu tudi pri biotičnem zatiranju nekaterih ostalih gospodarsko pomembnih škodljivih vrst metuljev. S prvo potrditvijo zastopanosti *T. brassicae* v Sloveniji je izpolnjen prvi pogoj za njeno uvrstitev na Seznam domorodnih vrst organizmov za namen biotičnega varstva rastlin in s tem za njeno praktično uporabo pri biotičnem zatiranju škodljivih metuljev pri nas.

Ključne besede: *Trichogramma brassicae*, jajčni parazitoidi, biotično varstvo rastlin, gosenice

ABSTRACT

FIRST RECORD OF PARASITIC WASP *Trichogramma brassicae* BEZDENKO, 1968 (Hymenoptera, Trichogrammatidae) IN SLOVENIA

The paper presents the parasitic wasp, which occurrence in Slovenia was first confirmed in August 2014 on egg layers of cabbage moth (*Mamestra brassicae*) from cabbage. The wasp *Trichogrammma brassicae* belongs among egg parasitoids and it is especially known as biological control agent of lepidopteran pests. In the beginning the wasp was used for controlling European corn borer (*Ostrinia nubilalis*), later it becomes an important biological control agent of some other economically important lepidopteran pests. With the first confirmation of occurrence of *T. brassicae* in Slovenia first condition for its placing on the List of indegenous biological control agents - it contains the organisms which practical use in Slovenia is allowed - is fulfilled.

Key words: *Trichogramma brassicae*, egg parasitoids, biological control, caterpillars

1 UVOD S POUDARKOM NA POMENU JAJČNIH PARAZITOIDOV IZ RODU *Trichogramma*

V rod *Trichogramma* uvrščamo jajčne parazitoide, ki so pomembni naravni sovražniki v varstvu gojenih rastlinskih vrst že več kot 70 let (Kuske *et al*., 2003), tudi masovno namnoževanje omenjenih parazitoidov z namenom ciljnega spuščanja na kmetijska zemljišča pa se v svetu pojavlja že skoraj 100 let (Eizaguirre *et al*., 1998; Lundgren in Heimpel, 2003). Parazitoidne ose iz omenjenega rodu so naravni sovražniki nekaterih škodljivcev vrtnin in poljščin, uporabne pa so lahko tudi pri zatiranju škodljivcev gozdnega drevja (Bai *et al*., 1995; Kuske *et al.*, 2003). Vrste iz rodu

[1] dr., Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za agronomijo, Jamnikarjeva 101, SI-1000 Ljubljana; mail: tanja.bohinc@bf.uni-lj.si

[2] dr., Zoologische Staatssammlung, Muenchhausenstr. 21, D-81247, München, Nemčija

[3] dr., Staatliches Museum für Naturkunde, Stuttgart, Nemčija

[4] prof. dr., Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za agronomijo, Jamnikarjeva 101, SI-1000 Ljubljana

Trichogramma so generalisti. Samice lahko odlagajo jajčeca v jajčeca približno 200 različnih gostiteljev. Tako parazitirajo jajčeca metuljev (Lepidoptera), dvokrilcev (Diptera), hroščev (Coleoptera), kožekrilcev (Hymenoptera), mrežekrilcev (Neuroptera) in velekrilcev (Megaloptera) (Kuske et al., 2003). Iz tržnega vidika so poleg vrste *Trichogramma brassicae* Bezdenko, 1968 zanimive še vrste *Trichogramma pintoi* Voegele, 1982, *Trichogramma evanescens* Westwood, 1833, *Trichogramma dendrolini* Matsumura, 1925 in *Trichogramma cacaeciae* Marchal, 1927 (List of biological …, 2014). Odrasli osebki iz rodu *Trichogramma* se prehranjujejo z nektarjem, pelodom, medeno roso, občasno pa tudi s jajčno vsebino njihovih gostiteljev (Coombs in Coombs, 2003). Vsako leto se biotično varstvo gojenih rastlin z uporabo parazitoidov iz rodu *Trichogramma* izvaja na več kot 32 milijonov ha (Chailleux *et al*., 2012).

2 OPIS IN RAZVOJNI KROG VRSTE *Trichogramma brassicae*

Obravnavana parazitoidna vrsta izhaja iz Moldavije. V Francijo so jo vnesli z namenom zatiranja koruzne vešče (*Ostrinia nubilalis* [Hübner, 1796]) in to metodo tam uspešno uporabljajo od osemdesetih let prejšnjega stoletja (Eizaguirre *et al*., 1998). Parazitoid *Trichogramma brassicae* prezimi kot predbuba v jajčecih gostiteljev (Babendreier *et al*., 2003). Jajčeca iz rodu *Trichogramma* so prosojna. Njihov premer je 0,04 mm, v dolžino pa merijo 0,14 mm. Po 24 urah se razvije ličinka, ki ima srpasto oblikovane čeljusti. Ličinka poje vso vsebino jajčeca gostitelja. Samica navadno parazitira sveže odložena jajčeca. Ličinke se štirikrat levijo, nato se zabubijo. Pri tem jajčece gostitelja počrni. Bube so svetle. Malo pred izvalitvijo odraslega osebka se na zadku pojavijo črte. Izvalitev odrasle žuželke (slika 1) se navadno zgodi v jutranjem času. Samice merijo v dolžino 0,6 mm. Njihova glava in oprsje sta črna (Malais in Ravensberg, 2012).

Slika 1: Parazitoid *Trichogramma brassicae* Bezdenko, 1968 pred DNA ekstrakcijo (foto: Stefan Schmidt)

Na razvojni krog in sposobnost parazitiranja parazitoidne ose *T. brassicae* ima pomemben vpliv temperatura (Chihrane in Laugé, 1996). Pri 25°C traja razvojni krog (od jajčeca do odraslega osebka) 10 dni, pri 23°C se razvojni krog sklene v 12 dneh (Chailleux *et al.*, 2012). Razvoj je tudi pri drugih vrstah iz rodu *Trichogramma* močno odvisen od temperature. Višja kot je temperatura, hitrejši je razvoj vrste (Coombs in Coombs, 2003).

Razvoj poteka pri samcih hitreje kot pri samicah. Ustrezne gostitelje išče *T. brassicae* od tal proti vrhu rastline. Najvišja temperatura, pri kateri razvoj še poteka, je 38°C. Poleg temperature okolja pa na uspešnost parazitiranja vpliva tudi vrsta gostitelja, dostopnost hrane in velikost jajčec. Če so slednja premajhna, do parazitiranja ne pride. V večja jajčeca pa lahko samica odloži več jajčec, lahko tudi do 30. V enem jajčecu gostitelja pride lahko do razvoja obeh spolov vrste *T. brassicae*. Parazitirana jajčeca postanejo pri 24°C črna po 4 dneh (slika 3).

Slika 2: Moški spolni organ vrste *Trichogramma brassicae* ventralno, mikroskopska slika (foto: Juan Carlos Monje)

Odlaganje jajčec samic *T. brassicae* je najbolj intenzivno v prvih dveh dneh po izletu iz gostitelja. Razmerje v spolih po izvalitvi je v prid samic. V jajčece iz družine sovk (Noctuidae) parazitoidna osa odloži 2-3 svoja jajčeca.

3 PARAZITOID *Trichogramma brassicae* V SLOVENIJI

18. avgusta 2014 smo na njivi z zeljem (*Brassica oleracea* L. var. c*apitata* L.f. *alba*) v bližini kraja Zvirče (zemljepisna širina: 46°19′49.09″N, zemljepisna dolžina: 14°16′46.41″E, nadmorska višina 496 m) v občini Tržič našli več različnih jajčnih legel sovk (Noctuidae). Liste zelja, na katerih so bila jajčna legla, smo nabrali in jih prenesli v Laboratorij za entomologijo Katedre za fitomedicino, kmetijsko mehanizacijo, poljedelstvo, pašništvo in travništvo Oddelka za agronomijo Biotehniške fakultete, kjer smo jih shranili v steklenem insektariju. Ugotovili smo, da jajčna legla pripadajo kapusovi sovki (*Mamestra brassicae* (Linaeus, 1758)). Jajčeca v nekaterih jajčnih leglih so bila temna – parazitirana (slika 3). Legla smo pustili v insektariju 3 tedne, nakar smo v njih pobrali izletele parazitoidne ose in jih shranili v mikrocentrifugirki z 70 % etanolom. Morfološko identifikacijo osebkov je v Zoologische Staatsammlung v Münchnu opravil

Dr. Stefan Schmidt, Dr. Juan Carlos Monje (Staatliches Museum fùr Naturkunde v Stuttgartu) pa je z genetsko analizo potrdil vrsto *T. brassicae*.

Slika 3: Parazitirana (črna) jajčeca v jajčnem leglu kapusove sovke (*Mamestra brassicae*), najdenem v Žvirčah na Gorenjskem (foto: J. Rupnik)

4 POMEN V VARSTVU RASTLIN S SKLEPI

V aplikativnem biotičnem varstvu se parazitoidna osa *T. brassicae* uporablja predvsem za zatiranje koruzne vešče (Hassan in Zhang, 2001). Pri tem poročajo tako o uspelih, kot tudi o neuspelih poskusih vnosa (Lundgren in Heimpel, 2003). Neuspeli poskusi so vezani predvsem na način razmoževanja obravnavanih parazitoidnih os. Znano je namreč, da se vrste iz rodu *Trichogramma* večinoma razmožujejo z arhenotokijo (iz neoplojenih jajčec se razvijejo samo samci). Populacije, ki so nastale z telitokijo (iz neoplojenih jajčec se razvijejo samo samice), pa vsebujejo endosimbiontsko vrsto bakterije iz rodu *Wolbachia*, ki vpliva na večjo smrtnost vrst iz rodu *Trichogramma* (Lundgren in Heimpel, 2003).

Glede na podatke Fauna Europea (2015) je bila osa *T. brassicae* v Evropi doslej najdena v Avstriji, Italiji, Franciji, Nemčiji, Romuniji, Moldaviji, Bolgariji, Belgiji, Švici, na Nizozemskem in v Ukrajini, po podatkih EPPO (List of biological…, 2015) pa parazitoida uporabljajo pri biotičnem zatiranju uporabljajo v Avstriji in Belgiji, na Češkem, Danskem, Finskem, v Franciji, Nemčiji, Grčiji, Italiji, Jerseyu, Jordaniji, na Nizozemskem, Slovaškem, v Španiji, Švici in Veliki Britaniji. Uspešnost vnosa parazitoidnih os iz rodu *Trichogramma* je pogojena z različnimi dejavniki, kot so uporaba fitofarmacevtskih sredstev, vremenske razmere, razvojni stadij gostitelja in število izpuščenih osebkov (Thomson *et al*., 2003).

Pri uporabi parazitoidov iz rodu *Trichogramma* v aplikativnem biotičnem varstvu lahko večjo učinkovitost dosežemo s časovno nadzorovanim izpuščanjem parazitoidov v okolje (Coombs in Coombs, 2003). Na različnih območjih sveta vrsto *T. brassicae* masovno namnožujejo in/ali tržijo različna podjetja. Od šestih podjetij sta dve locirani v Evropi.

Obravnavana koristna vrsta je učinkovita tako pri parazitiranju posameznih jajčec, kot na jajčnih leglih. Največkrat so parazitirana jajčeca, ki so jih gostitelji odložili zelo kratek čas pred parazitiranjem. Pri uporabi pripravkov na podlagi vrste *T. brassicae* je pomembno, da vnos opravimo že zelo zgodaj v rastni dobi, ko je populacija škodljivca majhna. Če prvi izpust opravimo prepozno, obravnavana koristna vrsta nima vpliva na zmanjševanje populacij škodljivih organizmov. Ker je vrsta *T. brassicae* jajčni parazitoid, mora biti ciljni škodljivi organizem v času vnosa naravnega sovražnika prisoten v razvojnem stadiju jajčec. Potrebno število izpuščenih osebkov parazitoidne ose pa je odvisno tudi od gostiteljske rastline.

Osa *T. brassicae* se v pripravkih nahaja v razvojnem stadiju bube v parazitiranih jajčecih močne vešče (*Ephestia kuehniella* Zeller, 1879). Uporabnost omenjene parazitoidne ose ima velik potencial pri zatiranju gospodarsko škodljivih vrst metuljev na poljščinah, vrtninah in ostalih gojenih rastlinah. EPPO (List of biological…, 2015) kot najustreznejšega gostitelja za vrsto *T. brassicae* navaja koruzno veščo. Zaradi velike občutljivosti na uporabo insekticidov (ki vsebujejo aktivne snovi iz skupine piretroidov) moramo biti pozorni, da je njena aplikacija povezana predvsem z ostalimi alternativnimi metodami v varstvu rastlin.

Naša raziskava omogoča uvrstitev obravnavane koristne vrste na Seznam domorodnih vrst organizmov za namen biotičnega varstva rastlin, kot določa Pravilnik o biotičnem varstvu rastlin (Uradni list RS, 45/06). Z navedeno razširitvijo seznama bo dovoljena uporaba vrste *T. brassicae* za zatiranje koruzne vešče (in morda tudi pri zatiranju nekaterih drugih vrst gospodarsko pomembnih škodljivih metuljev) v okoljsko sprejemljivih sistemih pridelovanja gojenih rastlin.

5 ZAHVALA

Prispevek je nastal s finančno pomočjo Ministrstva za kmetijstvo, gozdarstvo in prehrano – Uprave RS za varno hrano, veterino in varstvo rastlin v okviru strokovnih nalog s področja zdravstvenega varstva rastlin.

6 VIRI

Babendreier, D., Kuske, S., Bigler, F. 2003. Overwintering of the egg parasitoid *Trichogramma brassicae* in Nothern Switzerland. BioControl, 48: 261-273, DOI: 10.1023/A:1023661420247

Bai, B., Cobanoglu, S., Smith, S.M. 1995. Assessment of *Trichogramma* species for biological control of forest Lepidopteran defoliators. Entomologia Experimentalis et Applicata. 75:135-143, DOI: 10.1111/j.1570-7458.1995.tb01919.x

Chailleux, A., Desneux, N., Seguret, J., Khanh, H.D.T., Maignet, P., Tabone, E. 2012. Assessing European egg parasitoids as a mean of controlling the invasive south American tomato pinworm *Tuta absoluta*. PlosOne. 7: e48068. DOI:10.1371/journal.pone.0048068

Chihrane, J., Laugé, G. 1996. Loss of parasitization efficiency of *Trichogramma brassicae* (Hym.: Trichogrammatidae) under high-temperature conditions. Biological Control, 7: 95-99.

Coombs, J., Coombs, R.F. 2003. A dictionary of biological control & integrated pest management. 3. Izdaja. CPL Press. 200 str.

Eizaguirre, M., Albajes, R., Sans, A. 1998. Application of *Trichogramma brassicae* against *Ostrinia nubilais* in Catalonia. IOBC Bulletin, 21, 8: 181-191.

Fauna Europea. 2015. http://www.faunaeur.org/index.php (5.7.2015)

Kuske, S., Widmer, F., Edwards, P.J., Turlings, T.C.J., Babendreier, D., Bigler, F. 2003. Dispersal and persistence of mass released *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) in non-target habitats. Biological Control. 27:181-193, DOI: 10.1016/S1049-9644(02)00191-3

List of biological control agents widely used in the EPPO region. 2014. EPPO. EPPO Standards on safe use of biological control. PM 6/3. http://archives.eppo.int/EPPOStandards/biocontrol_web/bio_list.htm (5.7.2015)

Lundgren, J.G., Heimpel, G.E. 2003. Quality assessment of three species of commerially produced *Trichogramma* and the first report of thelytoky in commerciallly produced *Trichogramma*. Biological Control. 26:68-73, DOI: 10.1016/S1049-9644(02)00117-2

Malais, M.H., Ravensberg, W.J. 1992. Knowing and recognizing. The biology of glasshouse pests and their natural enemies. Koppert. Red Business Information, 288 str.

