# ACTA AGRICULTURAE SLOVENICA

111•3
2018

Biotehniška fakulteta Univerze v Ljubljani
Biotechnical Faculty University of Ljubljana

Acta agriculturae Slovenica • ISSN 1581-9175 • 111 – 3 • Ljubljana, december 2018

*Acta agriculturae Slovenica*
Volume / Letnik **111** · Number / Številka **3** · **2018**

# VSEBINA / CONTENTS

## Pregledni znanstveni članki / Review articles



## Kratke vesti / Short Communications

doi:10.14720/aas.2018.111.3.01 Original research article / izvirni znanstveni članek

# The response of corn (*Zea mays* L.) cultivars to row spacing under weed interference condition

Gholamreza MOHAMMADI[1]*, Mehdi NOOKANI[2], Hamidreza MOHAMMADDOUST[2], Danial KAHRIZI[1]

Received May 10, 2018; accepted October 23, 2018.
Delo je prispelo 10. maja 2018, sprejeto 23. oktobra 2018.

**ABSTRACT**

This study was carried out in order to study the response of corn cultivars to row spacing and weed interference at the Research Farm of Agricultural and Natural Resources Faculty, Razi University, Kermanshah, Iran in 2011. The experiment was a split block factorial based on a randomized complete block design with three replications. Factors consisted of three corn cultivars ('KSC 704', 'Simon' and 'Maxima') and three plant row spacings (45, 60 and 75 cm) under weeded and un-weeded conditions for all of the growing season. Results indicated that for all three corn cultivars, the highest weed dry mass occurred in the row spacing of 75 cm. Weed interference throughout the growing season reduced corn grain yield by 20 %. This condition also significantly decreased corn yield components except the 100-seed mass. Increasing plant row spacing increased weed density, while decreased corn yield by 16.5 %. Corn cultivars were significantly different in terms of the number of seed per ear and 100-seed mass, as 'KSC 704' and 'Simon' showed the highest values for these yield components, respectively. However, the number of ear per plant and grain yield were not significantly different between the corn cultivars under study.

**Key words:** corn; competition; 'KSC 704'; 'Maxima'; 'Simon'; weed; yield; yield component

**IZVLEČEK**

**ODZIV SORT KORUZE (*Zea mays* L.) NA RAZMIK MED SETVENIMI VRSTAMI IN VPLIV PLEVELA**

V raziskavi je bil preučevan vpliv razmika med setvenimi vrstami in zapleveljenostjo pri treh sortah koruze na Research Farm of Agricultural and Natural Resources Faculty, Razi University, Kermanshah, Iran, v letu 2011. Poskus je bil izveden kot popolni naključni bločni poskus z deljenkami s tremi ponovitvami. Faktorje so predstavljali tri sorte koruze ('KSC 704', 'Simon' in 'Maxima') in trije razmiki med setvenimi vrstami (45, 60 in 75 cm) v razmerah brez plevela in s plevelom v celotni rastni sezoni. Rezultati so pokazali, da je bila največja masa plevelov pri vseh treh sortah pri razmiku setvenih vrst 75 cm. Zapleveljenost skozi celotno rastno sezono je zmanjšala pridelek zrnja koruze za 20 %. Te razmere so tudi značilno zmanjšale posamezne komponente pridelka razen mase 100-zrn. Povečan razmik med vrstami je povečal gostoto plevela, kar je zmanjšalo pridelek koruze za 16.5 %. Sorte koruze so se značilno razlikovale v številu zrn na storž in v masi 100-zrn, pri čemer sta imeli 'KSC 704' in 'Simon' največji vrednosti teh dveh komponent pridelka.Kljub temu se število storžev na rastlino in pridelek zrnja nista statistično značilno raziskovala med sortami, preučevanimi v tej raziskavi.