Hassan, S.A., Zhang, W.Q. 2001. Variability in quality of *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) from commercial suppliers in germany. Biological Control, 22: 115-121, DOI: 10.1006/bcon.2001.0962

Pravilnik o biotičnem varstvu rastlin (Uradni list RS, št. 45/06), http://www.furs.si/law/slo/zvr/Biot_varstvo/Uradni_list_45_06_kazalo.pdf, 9 str., 2.9.2015

Thomson, L., Bennet, D., Glenn, D.A., Hoffman, A. 2003. Developing *Trichogramma* as a pest management tool. In: Predators and parasitoids. Koul, O., Dhaliwal, G.S. (ur.). Taylor and Francis: 191 str.

COBISS Code 1.04

DOI: 10.14720/aas.2015.105.2.16
Agrovoc descriptors: pests, *Scaphoideus titanus*, phytoplasmas, biological control, integrated pest management, biological pest control

Agris category code: h01, h10, h20

Možnosti okoljsko sprejemljivega zatiranja ameriškega škržatka (*Scaphoideus titanus* Ball, 1932)

Žiga LAZNIK[1], Stanislav TRDAN[2]

Received July 22, 2015; accepted August 31, 2015.
Delo je prispelo 22. julija 2015, sprejeto 31. avgusta 2015.

IZVLEČEK

Ameriški škržatek (*Scaphoideus titanus* Ball, 1932) je žuželka iz družine malih škržatkov (Cicadellidae), izvorno razširjena po Severni Ameriki. Vrsta je bila vnešena v Evropo, kjer napada vinsko trto in je znana predvsem kot prenašalec fitoplazme Grapevine Flavescence dorée (FD) (*Candidatus Phytoplasma vitis*), ki povzroča bolezen zlato trsno rumenico. Ameriški škržatek je bil v Sloveniji prvič najden leta 1983 na Primorskem. Zlata trsna rumenica je bila v Sloveniji potrjena leta 2005. Za zatiranje ameriškega škržatka se uporabljajo insekticidi, saj žuželka zaenkrat nima učinkovitih naravnih sovražnikov, ki bi lahko zmanjšali populacijo pod gospodarski prag škodljivosti. Nekateri raziskovalci so mnenja, da je na obeh celinah (Severna Amerika, Evropa) številčnost naravnih sovražnikov ameriškega škržatka zelo majhna. Iz ZDA poročajo, da nekateri parazitoidi (Drynidae: Hymenoptera in Pipunculidae: Diptera) parazitirajo ameriškega škržatka, vendar je odstotek učinkovitosti zelo majhen (od 1,3 do 0,8 %). Med načine integriranega varstva vinske trte pred množičnim pojavom ameriškega škržatka so doslej uporabljali metode zbeganja, termoterapije in privabilne posevke.

Ključne besede: ameriški škržatek, *Scaphoideus titanus*, biotično varstvo rastlin, integrirano varstvo rastlin

ABSTRACT

POSSIBILITIES OF ENVIRONMENTALLY ACCEPTABLE CONTROL METHODS OF AMERICAN GRAPEVINE LEAFHOPPER (*Scaphoideus titanus* Ball, 1932)

The American Grapevine Leafhopper (AGL) (*Scaphoideus titanus* Ball, 1932) is a small insect of the family leafhoppers (Cicadellidae), originally spread across North America. Specie has been introduced to Europe, where is known primarily as a vector of phytoplasma Grapevine flavescence dorée (FD), (*Candidatus Phytoplasma vitis*), a disease-causing grapevine yellows. AGL was first found in Slovenia in 1983. First occurrence of grapevine yellows was confirmed in Slovenia in 2005. Since no effective biological control agents are known to date, AGL populations are suppressed using insecticides during the host plant's growth period. Some researchers reported that it is in both continents (North America, Europe) abundance of natural enemies of the AGL very small. Researchers reported that some parasitoids (Drynidae: Hymenoptera and Pipunculidae: Diptera) parasitize the AGL, but the percentage of efficiency is very low (from 1.3 to 0.8 %). Among the methods of integrated pest management of AGL methods of mating disruption, thermotherapy, and cover crops are used.

Key words: The American Grapevine Leafhopper, *Scaphoideus titanus*, biological control, integrated pest management

1 UVOD

Ameriški škržatek (*Scaphoideus titanus* Ball, 1932) je glavni prenašalec zlate trsne rumenice na žlahtni vinski trti, ki jo povzroča karantenska fitoplazma Grapevine Flavescence dorée (FD), (*Candidatus Phytoplasma vitis*). Zlata trsna rumenica je bila v Sloveniji potrjena leta 2005 v

[1] doc. dr., univ. dipl. inž. agr, Jamnikarjeva 101, SI-1000 Ljubljana, e-mail: ziga.laznik@bf.uni-lj.si

[2] prof.. dr., Jamnikarjeva 101, SI-1000 Ljubljana, e-mail: stanislav.trdan@bf.uni-lj.si

okolici Kopra, v letu 2008 na Dolenjskem, v letu 2009 pa tudi na območju severovzhodne Slovenije (Štajerska in Prekmurje) (Matko in sod., 2013). Ameriški škržatek izvira iz Severne Amerike, od koder naj bi bil s sadilnim materialom, na katerem so bila odložena njegova jajčeca, zanesen v Evropo. V Evropi je bil ameriški škržatek prvič ugotovljen v Franciji (Decante in van Helden, 2006), od tam pa se je razširil v Španijo in na Portugalsko. Njegovo širjenje se je nadaljevalo preko Italije do držav na Balkanskem polotoku (Battle in sod., 2000; Angelini in sod., 2001; Krnjajić in sod., 2007; Delić in sod., 2011; Žežlina in sod., 2013). Prvič je bil ameriški škržatek v Sloveniji najden že leta 1983 na Primorskem, v letu 2003 pa tudi v severovzhodnem delu Slovenije (Seljak, 2008). Danes je razširjen v vseh vinorodnih deželah Slovenije (Matko in sod., 2013).

Bionomija ameriškega škržatka je dobro znana (Rigamonti in sod., 2011). Matko in sod. (2013) navajajo, da je škodljivec univoltilna vrsta, saj razvije le en rod na leto. Prezimi v stadiju jačec na dve- in triletnem lesu trte (Bertin in sod., 2007; Rigamonti in sod., 2011). Ličinke se iz jajčec izležejo v drugi polovici maja in se štirikrat levijo (do petostopenjske ličinke). Fitoplazme prenašajo navadno odrasli osebki ameriškega škržatka. Seljak (1993) sicer navaja, da so vektorji povzročiteljic zlate trsne rumenice lahko tudi ličinke tretje stopnje. Sprva so bili raziskovalci mnenja, da žlahnta vinska trta (*Vitis vinifera* L.) predstavlja edino gostiteljsko rastlino za ameriškega škržatka (Vidano, 1966), vendar so nekatere novejše raziskave pokazale, da lahko škodljivec del razvojnega kroga preživi tudi na drugih gostiteljskih rastlinah, kot so plazeča detelja (*Trifolium repens* L.) (Jermini, 2011), plazeča zlatica (*Ranunculus repens* L.) (Jermini, 2011) in navadni srobot (*Clematis vitalba* L.) (Angelini in sod., 2004).

Za zatiranje ameriškega škržatka se uporabljajo insekticidi, saj žuželka nima učinkovitih naravnih sovražnikov, ki bi lahko zmanjšali številčnost njegovih populacij pod gospodarski prag škodljivosti (Malausa in sod., 2003; Nusillard in sod., 2003; Boudon-Padieu in Maixner, 2007; Žežlina in sod., 2013). Prav tako v strokovni literaturi ni navedenih drugih možnosti okoljsko sprejemljivih načinov zatiranja škodljivca, ki bi bili dovolj učinkoviti. Žežlina in sod. (2013) navajajo, da je vzdrževanje majhnih populacij ameriškega škržatka ključnega pomena pri preprečevanju širjenja okužb rastlin vinske trte z zlato trsno rumenico. Seljak (2008) poroča, da je številčnost ameriškega škržatka močno zmanjšana predvsem pri uporabi insekticidov, katerih aktivne snovi predstavljajo klorpirifos-etil in klorpirifos-metil. Žežlina in sod. (2013) so v sorodni raziskavi, ki je potekala med leti 2010 in 2011 preučevali pet sintetičnih insekticidov za zatiranje ameriškega škržatka. Nanos je bil opravljen v juniju, saj so se raziskovalci želeli izogniti morebitnemu pojavu ostankov FFS v vinu (Bosio in sod., 2003). Rezultati njihove raziskave so pokazali, da je aktivna snov tiametoksam učinkovala najbolje, saj so potrdili kar 96 % smrtnost ličink ameriškega škržatka. Ostale preučevane aktivne snovi so delovale slabše (Žežlina in sod., 2013).

V prispevku želimo predstaviti dosedanje raziskave na področju preučevanja naravnih sovražnikov ameriškega škržatka in drugih okoljsko sprejemljivih načinov njegovega zatiranja.

2 BIOTIČNO VARSTVO

Znanstveni izsledki, ki navajajo možnosti biotičnega zatiranja ameriškega škržatka, so zelo skopi. Pregledni članek, ki sta ga objavila Chuche in Thiéry (2014) podaja nekaj informacij, ki so jih raziskovalci na omenjenem področju raziskav potrdili do sedaj. Populacija ameriškega škržatka na vinski trti je v Franciji znatno večja kot v ZDA (Maixner in sod., 1993). Omenjeno dejstvo je vodilo v hipotezo, da v ZDA obstajajo naravni sovražniki ameriškega škržatka, ki populacije te žuželčje vrste učinkovito zmanjšujejo. Maixner in sod. (1993) v svojem delu zaključujejo, da se v Evropo poleg ameriškega škržatka najverjetneje niso prenesli tudi njegovi naravni sovražniki, ki sicer obstajajo v ZDA. Nekateri drugi raziskovalci so mnenja, da je na obeh celinah (Severna Amerika, Evropa) številčnost naravnih sovražnikov ameriškega škržatka zelo majhna, v

primerjavi z naravnimi sovražniki nekaterih drugih škodljivih žuželčjih vrst, ki se pojavljajo na vinski trti (Schvester in sod., 1962; Bernard in Du Fretay, 1988; Malausa in Sentenac, 2011). Kot zgled navajajo gosenice križastega grozdnega sukača (*Lobesia botrana* (Denis & Schiffermüller, 1775)), katerih populacije lahko naravni sovražniki zmanjša od 50 do 80 %, seveda v odvisnosti od vinograda (Marchesini in Monta, 1994; Thiéry in sod., 2001; Bagnoli in Lucchi, 2006). Iz ZDA poročajo, da nekateri parazitoidi (Drynidae: Hymenoptera in Pipunculidae: Diptera) parazitirajo ameriškega škržatka, vendar je odstotek učinkovitosti zelo majhen (od 1,3 do 0,8 %) (Barnett, 1976). Malausa in sod. (2003) pa poročajo, da parazitoidi iz družin Mymaridae (Hymenoptera) in Trichogrammatidae (Hymenoptera) parazitirajo ameriškega škržatka, vendar je odstotek zelo majhen. Kot zanimivost naj navedemo posebno sožitje, ki sta ga razvila ameriški škržatek in plenilska stenica *Malacocoris chlorizans* (Panzer, 1794), Hemiptera: Miridae. Omenjena stenica se ne hrani z ameriškim škržatkom, vendar podobno kot v sožitju med mravljami in listnimi ušmi (Stadler in Dixon, 2005) omenjena stenica ob stiku z zadkom ali tipalkami ameriškega škržatka povzroči, da začne škržatek izločati medeno roso (Carle, 1965). Na podlagi opazovanj v vinogradih so ugotovili, da se omenjeni žuželčji vrsti zelo pogosto pojavljata v mešanih populacijah (Carle, 1965).

Potekali so tudi poskusi uporabe različnih biotičnih agensov z namenom zmanjšanja populacije ameriškega škržatka (preglednica 1). Poskusi so vključevali tako zglede klasičnega biotičnega varstva (načrten vnos tujerodne koristne vrste za zatiranje tujerodnega škodljivega organizma, ki se je razširil od drugod in v novem okolju nima učinkovitih naravnih sovražnikov) kot tudi varovalnega biotičnega varstva (varovanje domorodnih koristnih organizmov in vzpodbujanje njihovega razmnoževanja in naselitve). Obe strategiji sta se sicer izkazali kot neučinkoviti pri zatiranju ameriškega škržatka (Malausa in Sentenac, 2011). V Franciji so na dveh lokacijah vnesli parazitodino osico *Gonatopus clavipes* (Thunberg, 1827), Hymenoptera: Dryinidae. V triletnem poskusu so vnesli 368 osebkov parazitodine osice in preverili parazitiranost 46.000 ameriških škržatkov. Stopnja parazitiranja je znašala le 0,4 % (Malausa in Sentenac, 2011).

Tabela 1. Naravni sovražniki ameriškega škržatka (*Scaphoideus titanus* Ball, 1932)

Red	Družina	Vrsta	Tip naravnega sovražnika	Ciljni stadij	Država	Vir
Diptera	Pipunculidae	*Eudorylas* sp.	parazitoid	ličinka, imago	Francija	Malausa in Sentenac, 2011
	Syrphidae	-	plenilec	ličinka	Francija	Schvester in sod., 1962
Hemiptera	Reduviidae	-	plenilec	ličinka	Francija	Schvester in sod., 1962
Hymenoptera	Dryinidae	*Anteon masoni* Olmi, 1984	parazitoid	ličinka, imago	ZDA	Malausa in sod., 2003
		Anteon pubicorne (Dalman, 1818)	parazitoid	ličinka, imago	Francija	Malausa in Sentenac, 2011
		Esagonatopus niger (Fenton, 1924)	parazitoid	ličinka, imago	ZDA	Malausa in sod., 2003
		Esagonatopus niger	parazitoid	ličinka, imago	ZDA	Malausa in sod., 2003
		Esagonatopus perdebilis (Perkins, 1907)	parazitoid	ličinka, imago	ZDA	Malausa in sod., 2003
		Gonatopus audax (Olmi, 1984)	parazitoid	ličinka, imago	Francija	Malausa in Sentenac, 2011
		Gonotopus clavipes (Thunberg, 1827)	parazitoid	ličinka, imago	Francija	Malausa in Sentenac, 2011
		Gonatopus lunatus Klug, 1810	parazitoid	ličinka, imago	Francija	Malausa in Sentenac, 2011
		Gonatopus peculiaris Brues, 1903	parazitoid	ličinka, imago	ZDA	Malausa in sod., 2003
		Lonchodryinus flavus Olmi, 1984	parazitoid	ličinka, imago	ZDA	Malausa in sod., 2003
	Mymaridae	*Polynema* sp.	parazitoid	jajčece	ZDA	Malausa in sod., 2003
	Trichogrammatidae	*Oligosita* sp.	parazitoid	jajčece	Evropa	Malausa in sod., 2003
Acarina	Anystidae	*Anystis baccarum* (L.)	plenilec	ličinka	Francija	Bernard in Du Fretay, 1988
Araneae	Philodromidae	-	plenilec	ličinka	Italija	Chuche in sod., 2011
	Thomisidae	-	plenilec	ličinka	Italija	Chuche in sod., 2011

3 INTEGRIRANO VARSTVO

V sklop integriranega varstva sodi odstranjevanje/uničevanje rastlin žlahtne vinske trte v opuščenih vinogradih oziroma drugih vrst iz rodu *Vitis*, saj lahko take rastline služijo kot rezervoar tako zlate trsne rumenice kot tudi njenega vektorja *S. titanus*. Nekateri raziskovalci (Caudwell in sod., 1997; Dupraz in Schaub, 2007; Linder in sod., 2011) poročajo o možnosti tretiranja sadilnega materiala z vročo vodo (t.i. termoterapija). Znano je, da na ta način lahko uničimo tako zlato trsno rumenico kot tudi jajčeca ameriškega škržatka. Med načine integriranega varstva vinske trte pred množičnim pojavom ameriškega škržatka so doslej uporabljali metodo zbeganja in privabilne posevke (push-pull strategy) (Chuche in Thiéry, 2014).

Za ameriškega škržatka je značilno, da poteka parjenje z zaznavanjem vibracij, ki jih zaznava iz okolja. Raziskovalci so preučevali možnosti uporabe metode zbeganja s spreminjanjem vibracij, s katerimi bi otežili process parjenja ameriškega škržatka (Mazzoni in sod., 2009; Eriksson in sod., 2012). Rezultati so bili obetajoči predvsem v laboratorijskih poskusih, njihova implementacija za razmere na prostem, pa do danes še ni bila učinkovita (Mazzoni in sod., 2009; Eriksson in sod., 2012). Omenjena metoda se lahko uporablja, ko je ameriški škržatek v razvojnem stadiju odraslega osebka, saj v stadiju ličinke ne zaznava vibracij iz okolja (Chuche in sod., 2011).

Strategija "push and pull" vključuje manipulacijo obnašanja žuželk s kombinacijo rabe rastlinskih atraktantov in repelentov , ki povzročijo premik ciljne žuželke na območje, kjer jo zatremo (Cook in sod., 2007). V Izraelu so izvedli raziskavo, kjer so z omenjeno metodo učinkovito zatrli škržata *Hyalesthes obsoletus* Signoret, 1865 (Hemiptera: Cixiidae), ki prenaša fitoplazme na vinski trti (Zahavi in sod., 2007). Številni viri navajajo, da so ameriške vinske trte bolj dovzetne za napad ameriškega škržatka kot sorte evropske žlahtne vinske trte (Tubajika in sod., 2007; Marko in sod., 2008). Omenjeni raziskovalci navajajo možnost, da bi v vinograde s sortami žlahtne vinske trte posadili izmenično ameriške vrste trt, na katere bi se naselili ameriški škržatki iz evropskih trt. Skorjo ameriških vrst bi nato tretirali z mineralnimi olji (Chuche in Thiéry, 2014).

4 ZAKLJUČKI

Strategija pridelave hrane, ki sledi vse strožji okoljski politiki, vse bolj temelji na zmanjšani uporabi sintetičnih fitofarmacevtskih sredstev. Razvoj novih, okoljsko sprejemljivih načinov varstva rastlin, zato vse bolj pridobiva na pomenu. Na podlagi znanstvenih virov ugotavljamo, da je ameriškega škržatka brez uporabe sintetičnih insekticidov trenutno nemogoče zatirati. Zato bomo morali še naprej iskati bodisi ustreznejše biotične agense (entomopatogene ogorčice, entomopatogene glive, plenilce in parazitoide) oziroma druge okoljsko sprejemljive načine varstva žlahtne vinske trte, s katerimi se bo mogoče uspešneje zoprstaviti ameriškemu škržatku, ki kot prenašalec zlate trsne rumenice povzroča veliko težav gojiteljem žlahtne vinske trte po svetu.

5 ZAHVALA

Prispevek je nastal s finančno pomočjo Ministrstva za kmetijstvo, gozdarstvo in prehrano RS – Uprave RS za varno hrano, veterinarstvo in varstvo rastlin v okviru strokovnih nalog s področja zdravstvenega varstva rastlin.

6 VIRI

Angelini E., Clair D., Borgo M., Bertaccini A, Boudon-Padieu E. 2001. Flavescence dorée in France and Italy - Occurrence of closely related phytoplasma isolates and their relationships to Palatinate grapevine yellows and an elder yellows phytoplasma. Vitis, 40: 79-86

Angelini E., Squizzato F., Lucchetta G., Borgo M. 2004. Detection of a phytoplasma associated with grapevine Flavescence dorée in *Clematis vitalba*. European Journal of Plant Pathology, 110: 193-201, DOI: 10.1023/B:EJPP.0000015361.95661.37

Bagnoli B., Lucchi A. 2006. Parasitoids of *Lobesia botrana* (Den. & Schiff.) in Tuscany. IOBC/wprs Bulletin, 29: 139-142

Barnett D.E. 1976. A revision of the Neartic species of the genus *Scaphoideus* (Homoptera: Cicadellidae). Trans American Entomological Society, 102: 485-593

Battle A, Angeles Martinez M., Laviña A. 2000. Occurrence, distribution and epidemiology of Grapevine Yellows in Spain. European Journal of Plant Pathology, 106: 811-816, DOI: 10.1023/A:1008794905178

Bernard P., Du Fretay G. 1988. Dynamique de population de *Scaphoideus titanus*, vecteur de la Flavescence dorée dans l'Aude en 1987. Bulletin of Tech, 433: 457-464

Bertin S., Guglielmino C.R., Karam N., Gomulski L.M., Malacrida A.R., Gasperi G. 2007. Diffusion of the Nearctic leafhopper *Scaphoideus titanus* Ball in Europe: a consequence of human trading activity. Genetica, 131: 275-285, DOI: 10.1007/s10709-006-9137-y

Bosio G., Gremo F., Alliani N., Battaglia G., Rabino M., Bonifacino G., Tragni R. 2003. Residual performance of insecticides used against *Scaphoideus titanus* in vines. Inform Agrar., 59: 45-48

Boudon-Padieu E., Maixner M. 2007. Potential effects of climate change on distribution and activity of insect vectors of grapevine pathogens. Proceedings of the international and multi-disciplinary colloquium "Global warming, which potential impacts on the vineyards?". CHAIRE UNESCO "Culture et Traditions du Vin", Dijon (France): 8 p.

Carle P. 1965. Relations alimentaires entre *Malacocoris chlorizans* Pz (Hémipt. Hétérop. "Miridae") et *Scaphoideus littoralis* Ball. (Hémipt. Homopt. "Jassidae") sur les *Vitis* dans le Sud-Ouest de la France. Rev Zool Agr Appl, 7-9: 72-78

Caudwell A., Larrue J., Boudon-Padieu E., McLean G.D. 1997. Flavescence dorée elimination from dormant wood of grapevines by hot-water treatment. Australian Journal of Grape and Wine Research, 3: 21–25, DOI: 10.1111/j.1755-0238.1997.tb00112.x

Chuche J., Boursault A., Thiéry D. 2011. Preliminary study of the aggregative behaviour of *Scaphoideus titanus* larvae. IOBC/wprs Bull., 67: 239-244

Chuche J., Thiéry D. 2014. Biology and ecology of the Flavescence dorée vector *Scaphoideus titanus*: a review. Agronomy for Sustainable Development, 34: 381-403, DOI: 10.1007/s13593-014-0208-7

Cook S.M., Khan Z.R., Pickett J.A. 2007. The use of push-pull strategies in integrated pest management. Annual Review of Entomology, 52: 375-400, DOI: 10.1146/annurev.ento.52.110405.091407

Decante D., van Helden M. 2006. Population ecology of *Empoasca vitis* (Göthe) and *Scaphoideus titanus* (Ball) in Bordeaux vineyards: Influence of migration and landscape. Crop Protection, 25: 696-704, DOI: 10.1016/j.cropro.2005.09.016

Delić D., Contaldo N., Paltrinieri S., Lolić B., Urić Z., Hrnčić S., Bertaccini A. 2011. Grapevine yellows in Bosnia and Herzegovina: surveys to identify phytoplasmas in grapevine, weeds and insect vectors. Bulletin of Insectology, 64: 245-246

Dupraz P., Schaub L. 2007. Control of grapevine flavescence dorée phytoplasma: reinventing hot water! Revue Suisse de Viticulture, Arboriculture et Horticulture, 39(2): 113-115

Eriksson A., Anfora G., Lucchi A., Virant-Doberlet M., Mazzoni V. 2011. Inter-plant vibrational communication in a leafhopper insect. PLoS ONE, 6. DOI: 10.1371/journal.pone.0019692

Jermini M. 2011. Übertragungen der Goldgelben Vergilbung nicht nur auf Reben. Die Schweizer Zeitschrift für Obst- und Weinbau, 1: 16

Krnjajić S., Mitrović M., Cvrković T., Jović J., Petrović A., Forte V., Angelini E., Toševski I. 2007. Occurrence and distribution of *Scaphoideus titanus* in multiple outbreaks of "flavescence dorée" in Serbia. Bulletin of Insectology, 60: 197-198

Linder C., Schaub L., Klötzli-Estermann F., Calonnec A., Duso C., Gessler C., Kassemeyer H.H., Maixner M., Thiéry D., Zahavi T. 2011. Effectiveness of hot water treatments against the eggs of *Scaphoideus titanus* Bal. IOBC/WPRS Bulletin, 67: 17-20

Maixner M., Pearson R.C., Boudon-Padieu E., Caudwell A. 1993. *Scaphoideus titanus*, a possible vector of Grapevine Yellows in New York. Plant Disease, 77: 408-413, DOI: 10.1094/PD-77-0408

Malausa J.C., Nusillard B., Giuge L. 2003. Biological control against *Scaphoideus titanus*, the vector of flavescence dorée: assessment of research and collection of natural enemies conducted in its North America native area during 2001 and 2002. Phytoma, 565: 24-27

Malausa J.C., Sentenac G. 2011. Parasitoïdes de *Scaphoideus titanus*. In: Sentenac G (Editor). La faune Auxiliaire des vognobles de France. France Agricole, Paris: 143-146

Marchesini E., Monta L.D. 1994. Observations on natural enemies of Lobesia botrana (Den. &Schiff.) (Lepidoptera, Tortricidae) in Venetian vineyards. Bull Zool Agrar Bachic, 26: 201-230

Marko V., Blommers L.H.M., Bogya S., Helsen H. 2008. Kaolin particle films suppress many apple pests, disrupt natural enemies and promote wooly apple aphid. Journal of Applied Entomology,132: 26-35, DOI: 10.1111/j.1439-0418.2007.01233.x

Matko B., Miklavc J., Mešl M. 2013. Izkušnje z zatiranjem ameriškega škržatka (*Scaphoideus titanus* Ball) v obdobju 2008-2012 v severovzhodni Sloveniji. Zbornik predavanj in referatov 11. Slovenskega posvetovanja o varstvu rastlin z mednarodno udeležbo, Bled, 5.-6. marec 2013. Ljubljana, Društvo za varstvo rastlin Slovenije: 210-215

Mazzoni V., Prešern J., Lucchi A., Virant-Doberlet M. 2009. Reproductive strategy of the Neartic leafhopper *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae). Bulletin of Entomological Research, 99: 401-413, DOI: 10.1017/S0007485308006408

Nusillard B., Malausa J.C., Giuge L., Millot P. 2003. Assessment of a two years study of the natural enemy fauna of *Scaphoideus titanus* Ball in its North American native area. In: Lozzia C. (editor). Integrated protection and production in viticulture. IOBC/wprs Bulletin, 26: 237-240

Rigamonti I.E., Jermini M., Fuog D., Baumgärtner J. 2011. Towards an improved understanding of the dynamics of vineyard-infesting *Scaphoideus titanus* leafhopper populations for better timing of management activities. Pest Management Science, 67: 1222-1229, DOI : 10.1002/ps.2171

Schvester D., Moutous G., Carle P. 1962. *Scaphoideus littoralis* Ball. (Homop. Jassidae) cicadelle vectrice de la Flavescence dorée de la vigne. Rev Zool Agr Appl, 10-12: 118-131

Seljak G. 1993. Škodljivi škržati vinske trte; ameriški škržat (*Scaphoideus titanus* Ball). Sad, revija za sadjarstvo, vinogradništvo in vinarstvo, 4: 9-11

Seljak G. 2008. Distribution of *Scaphoideus titanus* in Slovenia: its new significance after the first occurrence of grapevine "flavescence dorée". Bulletin of Insectology, 61:201-202

Stadler B., Dixon A.F.G. 2005. Ecology and evolution of aphid-ant interactions. Annual Review of Ecology**,** Evolution**,** and Systematics, 36: 345-372, DOI: 10.1146/annurev.ecolsys.36.091704.175531

Thiéry D., Xuéreb A., Villemant C., Sentenac G., Delbac L., Kuntzman P. 2001. The parasites of grape tortricids: noticed on several species present in 3 French vine regions. IOBC/wprs Bulletin, 24: 135-141

Tubajika K.M., Civerolo E.L., Puterka G.J., Hashim J.M., Luvisi D.A. 2007. The effects of kaolin, harpin, and imidacloprid on development of Pierce's disease in grape. Crop Protection, 26: 92-99, DOI: 10.1016/j.cropro.2006.04.006

Vidano C. 1966. Scoperta della ecologia ampelofila del cicadellidae *Scaphoideus littoralis* Ball nella regione neartica originaria. Annali, Facoltà di Scienze Agrarie della Università degli Studi di Torino, 3: 297-302

Zahavi T., Peles S., Harari A.R., Soroker V., Sharon R. 2007. Push and pull strategy to reduce *Hyalesthes obsoletus* population in vineyards by *Vitex agnus* castus as trap plant. Bulletin of Insectology, 60: 297-298

Žežlina I., Škvarč A., Bohinc T., Trdan S. 2013. Testing the efficacy of single application of five insecticides against *Scaphoideus titanus* on common grapevines. International Journal of Pest Management, 59: 1-9, DOI: 10.1080/09670874.2012.735378

COBISS Code 1.04

DOI: 10.14720/aas.2015.105.2.17
Agrovoc descriptors: olives, olea europaea, irrigation, growth, crop yield, drought stress, evapotranspiration

Agris category code: f01, f06

Deficitni princip namakanja oljčnih nasadov v Slovenski Istri

Maja PODGORNIK[1,2], Dunja BANDELJ[1,2]

Received Juny 09, 2015; accepted July 20, 2015.
Delo je prispelo 09. junija 2015, sprejeto 20. julija 2015.

IZVLEČEK

Oljka je anatomsko-morfološko in fiziološko zelo dobro prilagojena na pomanjkanje vode v tleh. Kljub temu lahko vodni primanjkljaj vpliva na slabšo rast in rodnost oljk, v kolikor se pojavi v fazi razvoja (med rastjo poganjkov, razvojem cvetnih brstov, cvetenjem, nastavljanjem plodov, delitvijo in rastjo celic ter akumulacijo olja), ki je za sušni stres najbolj občutljiva. Slovenska Istra se sooča s povečanim tveganjem pojava suš, zaradi česar bo kontrolirano deficitno namakanje oljk postalo nepogrešljiv element kmetijske prakse.

Ključne besede: oljka, namakanje, rast, pridelek, evapotranspiracija, sušni stres

ABSTRACT

DEFICIT IRRIGATION PRINCIPLES APPLIED TO OLIVE ORCHARD IN SLOVENE ISTRIA

The olive tree has anatomical-morphological and physiological adaptations which enable it to cope well with dry conditions and water deficits. However, if water shortage occurs during the development phases (shoot growth, flower bud development, bloom, fruit set, cell division and enlargement and oil accumulation), which are the most susceptible to stress, it can also have a negative effect on the growth and productivity of olive trees. The Slovenian Istria is facing with increased risk of drought. Due to increased occurrence and intensity of agricultural droughts controlled deficit irrigation will become an inevitable element of agricultural practice in Slovene Istria.

Key words: olive, irrigation, growth, yield, evapotranspiration, drought stress

1 UVOD

Slovensko ozemlje zavzema 2.072.277 ha površine. Največji del ozemlja prekrivajo gozdovi 54 % (1.184.526 ha) in kmetijska zemljišča 23 % (479.653 ha) (Statistični letopis, 2013). V strukturi kmetijskih zemljišč prevladujejo travniki in pašniki (59 %). Manjši del kmetijskih zemljišč predstavljajo trajni nasadi 5,6 % (26.867 ha), od katerih 7,3 % (1.968 ha) predstavljajo oljčniki. Čeprav oljkarstvo zavzema le 0,38 % kmetijskih zemljišč, je pomembna kmetijska panoga slovenskega prostora, saj poleg ohranjanja kulturne dediščine Sredozemlja, omogoča razvoj turizma in zaposlovanje lokalnega prebivalstva Istrske regije ter prispeva tudi k večanju samooskrbe z rastlinskimi olji v Sloveniji.