**Ključne besede:** koruza; kompeticija; 'KSC 704'; 'Maxima'; 'Simon'; plevel; pridelek; komponente pridelka

## 1 INTRODUCTION

Corn is one of the most important crops which is extensively planted in Iran and Kermanshah province is proposed as a major region to produce this crop. Weed interference is a main limiting factor which can significantly reduce corn yield and economic return. The reduction may range from 30 to 70 % when weeds are not controlled during the growing season (Ford and Mt Pleasant, 1994; Teasdale, 1995; Mohammadi 2010; Mohammadi et al., 2012b).In many regions of Iran such as Kermanshah, farmers highly use chemical and mechanical methods to control weeds in their corn production systems. These methods usually have

[1] Dept. of Plant Production and Genetics, College of Agriculture and Natural Resources, Razi University, Kermanshah, Iran; *corresponding author: mohammadi114@yahoo.com

[2] Dept. of Crop Production and Breeding, Faculty of Agricultural Sciences, University of MohagheghArdabili, Ardabil, Iran

negative environmental consequences (such as soil erosion, water and soil pollution) and notably increase the cost of corn production.

Alteration of planting arrangement has been documented as an efficient approach to suppress weeds in agroecosystems (Mohammadiet al., 2015) which can be achieved by a reduced row spacing.Vera et al. (2006) suggested that narrower row spacings can cause an earlier canopy closure and allow the crop to shade weeds in their early developmental stages. Other researchers working with barley showed that increasing seeding rate and the use of high competitive cultivars improved crop competitiveness against weeds (Watson et al., 2006; Harker et al., 2009). This can be due toan increased resource use by crop which can lead to the reduced effects of weeds (Berkowitz, 1988; Mohler, 1996). Chauhain and Johnson (2011) reported that rice grown in narrower rows had higher grain yield and lower weed biomass than in wider rows and increasing row spacing caused more crop vulnerability to weed competition for the longest period. Drews et al. (2004) also found that the competitive ability of short-stature cultivars could be improved by reducing row spacing.

Weed competition to acquire limited resources is the primary cause for crop yield loss from weeds. Crop cultivars with high weed competitive abilities may be used in an integrated weed management (IWM) program (Lemerle et al., 1996; Lindquist and Kropff, 1996). Some studies have shown that corn cultivars differ in terms of their ability to suppress weeds (Mohammadi, 2007; So et al., 2009). Identification of these cultivars can significantly improve crop yield in the presence of weeds and reduce the cost and environmental consequences caused by weed control practices. According to McDonald (2003) the development of crop cultivars with high competitive abilities against weeds is an important aspect of IWM and can decrease the reliance of cropping systems to chemical herbicides.

The main objective of this study was to evaluate the response of some corn cultivars to different row spacings under weed interference condition at Kermanshah, west Iran.

## 2 MATERIALS AND METHODS

The study was carried out at the Research Farm of Agricultural and Natural Resources Faculty, Razi University, Kermanshah (latitude 34° 18′ N, longitude 47° 4′ E, altitude 1350 asl), Iranin 2011.Soil was a clay-loam with a pH of 8 and 1.4 % organic matter. The experiment was a factorial split block based on a randomized complete block design with three replications. The first factor was corn cultivars including 'KSC 704' (late matured), 'Simon' and 'Maxima' (both medium-matured cultivars) which are cultivars well-adapted to the environmental conditions of the region. The second factor was three plant row spacings including conventional (75 cm) and reduced (45 and 60 cm). To evaluate weed interference effect on corn plant traits each block was divided into two sections lengthways, which one of them was kept free of weeds and another was un-weeded for all of the growing season. There was a dense natural weed infestation in the experimental field.The list of dominant weed species is shown in Table 1.

The land was plowed then disked before planting. Fertilizers were applied according to the soil test recommendations. Corn was planted on May 2011. Each plot consisted of 6 rows (6 m per row) with a planting density of 6.5 plant $m^{-2}$. Weeds werehand weeded in weed-free section of each plot throughout the growing season. At maturity, the corn ears belong to the two center rows of each plot were harvested by hand, allowed to dry toa constant mass then threshed and grain yield was determined. Corn yield components including the number of ears per plant and the number of seeds per ear were determined on ten randomly selected plants of each plot. Additionally, 100-grainmass was calculated according to the recommendations of the International Seed Testing Association (ISTA) (Draper, 1985).