Oljčno olje Slovenske Istre je od leta 2007 kot prvi slovenski proizvod vpisan v evropski register »Register zaščitenih označ porekla in zaščitenih geografskih označb« zaščitenih izdelkov, za katere velja, da morajo biti pridelani, predelani in pripravljeni na določenem geografskem območju tako, da je zagotovljena stalna kakovost in sledljivost živila (Breznik in sod., 2007). Poimenovanje zaščitenega porekla »EDOOSI ZOP« velja izključno za »Ekstra deviška oljčna

[1] Univerza na Primorskem, Znanstveno-raziskovalno središče, Inštitut za oljkarstvo, Garibaldijeva 1, 6000 Koper, Slovenija, maja.podgornik@zrs.upr.si

[2] Univerza na Primorskem, Fakulteta za matematiko naravoslovje in informacijske tehnologije, Glagoljaška 8, 6000 Koper, Slovenija, dunja.bandelj@zrs.upr.si

olja Slovenske Istre«, ki ustrezajo višjim kakovostnim kriterijem v skladu z Uredbo Komisije (EGS) št. 2568/9 navedenim v Elaboratu za postopek priznanja označbe geografsko poreklo »EDOOSI« št. 324-01-7/2002/27. »EDOOSI ZOP« je olje iz različnih sort oljk ('Istrska belica', 'Leccino', 'Buga', 'Črnica', 'Maurino', 'Frantoio' in 'Pendolino') ali iz ene sorte oljk, pridelanih na območju Slovenske Istre. V sortni sestavi predelanih oljk mora biti najmanj 30 % sorte 'Istrska belica'. Sortna značilnost 'Istrske belice' je grenkoba, ki pa se v sušnih letih lahko pojavi v tako veliki intenzivnosti, da je oljčno olje pridelano iz nje izrazito neharmonično in potrošniku neprijetno (Bandelj, 2012).

Podatki kažejo, da ima Slovenija dovolj padavin, vendar zaradi neenakomerne razporeditve postaja slovenski prostor čedalje bolj ranljiv zaradi suše. V zadnjih 25 letih se v povprečju vsako tretje leto srečujemo s sušnimi razmerami. Tudi projekcije spremembe podnebja in potencialni vplivi na evapotranspiracijo in pogostnost kmetijskih suš niso obetavni. Pomanjkanje padavin ali njihova nepravilna časovna razporeditev že predstavljata problem, tveganje in veliko sušno ogroženost v jugozahodni Sloveniji (Sušnik, 2003), kjer so vodni viri za namakanje kmetijskih površin omejeni (Glavan in sod., 2012; Sušnik, 2003).

V Sloveniji se trenutno po podatkih Registra kmetijskih gospodarstev (RKG, 2014) namaka 19 ha oljčnikov, v katerih se večina poslužuje principa »kriznega namakanja«, ki pa je po mnenju strokovnjakov posledica pomanjkanja primarnih strategij rabe vode za potrebe rastlinske pridelave, saj gre za časovno in količinsko nekontrolirano dodajanje vode (Pintar in sod., 2010, Podgornik in sod., 2012a). Številne raziskave na območju Sredozemlja so pokazale, da je v pridelavi oljk, kjer so razpoložljivi vodni viri izjemno omejeni, primernejša uporaba principa »deficitnega namakanja«, kjer lahko kljub manjši količini dodane vode (kot je to optimalno potrebno) povečamo rodnost rastline in s tem hkrati zagotovimo večji pridelek ter trajnostno rabo vodnih virov (Patumi in sod., 1999; Melgar in sod., 2008; Gómez-Rico in sod., 2007; Fernandes-Silva in sod., 2010; Podgornik in sod., 2012b).

Z namenom, da bi opredelili pozitivne in negativne vplive sušnega stresa na rast in rodnost oljke ter sistematično predstavili deficitni princip namakanja oljk, smo izvedli pregled in vsebinsko analizo dostopnih znanstvenih in strokovnih publikacij. S primerjalno analizo pridobljenih virov je bil osnovan teoretični okvir in pridobljen osnovni vpogled v problematiko pomanjkanja vode in kmetijskih suš v oljčnih nasadih, ki bo v pomoč pri nadaljnji vzpostavitvi strategije namakanja slovenskih oljčnikov.

2 PREGLED DOSEDANJIH OBJAV

2.1 Izračun izgube vode iz tal in rastline

Na izgube vode iz tal v večji meri vpliva izhlapevanje vode s površine tal oz. proces evaporacije (E) in izhlapevanje vode z listne površine v atmosfero oz. proces transpiracije (T). Procesa evaporacija in transpiracija sestavljata pojav evapotranspiracije (ET), ki ga izražamo v debelini vodne plasti, ki izhlapi v določenem časovnem intervalu (npr. mm/mesec, mm/dan). V izogib težavam pri določanju stopnje evapotranspiracije za vsako posamezno rastlino in njeno razvojno fazo je bil definiran koncept referenčne evapotranspiracije, ki nam omogoča primerjavo evapotranspiracije med različnimi okolji in letnimi časi (Allen in sod., 1998; Habjan, 2008; Cesar, 2011). Referenčna evapotranspiracija (ETo) predstavlja količino vode, ki glede na lastnosti atmosfere in količine razpoložljive energije lahko pride v atmosfero z območja pokritega z referenčno kulturo - travnato rušo (višine 0,12 metra, s konstantno površinsko upornostjo 70 m/s, albedom 0,23) in dobro oskrbljenega z vodo.

Vrednosti referenčne evapotranspiracije (ETo) se lahko skupaj s koeficientom rastline (Kc), uporabi za izračun potreb po vodi za izbrano rastlino. Koeficient rastline nam pove, kolikšen je popravek referenčne evapotranspiracije za izbrano rastlino v posamezni razvojni fazi v določeni geografski regiji. V klimatskih razmerah Slovenske Istre se v izračunih za oljko upošteva koeficient rastline (zimski in pomladni meseci - 0,70; poletni mesecih

- od 0,50 do 0,58; jesenski meseci - od 0,65 do 0,69), ki ga je Organizacija za prehrano in kmetijstvo – FAO (Food and Agriculture Organization) določila za subhumidno podnebje Sredozemlja. Produkt referenčne evapotranspiracije in koeficienta rastline definira evapotranspiracijo rastline (ETc), ki predstavlja največjo količino vode, ki glede na lastnosti atmosfere in količine razpoložljive energije lahko pride v atmosfero z neprekinjenega območja, v celoti prekritega z izbrano rastlino in dobro oskrbljenega z vodo (Mikoš in sod., 2002) v standardnih razmerah ter izraža količino vode, ki jo rastlina potrebuje za njen nemoten razvoj (Allen in sod., 1998).

2.2 Prilagoditev in odziv oljke na sušni stres

Oljka je anatomsko-morfološko in fiziološko zelo dobro prilagojena na pomanjkanje vode v tleh, visoke temperature in intenzivno sončno obsevanje. Debeli usnjati listi z majhnimi listnimi ploskvami, ki imajo listno povrhnjico na zgornji strani prekrito s kutikulo, na spodnji strani pa s kratkimi zvezdastimi laski, zmanjšujejo izgubo vode skozi površino lista, kar rastlini omogoča boljše gospodarjenje z vodo v ekstremnih razmerah (Connor in Fereres, 2005).

Oljka se na pomanjkanje vode v tleh odzove tudi z zapiranjem listnih rež oz. zmanjšano stomatalno prevodnostjo, ki poveča učinkovitost izrabe vode (WUE – water use effciency) v ekstremno sušnih razmerah. V sušnih razmerah se v listu rastline močno zmanjša vodni potencial lista. Zaradi zmanjšanja tlačne komponente vodnega potenciala, turgorja, v listni povrhnjici pride do hidropasivnega zapiranja listnih rež in s tem do zmanjšanja stomatalne prevodnosti in izgube vode skozi listno površino. Ob zmanjšanju vsebnosti vode in vodnega potenciala v tkivih oljke je rastlina sposobna vzpostaviti velik gradient vodnega potenciala med listi in koreninami, zaradi česar je oljka v sušnih razmerah sposobna sprejemati tudi vodo, ki je v tleh vezana s silo do 2.5 MPa oz. 25 barov (Wahbi in sod., 2005). Xiloyannis in sod. (1999) ugotavljajo, da vodni stres pri oljki povzroča ustavljanje rasti poganjkov, ne vpliva pa na fotosintetsko aktivnost, kar omogoča kontinuirano sintezo asimilatov in njihovo akumulacijo v različnih delih rastline, še posebno v koreninskem sistemu. Pri zapiranju listnih rež v sušnih razmerah ima pomembno vlogo tudi zmanjšanje vodnega potenciala v tleh in dehidracija koreninskega sistema. V suhih tleh se v dehidriranem koreninskem sistemu sproži sinteza abscizinske kisline (ABA), ki se prenese v nadzemne dele rastline in v listih spodbudi hidroaktivno zapiranje listnih rež. S hidroaktivnim in hidropasivnim zapiranjem listnih rež se zmanjša stomatalna prevodnost, ki lahko ob manjšem sušnem stresu izboljša absorpcijo vode v rastlino ter poveča učinkovitost izrabe vode (WUE – water use effciency) (Bongi in Palliotti, 1994; Taiz in Zeiger, 2010).

Navkljub morfološkim, anatomskim in fiziološkim prilagoditvam oljke na omejene vodne zaloge v tleh, so daljša sušna obdobja v fazi rasti eden najpomembnejših vzrokov za pojav majhne in izmenične rodnosti (Cimato in sod., 1999).

Reakcija rastlin na sušni stres je odvisna tudi od fiziološkega stanja ter razvojne faze rastline (Štampar, 2006). V kolikor se sušni stres pojavi v času, ko oljka najbolj potrebuje vodo za rast in razvoj, torej med rastjo poganjkov, razvojem cvetnih brstov, cvetenjem, nastavitvijo plodov, rastjo plodov (intenzivna delitev in rast celic) in akumulacijo olja (Fernandez in Moreno, 1999), lahko sušni stres negativno vpliva na metabolne procese, sprejem hranil, fotosintezo, razvoj generativnih organov, venenje listov ter posledično na kakovost in količino pridelka (Orgaz in Fereres, 2004).

Poznavanje razsežnosti problematike vodnega stresa pri gojenju oljk, ki na eni strani pozitivno vpliva na akumulacijo olja v plodovih in na drugi strani v ekstremno sušnih razmerah privede do kolapsa metabolnih procesov v rastlini, je temelj za določitev optimalnega praga sušnega stresa, ki zagotavlja uravnoteženo razmerje med velikostjo pridelka in vsebnostjo olja (Bandelj, 2012).

2.3 Deficitni princip namakanja oljk in tehnologije dodajanja vode

Pomanjkanje vode v tleh nadomeščamo z ukrepom namakanja, s katerim z dodajanjem vode v času sušnega stresa zagotovimo količinsko in kakovostno primeren pridelek. Pri optimalnem namakanju z namakalnim obrokom nadomestimo izgubljeno količino vode, ki je enaka celotni dejanski evapotranspiraciji rastline in nam v času kmetijskih suš zagotovi konstantne in kakovostne

pridelke (Pintar in sod., 2010). Izhajajoč iz časovnih in količinskih omejitev razpoložljivih vodnih količin v sredozemskem prostoru in ob poznavanju problematike vpliva sušnega stresa na gojenje oljk, se uporaba principa »optimalnega namakanja« v oljkarstvu ne priporoča (Pintar in sod., 2010, Podgornik in sod., 2012 b).

Rezultati številnih raziskav so pokazali, da lahko v oljkastvu tudi z uporabo »deficitnega namakanja«, kjer namakalni obrok ne pokriva količine celotne evapotranspiracije rastline (ETc), pozitivno vpliva na količino pridelka (Patumi in sod., 1999; Melgar in sod., 2008; Gómez-Rico in sod., 2007; Fernandes-Silva in sod., 2010). Pri »deficitnem namakanju« oljko namerno oskrbimo z manj vode, kot je to optimalno potrebno. S tem zmanjšamo količino dodane vode na od 41 % do 85 % optimalnega namakalnega obroka (Pintar in sod., 2010, Podgornik in sod., 2012 b). Deficitno namakanje temelji na načelu, da obrok vode dodamo takrat, ko rastlina dodano vodo najbolj gospodarno uporabi. Kljub temu osnovnemu načelu pa se načini aplikacije vode pri deficitnem namakanju močno razlikujejo.

Dodajanje namakalnega obroka lahko izvedemo tako, da je vodni primanjkljaj enakomerno razporejen preko celotne rastne dobe (CDI – Continus deficit irrigation) (Giorio in sod., 1999), da vodo dodamo samo v kritični razvojni fazi (RDI – regulated deficit irrigation) (Iniesta in sod., 2009) ali da polovico korenskega sistema izbrane rastline izpostavimo sušnemu stresu, drugo polovico pa optimalno oskrbimo z vodo (PRD – Partial rootzone drying) (Fernández in sod., 2006).

Optimalno velikost namakalnega obroka je pri deficitnem principu namakanja oljk zelo težko ovrednoti, saj poleg lastnosti tal, meteoroloških parametrov ter agrotehničnih ukrepov na količino dodane vode močno vpliva tudi specifični odziv izbrane sorte na vodni deficit. Na podlagi rezultatov triletnega poskusa namakanja oljk 'Istrske belice' v Slovenski Istri izvedenega v okviru Ciljno raziskovalnega programa (CRP) »Konkurenčnost Slovenije 2006-2013« - Prilagajanje tehnologij pridelave vremenskim razmeram za doseganje visokih in kakovostnih pridelkov oljk in oljčnega olja (V4-0557) je bilo ugotovljeno, da je v ekstremno sušni rastni dobi (količina padavin od maj do novembra: 262 mm) za deficitno oskrbo oljk z vodo, kjer je namakalni obrok enak 33 % ETc, potrebno dodati 1.728 m^3/ha vode. V povprečni rastni dobi oljke (maj–november), ko količina padavin znaša 463 mm pa le 729 m^3/ha, kar je v skladu s količinami vode (500 - 2.500 m^3/ha) izračunane v okviru Slovenskega namakalnega projekta za povprečna leta. Znatno manjše količine vode za deficitno namakanje oljk z namakalnim obrokom enakim 33 % ETc priporočajo Patumi in sod. (2002), ki so s proučevanjem vpliva namakanja na sorto 'Kalamata' na jugu Italije ugotovili, da je med rastno dobo, ko količina padavin ne preseže 95 mm oz. 74 mm, potrebno dodati samo 290 m^3/ha oziroma 490 m^3/ha vode. Zgoraj navedene različne količine dodane vode pri namakalnem obroku enakem 33 % ETc narekujejo, da je potrebno optimalne odmerke vode za deficitno namakanje določiti za vsako pridelovalno območje posebej, saj poleg odziva sorte na vodni deficit, lastnosti tal, meteoroloških parametrov ter agrotehničnih ukrepov na optimalno velikost namakalnega obroka vpliva tudi prilagoditev sortnega izbora na pridelovalni okoliš.

Za izvedbo deficitnega principa namakanja oljk je poleg principa dodajanja vode potrebno izbrati tudi primerno tehnologijo namakanja, saj se te med seboj razlikujejo po delovanju namakalne opreme, načinu dodajanja in količini porabljene vode. Namakalna tehnika, ki omogoča najintenzivnejšo rastlinsko pridelavo ob največjem varovanju okolja je kapljično namakanje, ki poleg majhne porabe energije (delovanje pri nizkem tlaku), zagotavlja racionalno in gospodarno porabo vode. S kapljičnim namakanjem ne namakamo celotne površine nasadov, ampak vodo dodamo večkrat v manjših obrokih glede na dejanske potrebe rastline in to samo v območje korenin. Rastlina zato razvije koreninski sistem v manjšem volumnu tal, ki je ob morebitni okvari namakalnega sistema bolj izpostavljena suši. Celoten namakalni sistem je položen na površino ali vkopan v tla, zato se listne površine ne omočijo, zaradi česar ni nevarnosti pojava bolezni, kot je le to pri namakanju z oroševanjem (Pintar, 2003).

Namakanje z oroševanjem izvajamo z razpršilci, ki z delovanjem pri visokem tlaku omogočajo enakomerno razporeditev vode po celotni površini. Vodo pri tehniki oroševanja na namakano površino dodajamo čim manjkrat v čim večjih odmerkih

oziroma v tako velikih odmerkih kolikor to dopuščajo lastnosti tal in lastnosti izbrane rastline. Zaradi delovanja namakalne opreme pri visokih tlakih in večjega dela omočene površine je poraba energije in izgube vode pri namakanju z oroševanjem zelo velika (Pintar, 2003).

Na območjih, kjer razpolagamo z velikimi količinami vode in kjer so nakloni obdelovalnih površin majhni, se v oljkastvu poslužujejo namakanja s poplavljanjem in namakanja v brazde. Pri namakanju s poplavljanjem dodamo vodo na celotno površino nasada, tako da se tla popolnoma napojijo z vodo. Na podobnem principu temelji namakanje v brazde, kjer v namakalni kanal (globine 20-30 cm), ki smo ga predhodno zorali s plugom, dodamo vodo (Pintar, 2003; Çetin in sod., 2004).

2.4 Vpliv deficitnega namakanja na vegetativno rast in velikost pridelka

Izsledki proučevanja vpliva namakanja na sorte 'Chetoui', 'Chemlali', 'Coratina', 'Picholine' in 'Manzanilla' v Tuniziji (Aïachi-Mezghani in sod., 2012) so pokazali, da vegetativna rast oljk ni odvisna samo od velikosti namakalnega obroka ampak tudi od obremenitve drevesa in količine pridelka. Številni avtorji navajajo, da je v letih z veliko količino pridelka in veliko obremenitvijo dreves zmanjšana rast poganjkov (Lavee in sod., 1999; Melgar in sod., 2008; Martín-Vertedor in sod. 2011). Posledica je zmanjšano število socvetij in manjši pridelek v naslednjem letu. Manjša vegetativna rast oz. rast poganjkov pri večji obremenitvi drevesa in večja vegetativna rast pri manjši obremenitvi drevesa vpliva na nihanje količin pridelka med leti (izmenična rodnost), ki jo kljub optimalni velikosti namakalnega obroka (100 % ETc) ne moremo popolnoma odpraviti (Pierantozzi in sod., 2013). Pri tem pa avtorji ugotavljajo, da je ne glede na velikost pridelka in dolžino poganjka, razvoj socvetij in cvetov močno odvisen od prisotnosti sušnega stresa v času pred in med cvetenjem (Orgaz in Fereres, 2004). Z dodajanjem vode med oblikovanjem socvetij, cvetov, oploditvijo in začetno fazo razvoja plodov lahko povečamo število plodov na poganjek, število plodov na socvetje ter gostoto plodov na poganjek (Grattan in sod., 2006).

Ustrezno dodajanje vode med rastno dobo lahko zagotovi tudi večji prirast debla. Debelino debla, ki jo v vrhunsko dovršenih namakalnih sistemih spremljajo z dendrometri, uporabljajo kot parameter za dnevno uravnavanje količine namakalnega obroka (Moriana in sod., 2013). Rezultati poskusa namakanja sorte 'Cobrancosa' na Portugalskem (Fernandes-Silva in sod., 2010) in izsledki proučevanja vpliva namakanja na sorto 'Istrska Belica' v Slovenski Istri (Podgornik in sod., 2012b) so pokazali, da je povprečni prirast obsega debla za polovico manjši (letni povprečni prirast debla: 13 % oz. 17 %) pri nenamakanih drevesih, kot pri drevesih izpostavljenih različnim namakalnim režimom (povprečni prirast debla sorte 'Cobrancosa': 25 % - 100 % ETc, 20 % - 30 %ETc; povprečni prirast debla sorte 'Istrska Belica': 51 % - 100 % ETc; 47 % - 66 % ETc; 40 % - 33 % ETc).

Številne raziskave namakanja oljk so pokazale, da se pridelek oljk linearno povečuje s količino dodane vode (Patumi in sod., 1999; Melgar in sod., 2008; Moriana in sod., 2003; Gómez-Rico in sod., 2007, Ahmed in sod., 2007). Rezultati poskusa namakanja sorte 'Cornicabra' na jugu Španije (Gómez-Rico in sod., 2007) in izsledki proučevanja vpliva namakanja na sorto 'Chemlali' v Tuniziji (Ahmed in sod., 2007) so pokazali, da lahko z deficitnim namakanjem oljk dosežemo do 35 % večje pridelke, saj so pri nenamakanih drevesih izmerili manjše pridelke (39 kg/drevo; 26 kg/drevo), kot pri drevesih, ki so bila različno namakana (52-53 kg/drevo; 35-37 kg/drevo). Med različnimi namakalnimi režimi ni bilo statistično značilnih razlik. Podobni rezultati so bili ugotovljeni tudi v študiji proučevanja vpliva namakanja na pridelek oljk sorte 'Istrske belice' v Slovenski Istri, v kateri je bilo dokazano, da so pridelki pri deficitnem principu namakanja z namakalnim obrokom 33 % ETc (23 kg/drevo) in 66 % ETc (21 kg/drevo) za 25 % oz. 19 % večji kot pri kontroli, kjer voda ni bila dodana (17 kg/drevo). Večji pridelek pri 33 % ETc obravnavanju je najverjetneje posledica neheterogenih rastnih razmer, saj je bil poskus namakanja zastavljen na različnih terasah, kjer pogosto prihaja do robnih efektov. Večji povprečni pridelek (31 kg/drevo – 55 %) kot pri kontroli je bil ugotovljen tudi pri namakalnemu obroku 100 % ETc (Podgornik in sod., 2012b).

Z večanjem količine dodane vode se poleg pridelka linearno povečuje tudi število plodov na drevo

(Tognetti in sod., 2006; Grattan in sod., 2006) ter zmanjšuje zrelostni indeks plodov in akumulacija olja v plodovih (Gómez-Rico in sod., 2007; Grattan in sod., 2006). Rezultati raziskav Patumi in sod. (2002), Fernandes-Silva in sod. (2010) ter Podgornik in sod. (2012a) so pokazali, da lahko največjo vsebnost olja v plodovih pri sortah 'Kalamata', 'Cobrançosa' in 'Istrska belica' dosežemo z namakalnim obrokom 33 % ETc oz. 66 % ETc. Nekateri avtorji zaključujejo, da je ekstrakcija olja iz optimalno namakanih plodov (100 % ETc), ki zaradi pomanjkanja sušnega stresa dozorijo kasneje in imajo večjo vsebnost vode kot olja, težja kot iz plodov, ki so izpostavljeni zmernemu sušnemu stresu in dozorijo prej (Fernandes-Silva in sod., 2010). Zaradi kasnejšega dozorevanja in težje ekstrakcije olja iz optimalno namakanih plodov (100 % ETc) Grattan in sod. (2006) priporočajo, da pridelke izpostavljene sušnemu stresu poberemo pred pridelki, ki so optimalno oskrbljeni z vodo.

3 ZAKLJUČKI

Izhajajoč iz časovnih in količinskih omejitev razpoložljivih vodnih virov v sredozemskem prostoru in ob poznavanju problematike vpliva sušnega stresa na gojenje oljk, se v oljkarstvu za zagotavljanje boljših gospodarskih rezultatov izvaja ukrep »deficitnega namakanja«. Z »deficitnim principom namakanja«, kjer namakalni obrok pokriva le 30 % - 60 % evapotranspiracije (ETc), lahko ob sočasni zmanjšani porabi vode povečamo rodnost rastline in s tem zagotovimo večjo vegetativno rast, večji pridelek in trajnostno rabo vodnih virov ter zagotovimo boljšo kondicijo dreves. Kljub spodbudnim rezultatom številnih raziskav v sredozemskem prostoru, pa je potrebno optimalne odmerke vode za deficitno namakanje določiti za vsako pridelovalno območje posebej.

4 ZAHVALA

Avtorici se zahvaljujeta Ministrstvu za kmetijstvo, gozdarstvo in prehrano RS in Agenciji za raziskovalno dejavnost RS za finančno podporo ciljno raziskovalnega projekta V4-0557, ki je omogočal prve raziskave namakanja oljk v Sloveniji.

5 VIRI

Ahmed B.C., Rouina B.B., Boukhris M. 2007. Effects of water deficit on olive trees cv. Chemlali under field conditions in arid region in Tunisia. Scientia Horticulturae, 113: 267-277, doi: 10.1016/j.scienta.2007.03.020

Aïachi-Mezghani M., Masmoudi-Charfi C., Gouiaa M., Labidi F. 2012. Vegetative and reproductive behaviour of some olive tree cultivars (*Olea europaea* L.) under deficit irrigation regimes in semi-arid conditions of central Tunisia. Scientia Horticulturae, 146: 143-152, doi: 10.1016/j.scienta.2012.07.030

Allen R.G., Pereira L.S., Raes D., Smith M. 1998. Crop evapotranspiration. Guidelines for Computing Crop Water Requirements. FAO, Rome, Irrigation and Drainage Paper, 56

Bandelj D. 2012. Prilagajanje tehnologij pridelave vremenskim razmeram za doseganje visokih in kakovostnih pridelkov oljk in oljčnega olja. Zaključno poročilo o rezultatih ciljnega raziskovalnega projekta. Univerza na Primorskem, Znanstveno-raziskovalno središče. Koper: 17 str.

Bongi G., Palliotti A. 1994. Olive. V: Handbook of environmental physiology of fruit crops. Temperate Crops. Schaffer B., Andersen P.C. (eds). CRC Press Inc., USA: 165-178

Breznik B., Vöröš S., Bučar Miklavčič M. 2007. Oljčno olje. Ministrstvo za kmetijstvo, gozdarstvo in prehrano, Ljubljana: 10 str.

Cesar P. 2011. Primerjava različnih metod izračuna evapotranspiracije. Diplomska naloga. Ljubljana, Univerza v Ljubljani, Fakulteta za gradbeništvo in geodezijo: 133 str.

Cimato A., Baldini A., Moretti R. 1999. L' olio di oliva: cultivar, ambiente e tecniche agronomiche. Parte 2^{a}. ARSIA, Firenze, Regione Toscana: 88 str.

Connor D.J., Fereres E. 2005. The physiology of adaptation and yield expression in olive. Horticultural Reviews, 31: 155-229

Çetin B., Yazgan S., Tipi T. 2004. Economics of drip irrigation of olives in Turkey. Agricultural Water Management, 66: 145–151, doi: 10.1016/j.agwat.2003.10.004

Fernández J.E., Dias-Espejo A., Infante J.M., Duran P., Palomo M.J., Chamorro V., Giron I.F., Villagarcia L. 2006. Water relations and gas exchange in olive trees under regulated deficit irrigation and partial rootzone drying. Plant Soil, 284: 273–291, doi: 10.1007/s11104-006-0045-9

Fernández J.E., Moreno F. 1999. Water use by the olive tree. Journal of Crop Production 2:101-162, doi: 10.1300/J144v02n02_05

Fernandes-Silva A.A., Ferreira T.C., Correia C.M., Malheiro A.C., Villalobos F.J. 2010. Influence of different irrigation regimes on crop yield and water use efficiency of olive. Plant soil, 333: 35-47, doi: 10.1007/s11104-010-0294-5

Giorio P., Sorrentino G., d'Andria R. 1999. Stomatal behaviour, leaf water status and photosynthetic response in field-grown olive trees under water deficit. Environmental and Experimental Botany, 42: 95–104, doi: 10.1016/S0098-8472(99)00023-4

Glavan M., Tratnik M., Cvejić R., Pintar M. 2012. Geoprostorska analiza potencialne ogroženosti kmetijstva v primeru suše. V: Zbornik referatov 23. Mišičev vodarski dan 2012, Maribor, 5. december 2012. Vodnogospodarski biro Maribor d.o.o.; Drava Vodnogospodarsko podjetje Ptuj: 21-28

Gómez-Rico A., Desamparados Salvador M., Moriana A., Pérez D., Olmedilla N., Ribas F., Fregapane G. 2007. Influence of different irrigation strategies in a traditional Cornicabra cv. olive orchard on virgin olive oil composition and quality. Food Chemistry, 100: 568-578 10.1016/j.foodchem.2005.09.075

Grattan S.R., Berenguer M.J., Connell J.H., Polito V.S., Vossen P.M. 2006. Olive oil production as influenced by different quantities of applied water. Agricultural Water Management, 85: 133-140, doi: 10.1016/j.agwat.2006.04.001

Habjan M. 2008. Analiza vodne bilance v Vipavski dolini. Diplomsko naloga. Univerza v Ljubljani Biotehniška fakulteta, Oddelek za agronomijo: 45 str.

Iniesta F., Testi L., Orgaz F., Villalobos F.J. 2009. The effects of regulated and continuous deficit irrigation on the water use, growth and yield of olive trees. European Journal of Agronomy, 30: 258–265, doi: 10.1016/j.eja.2008.12.004

Lavee S., Rallo L., Rapoport H.F., Troncoso A. 1999. The floral biology of the olive II. The effect of inflorescence load and distribution per shoot on fruit set and load. Scientia Horticulturae, 66: 267-277

Martín-Vertedor A.I., Rodríguez J.M.P., Losada H.P. Castiel E.F. 2011. Interactive responses to water deficits and crop load in olive (*Olea europaea* L., cv. Morisca). I. Growth and water relations. Agricultural Water Management, 98: 941-949, doi: 10.1016/j.agwat.2011.01.002

Melgar J.C., Mohamed Y., Navarro C., Parra M.A., Benlloch M., Fernandez-Escobar R. 2008. Long term growth and yield responses of olive trees to different irrigation regimes. Agricultural Water Management, 95: 968-972, doi: 10.1016/j.agwat.2008.03.001

Mikoš M., Kranjc A., Matičič B., Muller J., Rakovec J., Roš M., Brilly M. 2002. Hidrološko izrazje = Terminology in hydrology. Acta hydrotehnica, 20/32: 3-324

Moriana A., Corella M., Girónb I.F., Conejerod W., Moralese D., Torrecillas A., Moreno F. 2013. Regulated deficit irrigation based on threshold values of trunk diameter fluctuation indicators in table olive trees. Scientia horticulturae, 164: 102-111, doi: 10.1016/j.scienta.2013.09.029

Moriana A., Orgaz F., Pastor M., Fereres E. 2003. Yield responses of a mature olive orchard to water deficits. Journal of the American Society for Horticultural Science, 128: 425-431

Orgaz F., Fereres E. 2004. Riego. V: El Cultivo del Olivo. Barranco D., Fernández-Escobar R., Rallo L. (eds.). Mundi-Prensa, Madrid, 285-306

Patumi M., D'Andria R., Fontanazza G., Morelli G., Giori P., Sorrentino G. 1999. Yield and oil quality of intensively trained trees of three cultivars of olive under different irrigation regimes. Journal of Horticultural Science & Biotechnology, 74: 729-737

Patumi M., D'Andria R., Marsilio V., Fontanazza G., Morelli G., Lanza B. 2002. Olive and olive oil quality after intensive monocone olive growing (*Olea europaea* L., cv. Kalamata) in different irrigation regimes. Food Chemistry, 77: 27-34, doi: 10.1016/S0308-8146(01)00317-X

Pierantozzi P., Torres M., Lavee S., Maestri D. 2013. Vegetative and reproductive responses, oil yield and composition from olive trees (*Olea europaea*) under contrasting water availability

during the dry winter-spring period in central Argentina. Annals of Applied Biology, 164: 116-127, doi: 10.1111/aab.12086

Pintar M. 2003. Osnove namakanja: s poudarkom na vrtninah in sadnih vrstah v severovzhodni Sloveniji. Ljubljana: Ministrstvo za kmetijstvo, gozdarstvo in prehrano: 49 str.