At the end of the growing season, weed density and dry masswere determined by harvesting weeds at ground level in two random 0.5 × 0.5 m squaresin un-weeded section of each plot. Weeds were initially counted then dried at 80 ºC to a constant mass then weighed. Data analyses including analysis of variance and mean comparison were carried out using SAS software (SAS Institute, 2003). Means were compared using Duncan test at the 0.05 level of probability.

**Table 1:** The list of dominant weed species emerged in the experimental field

| Common name | Scientific name |
|---|---|
| Johnsongrass | *Sorghum halepense* (L.) Pers. |
| Redroot pigweed | *Amaranthus retroflexus* L. |
| Lamb'squarters | *Chenopodium album* L. |
| Common cocklebur | *Xanthium strumarium* L. |
| Field bindweed | *Convolvulus arvensis* L. |
| Common purslane | *Portulaca oleracea* L. |

## 3 RESULTS AND DISCUSSION

### 3.1 Weed density

Analysis of variance (data not shown) revealed that weed density (WD) was significantly influenced by row spacing. Weed density reduced in response to decreasing row spacing as the highest WD occurred in the conventional row spacing (75 cm). Although, there was no significant difference between the two reduced row spacings (45 and 60 cm) in terms of WD (Fig. 1).Reduction of WD in narrower rows can be attributed to an earlier canopy closure and consequently a less light level intercepted by weed seeds. Some studies showed that an earlier canopy closure can notably reduce weed germination and emergence by decreasing light quantity (intensity) and quality (spectrum)reaching the soil surface under the canopy (Bradley, 2006; Ghadiri and Bayat, 2004; Rajcan and Swanton, 2001).

Lindquist and Mortensen (1999) also reported that a reduced row spacing can improve weed control in corn due to an earlier crop canopy closure. According to Porter et al. (1997) compared to wider rows (76.2 cm) a reduced row spacing (50.8 cm) decreased the light penetrated into the crop canopy by 10 % causing a 35 % decrease in weed infestation.

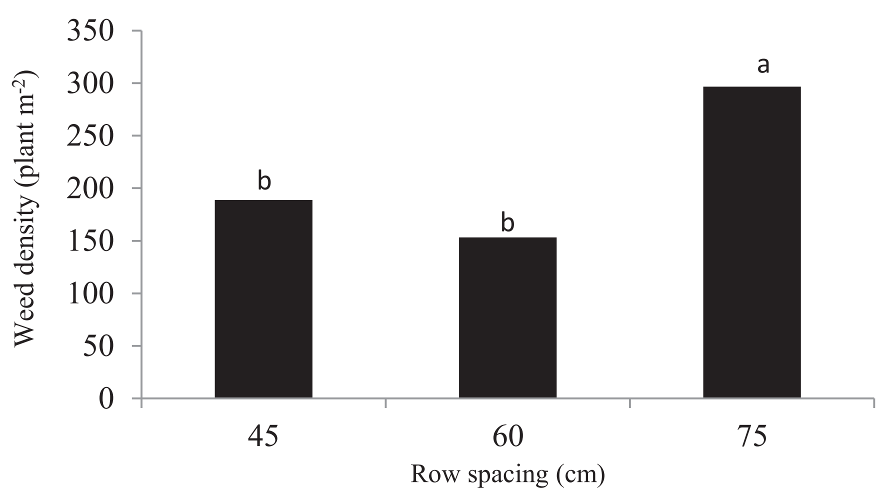



**Figure 1:** The effect of corn planting row spacing on weed density. The same letters show non significant difference at the 0.05 level of probability

### 3.2 Weed dry mass

There was a significant two-way interaction (cultivar×row spacing) for weed dry mass (WDM). A notable higher WDM was observed in the conventional row spacing (75 cm) compared to the narrower rows (45 and 60 cm) (Fig. 2). Although, there was no significant differences between these two reduced row spacings with regard to WDM. It seems that reduced row spacing can cause a more equidistant plant distribution in the field and consequently higher efficient use of the environmental resources by crop plants which lead to a higher weed suppressing ability. In contrast, wider rows can intensify intra-specific competition between crop plants and provide a suitable space to weed growth between rows (Akbari et al., 2011). A reduced weed dry massproduced due to narrower rows was reported by Johnson and Haverstad (2002). Mohammadi et al. (2012a) also observed a 49.4 % decrease in weed biomass in response to reduced row spacing and increased planting density.

Corn cultivars also showed different negative effects on WDMin relation to row spacing (Fig. 2). At the lowest row spacing (45 cm) 'Maxima' had higher weed suppressing effect than other two cultivars. However, there were no significant differences between the cultivars at the wider rows in terms of WDM (Fig. 2). According to Mohammadi (2007) corn cultivars differed in their competitive ability against weeds and the cultivars with higher relative growth rate and specific leaf area performed better than others. Similar results have been reported by So et al. (2009) working with sweet corn.

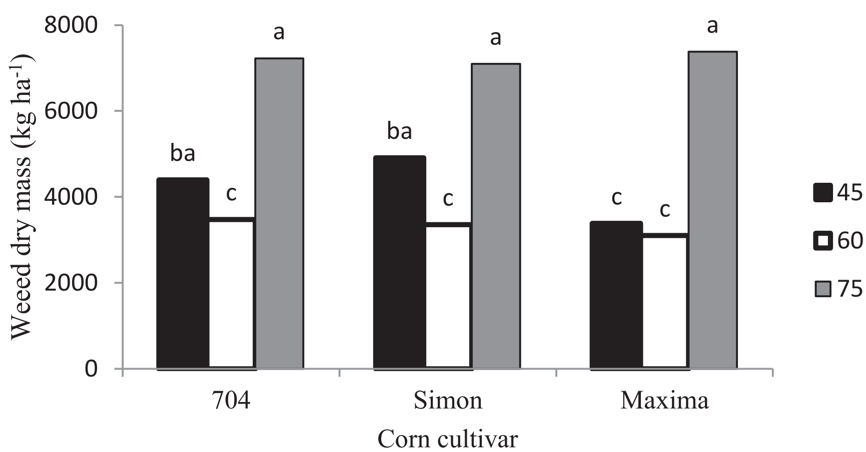



**Figure 2:** The effect of corn cultivar on weed dry mass under different row spacing. The same letters show non significant difference at the 0.05 level of probability

### 3.3 Corn yield components

The number of ear per plant (NEP) was significantly influenced by weed interference. However, cultivar and row spacing didn't show significant effects on this trait. Full season weed interference reduced NEP by 4.9 % (Fig. 3). This occurred due to the failure of some corn plants to form ear in the presence of competing weeds. However, NEP didn't show significant response to row spacing or cultivars. Other workers also suggested that row spacing didn't have a notable effect on the number of ear per plant (Turgut et al., 2005; Mohammadi etal., 2012a).

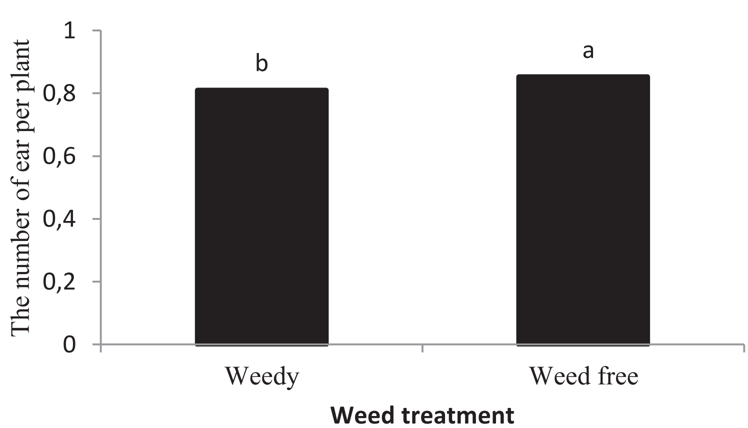



**Figure 3:** The effect of weed interference on the number of ears per plant of corn. The same letters show non significant difference at the 0.05 level of probability