Pintar M., Tratnik M., Cvejić R., Bizjak A., Meljo J., Kregar M., Zakrajšek J., Kolman G., Bremec U., Drev D., Mohorko T., Kodre N., Steinman F., Kozelj K., Prešeren T., Kozelj D., Urbanc J., Mezga K. 2010. Ocena vodnih perspektiv na območju Slovenije in možnosti rabe vode v kmetijski pridelavi: Ciljni raziskovalni program: končno poročilo. Biotehniška fakulteta. Ljubljana: 159 str.

Podgornik M., Pintar M., Arbeiter A., Bandelj D. 2012b. Vpliv sušnega stresa na rast in rodnost oljke (*Olea europaea* L.) sorte 'Istrska belica' v Slovenski Istri. V: Zbornik referatov 3. Slovenskega sadjarskega kongresa z mednarodno udeležbo. Hudin M. (eds.). Krško, 21.-23. november 2012. Ljubljana: Strokovno sadjarsko društvo Slovenije: 271-275

Podgornik M., Pintar M., Korpar P., Vuk I, Arbeiter A., Klančar U., Bandelj D. 2012a. Vpliv deficitnega namakanja na pridelek oljk (*Olea europea* L.) sorte 'Istrska belica'. V: Novi raziskovalni pristopi v oljkarstvu: zbornik znanstvenih prispevkov z mednarodnega posveta. Bandelj D., Podgornik M., Arbeiter A. (eds.). Koper, 16. – 17. februar 2012. Univerza na Primorskem, Znanstveno-raziskovalno središče, Univerzitetna založba Annales: 87-93

Register kmetijskih gospodarstev 2014. Ljubljana, Ministrstvo za kmetijstvo in okolje RS. http://rkg.gov.si/ (16. sep. 2014)

Statistični letopis Republike Slovenije 2013. Statistični urad Republike Slovenije, Ljubljana, 52: 61 str. http://www.stat.si/StatWeb/doc/letopis/2013/16_13/16-06-13.html (15.mar. 2015)

Sušnik A. 2003. Dinamika primanjkljaja vode za kmetijske rastline včeraj, danes in jutri. V:14. Mišičev vodarski dan 2003, zbornik referatov, Maribor, 5. december. 2003. Vodnogospodarski biro Maribor, Vodnogospodarsko podjetje Drava: 84–92.

Štampar F. 2006. Namakanje v sadjarstvu. Ljubljana, Ministrstvo za kmetijstvo, gozdarstvo in prehrano: 23 str.

Taiz L., Zeiger E. 2010. Plant physiology. Massachusetts, Publishers Sunderland: 777 str.

Tognetti R., d'Andria A., Lavini G., Morelli B. 2006. The effect of deficit irrigation on crop yield and vegetative development of Olea europaea L. (cvs. Frantoio and Leccino). European Journal of Agronomy, 25: 356–364, doi: 10.1016/j.eja.2006.07.003

Wahbi S., Wakrim R., Aganchich B., Tahi H., Serraj R. 2005. Effects of partial rootzone drying (PRD) on adult olive tree (*Olea europaea* L.) in field conditions under arid climate: I. Physiological and agronomic responses. Agriculture, Ecosystems & Environment, 106: 289–301, doi: 10.1016/j.agee.2004.10.015

Xiloyannis C., Dichio B., Nuzzo V., Celano G. 1999. Defence strategies of olive against water stress. Acta Horticulturae, 474: 423–426, doi: 10.17660/actahortic.1999.474.86

COBISS Code 1.02

DOI: 10.14720/aas.2015.105.2.18
Agrovoc descriptors: genetically modified organisms, risk assessment, environmental impact assessment, environment, risk, legislation, Slovenia

Agris category code: d50, f30

Namerno sproščanje gensko spremenjenih rastlin v okolje v Sloveniji

Zlata LUTHAR[1]

Received September 18, 2015; accepted October 15, 2015.
Delo je prispelo 18. septembra 2015, sprejeto 15. septembra 2015.

IZVLEČEK

Namerno sproščanje gensko spremenjenih višjih rastlin (GSVR) v okolje je v Sloveniji regulirano z Zakonom o ravnanju z gensko spremenjenimi organizmi (ZRGSO) Ur. l. RS 23/2005 in 21/2010, III. poglavje. Za vsako namerno sproščanje GSR v okolje je potrebno pridobiti dovoljenje, ki ga izda Ministrstvo za okolje in prostor (MOP). Vloga oz. prijava mora vsebovati zelo natančen in kompleksen opis GSR, njivo, kjer bo sproščena GSR in širšo okolico oz. okolje. Prijavo sestavlja Priloga 2 z dodatki: 1. Del A (tehnični podatki za izdajo dovoljenja za namerno sproščanje GSR v okolje); 2. Del B (ocena okoljskega tveganja); 3. Povzetek prijave za sproščanje GSR v slovenskem in angleškem jeziku, ki ga MOP posreduje v Bruselj in 4. Izpis za njivo iz zemljiškega katastra, na katero se bo sproščalo GSR. Postopek sproščanja do tu teče po omenjenem zakonu, ki velja že nekaj let in v katerem je jasno opredeljeno, da je sproščanje v Sloveniji mogoče. V konkretnem primeru GS riža iz leta 2011 je zakon veljal do izbire lokacije poskusa. Tu zakon ni bil upoštevan. Obveljal je pravilnik Sklada kmetijskih zemljišč in gozdov ter občinski sklep, ki sta bila močnejša od nacionalnega zakona in sta onemogočila gojenje GS riža na površini, ki je po zakonu primerna za sproščanje v okolje. Riž se v Sloveniji ne goji in nima divjih prednikov oz. bližnjih sorodnikov, s katerimi bi se lahko križal. Najbližje območje pridelovanja je v sosednji Italiji, ki je od potencialno izbrane lokacije v Sloveniji oddaljeno več kot 70 km.

Ključne besed: gensko spremenjene rastline, namerno sproščanje, okolje, tveganje, zakonodaja

ABSTRACT

DELIBERATE RELEASE OF GENETICALLY MODIFIED PLANTS INTO THE ENVIRONMENT IN SLOVENIA

Deliberate release of genetically modified higher plants (GMHPs) into the environment in Slovenia is regulated by the Law on the Management of Genetically Modified Organisms (ZRGSO) Ur. l. RS 23/2005 and 21/2010, III chapter. For each deliberate release of GMPs into the environment a license issued by the Ministry of Environment and Spatial Planning (MESP) must be acquired. The application or notification should contain a very accurate and complex description of the GMP, of the field where it will be released and of wider surroundings or environment. The application consists of Annex 2 with accessories: 1. Part A (technical data for the authorization of deliberate GMP release into the environment); 2. Part B (environmental risk assessment); 3. Application summary in Slovenian and English language) for the release of GMP into environment, which is transmitted to Brussels by MESP; 4. Extract from the Land Cadastre of the field to which the GMP will be released. The release procedure runs (till here) under the above mentioned Law, which has been in place for several years and which clearly defines that it is possible to release GMP in Slovenia. In the case of GM rice in 2011, the law applied till the site selection of the experiment. Here, the law was not sufficiently taken into account. It was prevailed by the regulation of Farmland and Forest Fund of the Republic of Slovenia and municipal decision, which was stronger than the national law and prevented the cultivation of GM rice in an area that is legally suitable for release of GMO into the environment. Rice is not grown in Slovenia and does not have wild ancestors or close relatives with whom it might mate. Nearest area of cultivation is in neighboring Italy, which is from potentially selected location in Slovenia more than 70 km away.

Keey words: genetically modified plants, deliberate release, environment, risk, legislation

[1] Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, e-mail: zlata.luthar@bf.uni-lj.si

1 UVOD

Gensko spremenjeni organizmi (GSO) so rezultat sodobne biotehnologije, katere tehnike in metodologije so omogočile proučevati in uporabljati biološke sisteme v koristne namene človeštva. Moderna biotehnologija ima svoje korenine v bazičnih raziskavah osnovnih bioloških mehanizmov, ki so vplivali na razvoj orodij za manipulacijo z DNA ter prenos genetskega materiala med različnimi vrstami organizmov. GSO so pridobljeni z izolacijo molekul nukleinske kisline enega organizma in vnosom teh molekul v drugi organizem tako, da postanejo sestavni del njegove dedne informacije, ki je sposobna dedovanja. GSO so odprli nove možnosti v bioloških raziskavah, vplivali so na proučevanje funkcije določenih genov, mehanizme kontrole izražanja genov in ponudili veliko aplikativno vrednost. Njihovi produkti se že desetletja uporabljajo v medicini za proizvodnjo biofarmacevtikov, kjer so vključeni GS mikroorganizmi, evkariontske celične linije in višji organizmi. Genska terapija se ukvarja in poskuša nadomestiti manjkajoče ali okvarjene gene. GSO se uporabljajo tudi v drugih vejah gospodarstva, predvsem v kmetijstvu in prehrambeni industriji (Seidman in Moore, 1999).

Uporaba GSO, tako kot kakršnihkoli, lahko pomeni tudi potencialne nevarnosti za okolje ter zdravje ljudi in živali. Te nevarnosti izhajajo iz dejstva, da GSO pomenijo novost v naravi in niso bili podvrženi naravni selekciji in evoluciji ter iz tega izhaja negotovost glede njihovih kratkoročnih in dolgoročnih vplivov na okolje ter zdravje ljudi in živali. Zaradi kompleksnosti ni mogoče predvideti in utemeljiti vseh negotovosti, ki so povezane z obnašanjem organizmov in bioloških sistemov, katerih del so (Wheelis in sod., 1998).

V Evropi je uveljavljeno načelo previdnosti pri ravnanju in uporabi proizvodov moderne biotehnologije. Previdnostni pristop izhaja iz predpostavke, da GSO predstavljajo morebitno nevarnost za okolje ter zdravje ljudi in živali. Zato sta uporaba in delo z GSO regulirana z evropskim zakonom in direktivami, ki jih imajo v svojo zakonodajo vključene tudi ostale članice na nacionalni ravni. Zakonodaja, kot bistveni del, zahteva oceno tveganja, ki ga novonastali GSO predstavlja za okolje, zdravje ljudi in živali ter upravljanje s tveganjem, to je izvajanje ukrepov, ki zmanjšujejo tveganje na najnižjo možno raven (Directive 2001/18/EC, 2001).

V Sloveniji je v veljavi Zakon o ravnanju z GSO (ZRGSO, Ur. l. RS 23/2005 in 21/2010), ki ureja delo z GSO in določa ukrepe za preprečevanje in zmanjševanje možnih škodljivih vplivov na okolje in zdravje ljudi ter živali. Zakon regulira delo z GSO v zaprtih sistemih, namerno sproščanje GSO v okolje in dajanje GSO oz. njihovih izdelkov na trg.

2 DELO Z GSO JE ZAKONSKO REGULIRANO

Delo z GSO se odvija na prej omenjenih treh ravneh. Njihovo ravnanje mora biti prijavljeno, za katero poskrbi prijavitelj, ki je fizična ali pravna oseba. Ta v okviru svoje dejavnosti izvaja delo z GSO v zaprtem sistemu, namerava namerno sproščati GSO v okolje ali dati izdelek na trg. Pred pričetkom dela mora oddati vlogo s predpisanimi podatki na Ministrstvo za okolje in prostor (MOP), na podlagi katere pridobi potrdilo oz. dovoljenje za delo z GSO.

Delo z GSO v zaprtih sistemih, tudi prostor, kjer se to delo izvaja, je opredeljeno z ZRGSO. Zaprti sistem je fizično omejen prostor, lahko je laboratorij, proizvodni oddelek, rastlinjak ali drug zaprti prostor, kjer se opravlja delo z GSO. Delo z GSO v zaprtem sistemu zajema gojenje GSO, razmnoževanje, shranjevanje, prevažanje, premeščanje, odstranjevanje in uničevanje, pri katerem se izvajajo zadrževalni ukrepi. Zadrževalni ukrep je fizična zapora ali kombinacija fizične zapore s kemično ali biološko omejitvijo, ki upošteva načela dobre laboratorijske in proizvodne prakse, za omejitev stika GSO z okoljem in prebivalstvom ter izključuje ali zmanjšuje sposobnost razmnoževanja GSO ali prenosa spremenjenega genskega materiala izven zaprtega sistema.

Delo z GSO v zaprtem sistemu je uvrščeno v štiri varnostne razrede, ki izhajajo iz ravni tveganja, ki ga GSO in delo z njimi predstavljajo za zdravje ljudi, živali in okolje. Merila, po katerih se delo z določenim GSO uvrsti v posamezni varnostni razred, so določena z Uredbo o merilih za uvrstitev dela z GSO v zaprtem sistemu v varnostni razred in o zadrževalnih ter drugih varnostnih ukrepih za posamezen varnostni razred (Ur. l. RS, št. 71/2011).

2.1 Namerno sproščanje GSO – rastlin v okolje

Po zakonu (ZRGSO 23/2005, 21/2010) in direktivi (Directive 2001/18/EC) je namerno sproščanje GSO v okolje vsak nameren vnos, ki zagotavlja veliko stopnjo varnosti, razen dajanja izdelkov na trg, pri katerem se ne izvajajo zadrževalni ukrepi za omejitev stika GSO z okoljem in prebivalstvom. Izdelek je GS ali kombinacija GS, ki je sestavljen ali vsebuje GSO ali kombinacijo GSO in je dan na trg. Dajanje na trg je posredovanje oz. dajanje izdelkov osebam proti plačilu ali brez njega. Za dajanje na trg se šteje tudi uvoz izdelkov na carinsko območje Evropske skupnosti. Za dajanje na trg se ne šteje dajanje GSO osebam za delo z GSO v zaprtih sistemih ali za namerno sproščanje GSO v okolje skladno z zakonom.

V primeru namernega sproščanja GSR v okolje, vlogo sestavlja obrazec t.i. Priloga 2 in dodatki. Obrazec Priloga 2 je razdeljen na poglavja oz. dele, ki v strnjeni obliki zajemajo tehnične podatke o GSR ter priloge. Obvezne priloge so: 1. Ocena tveganja namernega sproščanja GSR v okolje (krajše: ocena okoljskega tveganja); 2. Izpis za njivo iz zemljiškega katastra, na katero se bo sproščalo GSR; 3. Povzetek tehnične dokumentacije; 4. in druge priloge, ki so vezane na specifičnosti sproščanja (zaupni podatki itd.).

Dodatki so napisani obširno in temeljito ter so podkrepljeni z literaturnimi navedbami. Sestavlja jih: 1. Del A - tehnični podatki za izdajo dovoljenja za namerno sproščanje GSR v okolje; 2. Del B - ocena okoljskega tveganja, ki je istočasno tudi obvezna priloga obrazca Priloga 2; 3. Povzetek prijave za sproščanje GSVR v slovenskem in angleškem jeziku.

1. Tehnični podatki so zbrani v delu A, strnjena verzija se vpiše v obrazec Priloga 2, razširjena in podkrepljena z literaturnimi navedbami se pripravi posebej kot dodatek in je v grobem sestavljena iz 7 informacijskih poglavij:

A. Splošne informacije o prijavitelju: ime, strokovna izobrazba, poklicne izkušnje odgovornih in naslov projekta;

B. Informacije o prejemnem organizmu: vrsti, sorti, ki je bila predmet GS; dejavniki, ki vplivajo na razmnoževanje; obdobje oz. trajanje ciklusa razmnoževanja, ki je povezano z okoljskimi in genetskimi dejavniki; spolna kompatibilnost z drugimi gojenimi in divjimi vrstami ter razširjenost kompatibilnih vrst; sposobnost preživetja; razširjenost prejemne ali starševske rastline; morebitni toksični učinki za človeka, živali in druge organizme;

C. Informacije o genski spremembi: opis uporabljenih metod za GS; narava in izvor uporabljenega vektorja; velikost in ime donorjev in funkcija fragmentov (insertov oz. vključkov) predvidenih za vključitev;

D. Informacije o GSR: opis uvedenih ali spremenjenih lastnosti in značilnosti ter namen uvedbe; informacije o vsakem vključenem ali odstranjenem nukleotidnem zaporedju; velikost in struktura ter število kopij vključenega zaporedja; metode vnosa; izražanje vključka, čas in način ter mesto izražanja; metode spremljanja; informacije o razlikah med GSR in prejemno rastlino glede razmnoževanja, sposobnosti preživetja, genske in fenotipske stabilnosti vključka, morebitne spremembe sposobnosti GSR za prenos genskega materiala na druge rastline in bakterije, možna toksičnost, alergenost ali drugi škodljivi učinki za zdravje ljudi in živali, interakcije z abiotskim okoljem;

E. Informacije o kraju namernega sproščanja GSR: lega in velikost kraja sproščanja; opis lokalnega ekosistema, vključno s podnebjem, floro, favno in analizo tal ter vode v primeru zalivanja posevka; prisotnost naravnih ali gojenih kompatibilnih vrst in sort; navede se tudi bližnje uradno priznane biotope;

F. Informacije o namernem sproščanju: namen, metoda, začetek in trajanje sproščanja; metoda priprave in upravljanja z mestom sproščanja pred, med in po sproščanju vključno s prisotnimi

kulturami in načini pobiranja oz. žetve; ocenjeno število sproščenih GSR;
G. Informacije o načrtih monitoringa, kontrole kraja in odpadkov po sproščanju: morebitni varnostni ukrepi; potrebna razdalja od drugih divjih in gojenih kompatibilnih vrst; ukrepi za zmanjšanje širjenja reproduktivnih organov, pelod, semena, gomolji itd., metode in ukrepi čiščenja ter požig ostankov takoj po žetvi, obdelava tal; opis načrtov monitoringa in pripadajočih tehnik; opis načrtov ob izrednih stanjih; metode in zaščitni postopki za zavarovanje kraja sproščanja;
H. Uporabljena literatura in priloge: biografija in bibliografija prijavitelja; nukleotidna zaporedja vključkov; restrikcijska analiza vektorja itd.
Povzetek tehničnih podatkov prijave sproščanja, ki ga sestavljajo prej omenjena ključna poglavja v strnjeni obliki.

2. Ocena okoljskega tveganja je priloga oz. dodatek obrazca Priloga 2 in je predstavljena v t.i. delu B, ki ga sestavljajo štiri poglavja:

1. Karakteristike GSR in sproščanje v okolje: informacije o prejemnem organizmu (sistematika, izvor, sorta); opis genske spremembe in GSR (tkivna lokacija in raven izražanja GS lastnosti); predvideno sproščanje (kraj, namen, specifični pogoji); pridelovalne tehnike; aktivnosti monitoringa med in po sproščanju; opis prejemnega okolja, interakcije med GSR in okoljem;
2. Ocena okoljskega tveganja: uvod, kratka predstavitev genske spremembe in prejemne rastline (običajno je to že uveljavljena sorta); možnosti in ocena širitve GSR v okolje s cvetnim prahom, semeni in ostalimi deli rastline, širitev oz. premiki, ki jih povzročajo živali, ptice in človeške napake – nesreče; preživetje v okolju; potencialni drugi negativni učinki; ocena tveganja širitve za človeško in živalsko zdravje ter okolje; izvajanje strategij za obvladovanje tveganj zaradi sproščanja v okolje; možnosti in ocena prenosa genskega materiala med spolno kompatibilnimi in nekompatibilnimi organizmi tudi tistimi, ki pripadajo različnim taksonomskim skupinam in potencialni negativni učinki; ocena potencialnih posledic prenosa genskega materiala; ocena tveganja vezanega na genski prenos za človeka, živali in okolje; izvajanje strategij za obvladovanje tveganj pri sproščanju v okolje; potencialni negativni učinki izpostavljenosti GSR na kraju sproščanja z drugimi prisotnimi organizmi: s človekom, drugimi vretenčarji (ptice, glodalci), nevretenčarji (žuželke, mikroorganizmi); izvajanje strategij za obvladovanje tveganj je vezano na velikost in naravo sproščanja, na tehnologijo pridelave ter na genetsko in fenotipsko stabilnost lastnosti, na mesto izražanja lastnosti ali je po celi rastlini, ali samo v posameznem organu oz. tkivu;
3. Zaključke o potencialnem okoljskem učinku sproščene GSR: posebej se izpostavi morebitne možnosti, da GSR postane invazivnejša od gostiteljske ali sorodnih rastlin na območju sproščanja ali v naravnih habitatih; morebitne selektivne prednosti ali slabosti GSR; potencialne možnosti genskega prenosa GSR v razmerah pridelave na spolno kompatibilne rastlinske vrste ter morebitne selektivne prednosti ali slabosti, ki jih pridobijo omenjene rastline z genskim prenosom; potencialne takojšnje ali poznejše okoljske učinke, ki so rezultat posrednih in neposrednih interakcij med GSR in ciljnimi organizmi (roparice, paraziti, patogeni) ter neciljnimi organizmi, tudi tistimi, ki medsebojno delujejo s ciljnimi organizmi na ravni populacij tekmecev, rastlinojedov, simbiotov, ki so parazitoidni in patogeni; možni vplivi na človekovo zdravje izhajajoč iz posrednih in neposrednih interakcij GSR z osebami, ki jih uporabljajo, pridejo v stik z njimi ali se nahajajo v bližini krajev sproščanja; možni vplivi na živali in posledice za prehransko verigo, če je GSR in iz nje pridobljen produkt namenjen za živalsko prehrano; možni vplivi na biokemične procese, ki izhajajo iz interakcij GSR in ciljnih in ne-ciljnih organizmov na kraju in v bližini izpusta;
4. Uporabljena literatura: za pripravo ocene okoljskega tveganja;
Povzetek ocene okoljskega tveganja, ki ga sestavljajo ključna prej omenjena poglavja v strnjeni obliki.

3. Povzetek prijave za sproščanje GSR tudi v angleškem jeziku (Summary notification information format for the release of genetically modified higher plants - SNIF), ki ga MOP posreduje v Bruselj.

2.2 Ocena tveganja pri delu z GSO - rastlinami

Ocena tveganja, ki ga novonastali GSO predstavlja za okolje, zdravje ljudi in živali ter izvajanje ukrepov, ki zmanjšujejo tveganje na najnižjo možno raven, je osrednjega pomena pri delu z GSO. Tveganje pri delu z GSO je verjetnost, da bo ravnanje z GSO posredno ali neposredno, takoj ali kasneje ali dolgoročno kumulativno škodljivo vplivalo na zdravje ljudi, živali in okolje. Predvsem glede ohranjanja biotske raznovrstnosti, ohranjanja avtohtonih rastlinskih populacij, sort in živalskih pasem, rodovitnosti plodne zemlje, prehranjevalne verige ali zdravja človeka in živali. Lastnosti nevarnih dejavnikov, ki so lahko kemični, fizični ali biološki, so določene glede na naravo in glede na škodljivost posledic, ki jih lahko povzročijo ljudem, živalim ali okolju. Nevarne dejavnike se poskuša omejiti z različnimi zadrževalnimi ukrepi, tako da vsaka potencialna nevarnost ne pomeni avtomatično tudi tveganja. Nevarnost in tveganje sta različna, vendar soodvisna pojma, ki opredeljujeta stopnjo tveganja (Kinderlerer, 1997; Levin, 1997; McColl in sod., 2000; Jardine in sod., 2003).

Tveganje je torej določeno z verjetnostjo uresničitve nevarnosti in z resnostjo potencialnih škodljivih posledic. Tveganje pri delu z biološkimi dejavniki pomeni, kakšna je verjetnost, da pridemo v stik z mikroorganizmom (kontaminacija), rastlino (kontaktne spremembe zaradi izločanja rastlinskih snovi) in kakšne so lahko posledice stika. Te so lahko zanemarljive, lahko pa v primeru patogenega biološkega organizma ta povzroči infekcijo, obolenje človeka in v najhujši obliki tudi smrt.

Tveganje zaradi uporabe GSR je običajno predstavljeno z nekim splošnim vzorcem in je opredeljeno z nevarnostjo in izpostavljenostjo GSR. Nevarnost je potencialni pojav škodljivih vplivov zaradi dejanskih lastnosti organizma, medtem ko je izpostavljenost potencial, da bo prejemnik (osebek, populacija, sistem) v stiku s tem organizmom. Določitev nevarnosti (ang. hazard identification) je povezana z lastnostjo GSR, ki lahko v okolju povzroči škodo (npr. toksičnost, invazivnost). Ocena izpostavljenosti (ang. exposure assessment) je mera verjetnosti, da bo prišlo do izpostavljenosti okolja in ekosistema z morebitnimi nevarnimi lastnostimi GSR. Presoja vpliva (ang. effects assessment) je verjetnost, da bo zaradi izpostavljenosti prišlo do nezaželenih vplivov in je predstavljena s funkcijo nevarnosti in izpostavljenosti, ki predstavlja merilo vpliva. Določitev stopnje tveganja (ang. risk characterization) je povezano z oceno tveganja na osnovi presoje vpliva in doda se kriterij, ki določi različne stopnje tveganja, npr. nizka, srednja, visoka stopnja tveganja (Pravilnik o oceni tveganja za delo z GSO v zaprtem sistemu, Ur. l. RS 45/2004; Pravilnik o oceni tveganja za namerno sproščanje GSO v okolje, Ur. l. RS 4/2006; Pravilnik o oceni tveganja za dajanje izdelka, ki vsebuje GSO, na trg, Ur. l. RS 13/2006).

Velikost tveganja se lahko določi na dva načina. Prvi način je izkustveni, kjer stopnjo tveganja določimo na osnovi znanih primerov, ki so se že zgodili v preteklosti. Drugi način ocene tveganja je posreden, kjer se oceni teoretične možnosti za uresničitev morebitne izpostavljenosti nevarnim dejavnikom. Potencialne posledice pa se določi s pomočjo opravljenih eksperimentalnih študij in povzame literaturne navedbe.

Ocena tveganja vključuje ugotavljanje in ovrednotenje tveganja, ki bi lahko nastalo zaradi dela z GSR v zaprtem sistemu, namernem sproščanju GSR v okolje ali dajanja izdelka na trg, za vsak primer posebej. Ocena tveganja zelo temeljito ožje in širše ter z interakcijami neživega in živega okolja predstavlja in umešča GSR v okolje in opozarja na morebitne škodljive učinke na ljudi in živali.

Pri tveganju se išče ravnotežje med sprejemljivim tveganjem in koristjo, ki ga prinaša določen nevaren dejavnik. GSO prinašajo določene koristi za človeško družbo, po drugi strani pa tako kot vse ostalo, predstavljajo določene potencialne nevarnosti za ljudi in okolje. Zato poskuša družba najti ustrezno ravnovesje pri uporabi GSO, med tveganjem in koristmi ter uporabo GSO regulira z zakoni (ZRGSO, 2005 in 2010).

2.3 Nesreča in monitoring pri delu z GSO – rastlinami

Zakon opredeljuje tudi nesrečo oz. nenamerno sproščanje GSO v okolje. To je vsak izreden dogodek ali vrsta dogodkov, kadar pri delu z GSO v zaprtem sistemu in tudi pri namernem sproščanju pride do nepredvidenega sproščanja GSO v okolje, ki lahko pomeni takojšnjo ali kasnejšo nevarnost

za okolje ali zdravje ljudi in živali. Vsako delo z GSO, predvsem namerno sproščanje, zahteva monitoring oz. spremljanje in nadzorovanje GSO in prejemnega okolja, procesov in postopkov ter možnih škodljivih vplivov skladno s predpisi. Posebno težo ima monitoring pri namernem sproščanju GSR v okolje, ki naj bi se izvajal vsaj dve do tri leta po končanem sproščanju v okolje (Gebhard in Smalla, 1999).

3 POSKUSNO NAMERNO SPROŠČANJE GS RIŽA V OKOLJE

Italijanska industrijska sorta riža CR W3 je bila požlahtnjena z biotehnološkimi postopki v zaprtem sistemu, v namene pridobivanja farmakološke učinkovine, encima β-glukozidaze za zdravljenje dedne presnovne bolezni. V laboratoriju in rastlinjaku so bile opravljene potrebne študije. Za nadaljnja proučevanja, ki se zaključijo z registracijo zdravila, bi bilo potrebno vsaj dveletno poskusno gojenje na polju (Patti in sod., 2012).

3.1 Genska sprememba riža sorte CR W3 in uporabnost rekombinantnega encima

CR W3 je italijanska potrjena, a ne gojena industrijska sorta riža za pridobivanje škroba z okroglimi semeni, razmerje dolžina/širina je pod 1,75. Je zelo rana in na bolezni odporna sorta, predvsem na rjo. Zaradi majhne vsebnosti amiloze, slabe konsistentnosti in velikega indeksa lepljivosti, ki jo določa voščen - waxy tip endosperma, je popolnoma neprimerna za prehrano. Semena se pri 10 do 15 minutnem kuhanju popolnoma razkuhajo, postanejo lepljiva in neuporabna. Zaradi navedenih lastnosti je bila predmet genske spremembe. Na Univerzi v Vidmu (Udine) so, s posredno metodo z bakterijo *Agrobacterium tumefaciens*, uspeli v omenjeno sorto vnesti gen, katerega produkt, encim β-glukozidaza se izraža v osrednjem endospermu. Transgeni riž sorte CR W3 se od netransgene sorte loči samo po sintezi encima β-glukozidaze. V vseh ostalih lastnostih je popolnoma enak sorti CR W3. Transgeni riž je izključno namenjen farmakološki pridelavi učinkovine za medicinske potrebe oz. zdravljenje in ne za prehrano, saj že osnovne lastnosti sorte to ne dopuščajo (Patti in sod., 2012).