Weed interference and corn cultivar showed significant effects on the number of seeds per ear (NSE). 'KSC 704' had a significant higher NSE (600 seeds per ear) followed by 'Maxima' and "Simon'which showed lower and non significantdifferent NSE (Fig. 4). Weed interference for the entire growing season reduced NSE by 15.8 % as compared with weed free condition (Fig. 5). Similar result was reported by Mohammadi et al. (2012b) who reported a 23 % reduction in NSE resulted from a full season weedy condition. This may be due to a less number of fertilized florets per ear caused by weed competition. Evans et al. (2003) found a sigmoidal reduction in the number of seeds per ear in response to increasing weed interference period in corn.

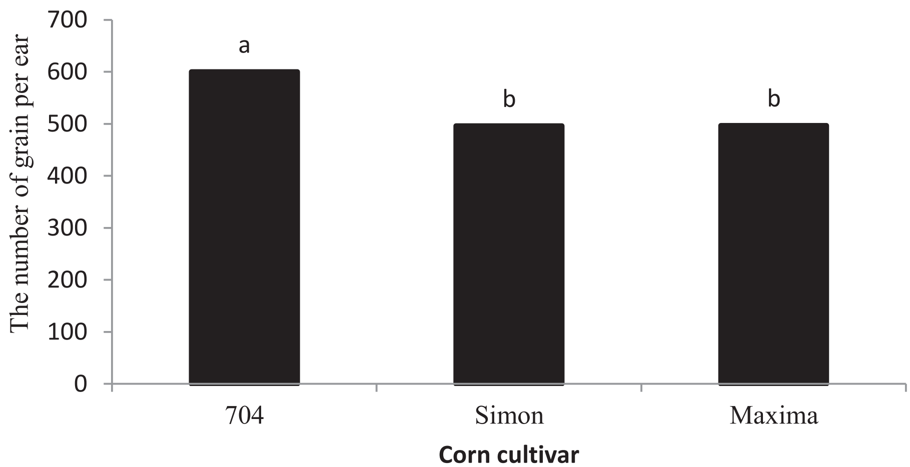



**Figure 4**: The effect on the number of grain per ear of corn. The same letters show non significant difference at the 0.05 level of probability

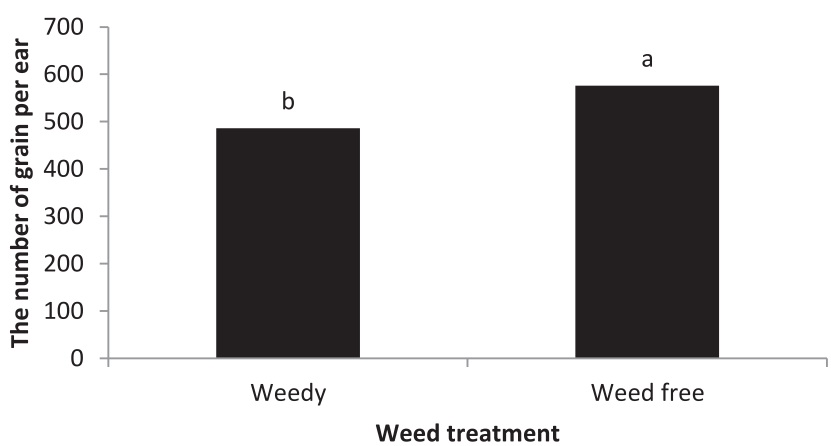



**Figure 5:** The effect of weed interference on the number of grain per ear of corn. The same letters show non significant difference at the 0.05 level of probability

100-grain yield (100-GM) was significantly affected by corn cultivar and row spacing. The highest 100-GM belonged to 'Simon' followed by 'Maxima' and 'KSC 704' (Fig. 6). However, there was no significant difference between 'Maxima' and other two cultivars in terms of 100-grain mass (Fig. 6). 100-grain mass showed a positive response to decreasing row spacing as this yield component was notably higher in the narrower rows (45 and 60 cm) compared to conventional row spacing (75 cm) (Fig. 7). This can be resulted from a more equidistantdistribution of corn plants and a lower intra-specific competition between them which consequently led to a more efficient use of the environmental resources.