Rekombinantni encim β-glukozidaza se uporablja za nadomestno zdravljenje genske avtosomno-recesivne Gaucherjeve bolezni. Ta način dedovanja se pojavlja pretežno pri presnovnih boleznih, med katerimi je tudi Gaucherjeva bolezen. To je redka genska okvara, ki prizadene približno 40.000 prebivalcev na svetu, 3.000 v Evropi in v Sloveniji je trenutno registriranih 19 bolnikov tipa 1 od treh poznanih. Pri tipu 1 je zmanjšana encimska aktivnost, znaki so anemija, trombocitopenija, levkopenija, povečana jetra in vranica, zmanjšanje kostne mase se lahko pokažejo šele v odrasli dobi. Pri tipu 2 se poleg povečanja jeter in vranice pojavijo še okvare centralnega živčnega sistema, zaradi česar bolniki redko živijo več kot dve leti. Pri tipu 3 so znaki podobni kot pri tipu 1, prisotne so tudi nevrološke motnje, ki se z leti večajo. Močno prevladuje tip 1, nevropatološki simptomi se pojavljajo le pri 5-10 % bolnikov. Pri vseh treh oblikah je prisotno pomanjkanja encima glukocerebrozidaze (β-glukozidaze), kar se odraža v nepravilni razgradnji maščobe glukocerebrozida, ki je produkt odmrlih rdečih in belih krvnih celic. Zaradi odsotnosti oz. nefunkcionalnosti encima glukocerebrozidaze se glukocerebrozid ne razgrajuje, ampak se kopiči v celicah - v tkivnih makrofagih. Ko je nerazgrajene maščobe v celicah zelo veliko, se te povečajo, postanejo okrogle in niso več sposobne opravljati svojih nalog - imenujejo se Gaucherjeve celice. Največ jih je v kostnem mozgu, vranici in jetrih, kjer onemogočajo normalno delovanje teh organov. To ima za posledico povečanje in odpoved jeter, vranice, slinavke in težave z okostjem. Zelo pomembno je, da se zdravljenje začne čim prej v otroštvu (Grabowski in sod., 1998). Bolezen se ne da ozdraviti, možni so trije načini zdravljenja, presaditev kostnega mozga, genska terapija in encimska terapija, ki se izvaja tudi v Sloveniji (Grabowski in sod., 1995; Benedik-Dolničar in Kitanovski, 2003). Terapija z injekcijami, ki nadomeščajo pomanjkanje encima je za pacienta nujna in doživljenjska ter draga. Zaradi visokih stroškov je terapija nedosegljiva pacientom iz številnih afriških, azijskih, južnoameriških, srednjevzhodnih in evropskih držav. Zato se iščejo načini za nadomestno zdravljenje.

3.2 Pridobivanje encima β-glukozidaze in nadomestna encimska terapija

Prvi klinični poskusi nadomestne encimske terapije so se pojavili sredi 70. let, v splošni uporabi pa je od leta 1991. Encim β-glukozidaza se je do leta 1994 pridobival iz človeških placent, sedaj se v Evropi pridobiva z rekombinantno celično linijo ovarijev kitajskih hrčkov, v katere je bil vnešen človeški gen za glukocerebrozidazo. Povprečni strošek zdravljenja je 200.000 evrov letno z minimalno dozo, ki pa vsem pacientom ne zadostuje (Pentchev in sod., 1973; Reddy in sod., 1985). Večkrat se zgodi, da je zaradi okvar bioreaktorjev ali mutacij celičnih linij, na trgu pomanjkanje encima, kar ima za posledico zmanjšanje minimalno potrebnih doz. Zdravljenje oz. potreben nivo encima v organizmu se vzdržuje z intravenoznim dodatkom vsak drugi teden. Leta 2012 je ameriška administracija za hrano in zdravila (FDA) odobrila pridobivanje encima, prav tako v bioreaktorju, s celično linijo gensko spremenjenega korenja (Morrow, 2012). Da bi se zadostilo potrebnim letnim količinam encima za vse paciente, se s ponujeno gensko spremembo riža poskuša dvigniti proizvodnjo β-glukozidaze.

3.3 Opravljene raziskave z GS rižem v zaprtem sistemu in poskusi namernega sproščanja v okolje

V Italiji je bilo že opravljeno gojenje v rastlinjaku in pridobivanje oz. ekstrakcija encima β-glukozidaze na manjšem vzorcu semen ter čiščenje in priprava zdravila. Te preliminarne študije in raziskave so pokazale, da je sintetizirana in naložena β-glukozidaza zelo čista in zato enakovredna oz. primernejša za zdravljenje kot pridobljena z do sedaj uveljavljenim postopkom. Za klinično testiranje na večjem vzorcu in za pridobitev registracije zdravila ter potrditev stabilnosti izražanja lastnosti v okoljskih razmerah, bi bilo potrebno opraviti vsaj dveletno poskusno gojenje GS riža na prostem.

Zaradi negativnega javnega mnenja v Evropi, ki je proti sproščanju GSO v okolje, in dolgih postopkov pridobitve dovoljenja za gojenje na prostem, tega niso opravili v prvotno izbrani Italiji in ne v Španiji. Eden od razlogov, ki je preprečil namerno sproščanje GS riža je bil tudi ta, da se v obeh državah goji riž in da bi lahko prišlo do križanja. Drugi razlog pa je bil v dolgotrajnih postopkih za pridobitev dovoljenja. V Italiji postopek traja 2 do 3 leta, v Španiji je krajši in traja 6 do 8 mesecev, v Sloveniji je najkrajši in traja 1,5 do 2 meseca. Zaradi tega se je poskušalo pridobiti dovoljenje pri nas. Riž se v Sloveniji ne prideluje in nima generativno kompatibilnih divjih sorodnikov ter vrst, s katerimi bi se lahko križal. Najbližja pridelava riža je v sosednji Italiji, ki je od izbrane lokacije oz. polja oddaljena približno 70 km. Na območjih, kjer se riž prideluje, se pojavljajo divji tipi s katerimi se kultiviran riž lahko križa. Slovenija ni pridelovalno območje riža, zato pri nas niso prisotne kompatibilne endemične, spontane ali divje vrste, niti ne naravne ali gojene sorte, s katerimi bi se lahko GS riž križal.

Običajno so poskusna polja z GSR nižje rasti obdana z naravni fizičnimi pregradami, s pasom koruze ali industrijske konoplje, ki zraste tudi do 3 m in s tem zadrževalnim ukrepom se preprečuje genski prenos s cvetnim prahom. Riževa pelodna zrna se v običajnih okoljskih razmerah ne dvignejo višje od 1,5 m in prej omenjene fizične pregrade lahko delno varujejo pred širitvijo transgenega peloda (Jackson in Lyford, 1999; Song in sod., 2001; Song in sod., 2004).

3.4 Možnosti namernega sproščanja GSR v Sloveniji in obstoječe ovire

Vloga za namerno sproščanje GS riža je bila temeljito pripravljena po členih III. poglavja ZRGSO, Ur. l. RS 23/2005 in 21/2010. Manjkal ji je samo katastrski izpis za izbrano njivo. Pred uradno oddajo na MOP je bila vloga na MOP pregledana in pozitivno ocenjena. Izbrana 0,2 ha njiva je del 16 ha polja oz. kompleksa, ki je obdano z ograjo in urejeno je tudi namakanje, ki je potrebno v obdobju cvetenja riža in formiranja semen. Dostop na polje je možen samo zaposlenim in pooblaščenim osebam. To je fizični zadrževalni ukrep in razlog, razen urejenega namakanja, zakaj smo izbrali to njivo. Poskusno naj bi bilo posajenih 20 rastlin/m^2 oz. približno 40.000 rastlin/0,2 ha, kar bi zadoščalo za klinične študije in razvoj industrijskega načrta pridobivanja encima.

Preden smo zaprosili za katastrski izpis, smo o nameri seznanili občino v kateri se nahaja njiva. Večina slovenskih občin je podpisala peticijo Inštituta za trajnostni razvoj proti gojenju GSR na njihovem ozemlju. Izbrana lokacija je v eni od teh

občin in v lasti Sklada kmetijskih zemljišč in gozdov RS, katerega smo tudi obvestili, da nameravamo gojiti GS riž na njihovi njivi. Z obeh strani smo dobili odklonilni odgovor. S pogovori na MOP in prisotnimi iz MKGP ter veljavnem nacionalnem Zakonu o ravnanju z GSO nismo uspeli, saj je bil močnejši občinski sklep in pravilnik Sklada kmetijskih zemljišč in gozdov RS.

4 ZAKLJUČEK

Ponesrečena izbira njive za katero sta bila lokalni, občinski sklep o razglasitvi območja brez GSO in pravilnik Sklada kmetijskih zemljišč in gozdov RS, ki zavezuje zakupnika, da ne bo sejal GSR, močnejša od nacionalnega zakona in verjetno edina ovira, zakaj gojenje GS riža pri nas ni uspelo. Čeprav so bile s predhodnim gojenjem v rastlinjaku simulirane naravne razmere pridelovanja v manjšem obsegu, je potrebno vsaj dve letno poskusno gojenje na prostem za potrditev stabilnosti izražanja lastnosti v okoljskih razmerah in za pripravo programa širitve površin ter tehnološkega procesa pridobivanja zdravila. Približno 7 kg GS riža sintetizira in naloži v endospermu semen zadostno količino encima β-glukozidaze za celoletno intravenozno zdravljenje enega bolnika, kar bi bistveno pocenilo trenutne stroške zdravljenja in dostopnost zdravila v optimalni koncentraciji večjemu številu pacientov z Gaucherjevo boleznijo tipa 1.

5 LITERATURA

Benedik-Dolničar M., Kitanovski L. 2003. Obravnava bolnikov z Gaucherjevo boleznijo tip 1. Zdravstveni vestnik 72: 701-704

Directive 2001/18/EC of the European Parliament & of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms & repealing Council Directive 90/220/EEC

Gebhard F., Smalla K. 1999. Monitoring field releases of genetically modified sugar beets for persistence of transgenic plant DNA and horizontal gene transfer. FEMS Microbiology Ecology 28: 261-272, doi: 10.1111/j.1574-6941.1999.tb00581.x

Grabowski G.A., Barton N.W., Pastores G., Dambrosia J.M., Banerjee T.K., McKee M.A., Parker C., Schiffmann R., Hill S.C., Brady R.O. 1995. Enzyme therapy in type 1 Gaucher disease: comparative efficacy of mannose-terminated glucocerebrosidase from natural and recombinant sources. Ann Intern Med 122: 33-39, doi: 10.7326/0003-4819-122-1-199501010-00005

Grabowski G.A., Leslie N., Wenstrup R. 1998. Enzyme therapy for Gaucher disease: the first 5 years. Blood Rev 9, 12: 115-133, doi: 10.1016/S0268-960X(98)90023-6

Jackson S.T., Lyford M.E. 1999. Pollen dispersal models in quarternary plant ecology: assumptions, parameters, and prescriptions. Botany Review 65: 39-75, doi: 10.1007/BF02856557

Jardine C.G., Hrudey S.R., Shortreed J.H., Craig L., Krewski D., Furgal C., McColl R.S. 2003. Risk Management Frameworks for Human Health and Environmental Risks. J. Toxicol Environ Health Part B 6: 569-641, doi: 10.1080/10937400390208608

Kinderlerer J. 1997. Tools of Regulation. Risk Assessment. Guide to Risk Assessment and Biosafety in Biotechnology. An Initiative of the United Nations Environment Programme: 78 p.

Levin M. 1997. A Primer on Risk Assessment. Concepts and Theory of Risk Assessment. Guide to Risk Assessment and Biosafety in Biotechnology. An Initiative of the United Nations Environment Programme: 94 p.

McColl S., Hicks J., Craig L., Shortreed J. 2000. Environmental Health Risk Management; A Primer for Canadians. NERAM, Institute for Risk Research: 210 p.

Morrow T. 2012. Gaucher's disease treatment option rides on carrot cells' biologic power. Manag Care 21, 6: 45-46

Patti T., Bembi B., Cristin P., Mazzarol F., Secco E., Pappalardo C., Musetti R., Martinuzzi M., Versolatto S., Cariati R., Dardis A., Marchetti S. 2012. Endosperm-specific expression of human acid beta-glucosidase in a waxy rice. Rice 5, 34: 1-15, doi: 10.1186/1939-8433-5-34

Pentchev P.G., Brady R.O., Hibbert S.R., Gal A.E., Shapiro D. 1973. Isolation and characterization of

glucocerebrosidase from human placental tissue. J Biol Chem 248, 15: 5256-5261

Pravilnik o oceni tveganja za delo z gensko spremenjenimi organizmi v zaprtem sistemu. Uradni list RS, št. 45/2004

Pravilnik o oceni tveganja za namerno sproščanje gensko spremenjenih organizmov v okolje. Uradni list RS, št. 4/2006

Pravilnik o oceni tveganja za dajanje izdelka, ki vsebuje gensko spremenjene organizme, na trg. Uradni list RS, št. 13/2006

Reddy P.U., Murray G.J., Barranger J.A. 1985. Purification and characterization of bovine brain glucocerebrosidase. Biochem Med. 33, 2: 200-210, doi: 10.1016/0006-2944(85)90028-6

Seidman L.A., Moore C.J. 1999. Basic laboratory methods for biotechnology: Textbook and laboratory reference. New Jersey, Prentice – Hall, Inc., 751 p.

Song Z.P., Lu B.R., Chen J.K. 2001. A study of pollen viability and longevity in Oryza rufipogon, O. sativa and their hybrid. International Rice Research Notes 26: 31-32

Song Z, Lu B.R., Chen J. 2004. Pollen flow of cultivated rice measure under experimental conditions. Biodiversity and Conservation 13: 579-590, doi: 10.1023/B:BIOC.0000009491.24573.1d

Uredba o merilih za uvrstitev dela z gensko spremenjeni organizmi v zaprtem sistemu v varnostni razred in zadrževalnih ter drugih varnostnih ukrepih za posamezen varnostni razred (Uradni list RS, št. 71/2011).

Wheelis M., Kapuscinski A.R., Spielman A., Istock C., Regal P.J., Ingham E., Ellstrand N., Letourneau D., Bhargava P.M., Klinger T., Akabas S. 1998. Manual for assessing ecological and human healt effects of genetically engineered organisms. Edmunds Institute, Edmunds, Washington: 245 p.

Zakon o ravnanju z gensko spremenjenimi organizmi (Uradni list RS, št. 23/2005 – uradno prečiščeno besedilo, 21/2010)

CONTENT ANALYSIS OF THE PAPERS IN THE ACTA AGRICULTURAE SLOVENICA

VSEBINSKA OBDELAVA PRISPEVKOV V ACTA AGRICULTURAE SLOVENICA let. 105 št. 2

Tomaž BARTOL[a], Karmen STOPAR[b],

SUBJECT INDEX BY AGROVOC DESCRIPTORS
PREDMETNO KAZALO PO DESKRIPTORJIH AGROVOC

a, b: Ph. D., M. Sc.., B. Sc., Jamnikarjeva 101, SI-1000 Ljubljana, P. O. Box 95

VSEBINSKO KAZALO PO SKUPINAH ZNANJA (PREDMETNIH KATEGORIJAH)

NAVODILA AVTORJEM

(letniki z liho številko - rastlinska proizvodnja)

Prispevki

Sprejemamo izvirne znanstvene članke s področja agronomije, hortikulture, rastlinske biotehnologije, raziskave živil rastlinskega izvora, agrarne ekonomike in informatike ter s sorodnih področij - **letniki z liho številko** (npr. 97, 99) - v slovenskem in angleškem jeziku; pregledne znanstvene članke samo po poprejšnjem dogovoru. Objavljamo tudi izbrane razširjene znanstvene prispevke s posvetovanj, vendar morajo taki prispevki zajeti najmanj 30 % dodatnih originalnih vsebin, ki še niso bile objavljene. O tovrstni predhodni objavi mora avtor obvestiti uredniški odbor. Če je prispevek del diplomske naloge, magistrskega ali doktorskega dela, navedemo to in tudi mentorja na dnu prve strani. Navedbe morajo biti v slovenskem in angleškem jeziku.
Prispevke sprejemamo vse leto.
Podrobnejša navodila: http://aas.bf.uni-lj.si/navodila.htm

INSTRUCTIONS FOR AUTHORS

(Odd-numbered volumes - plant production)

Articles

The Journal *accepts original scientific* articles from the fields of agronomy, horticulture, plant biotechnology, plant-related food-and-nutrition research, agricultural economics, information-science, and related research - odd-numbered volumes (for example: 97, 99) - in Slovenian or English language. Review articles are published in advance agreement with the editorial board.
Extended versions of selected proceedings-papers can also be considered for acceptance, provided they include at least 30% of new original content, but the editorial board must be notified beforehand. If the article is based on a thesis or dissertation, the thesis-type must be indicated (BSc, MSc, PhD...), along with the role of the candidate and advisor, at the bottom of the first article page.
Manuscripts are accepted throughout the year.
Detailed instructions: http://aas.bf.uni-lj.si/instructions.htm