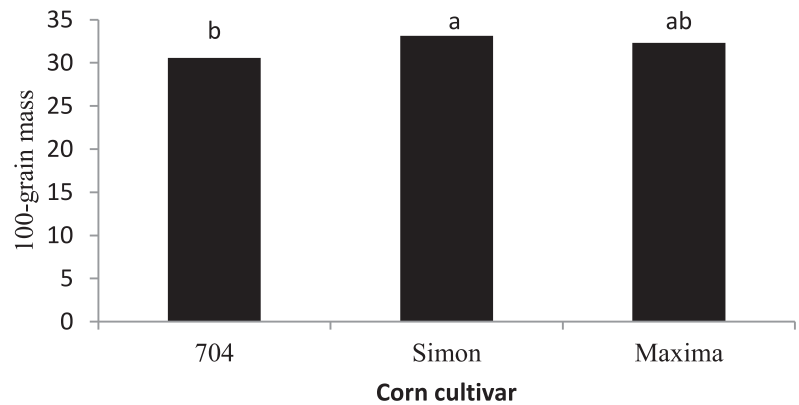



**Figure 6:** The effect cultivar on corn grain mass. The same letters show non significant difference at the 0.05 level of probability

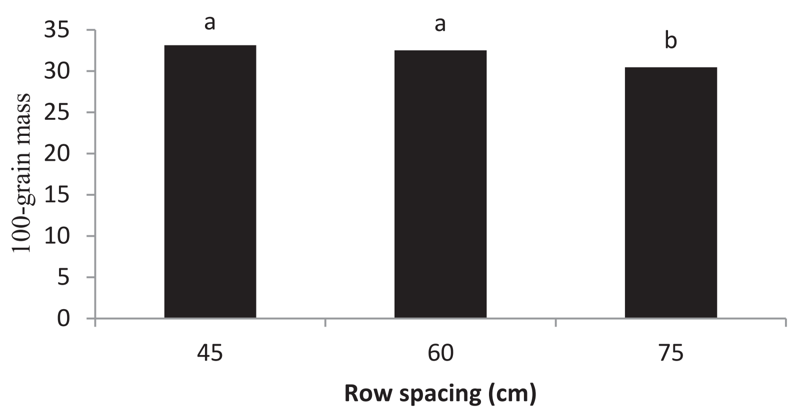



**Figure 7:** The effect of row spacing on 100-grain mass of corn. The same letters show non significant difference at the 0.05 level of probability

Weed interference had no significant effect on 100-GM. This is in contrast with Mohammadi (2010) who reported that 100-GM was negatively affected by competing weeds. No significant influence of weeds on 100-GM can be due to a lower grains produced per ear under weed stress condition (Fig. 5). In other words, it can be expected in this condition the number of grains formed in each ear to be balancedwith the available resources which led to a less vulnerability of 100-GM to weed interference

### 3.4 Corn grain yield

Corn grain yield showed significant responses to row spacing and weed interference. A 1000 kg ha$^{-1}$ increase in grain yield was observed when row spacing decreased from 75 to 45 cm (Fig. 8). This is compatible with Mohammadi et al. (2012a) who reported a 19.7 % increase in corn yield in response to decreasing row spacing from 75 to 50 cm. This can be explained by a more equidistant plant arrangement in narrower rows which consequently lead to a lower intra-specific competition and improved use of environmental resources by corn plants (Andrade et al., 2002; Barbieri et al., 2008). Bullock et al. (1988) also reported that increasing corn grain yield in reduced row spacings was due to more suitable plant distribution perunit area and consequently a lower competition between plants to acquire light, soil nutrient and moisture.

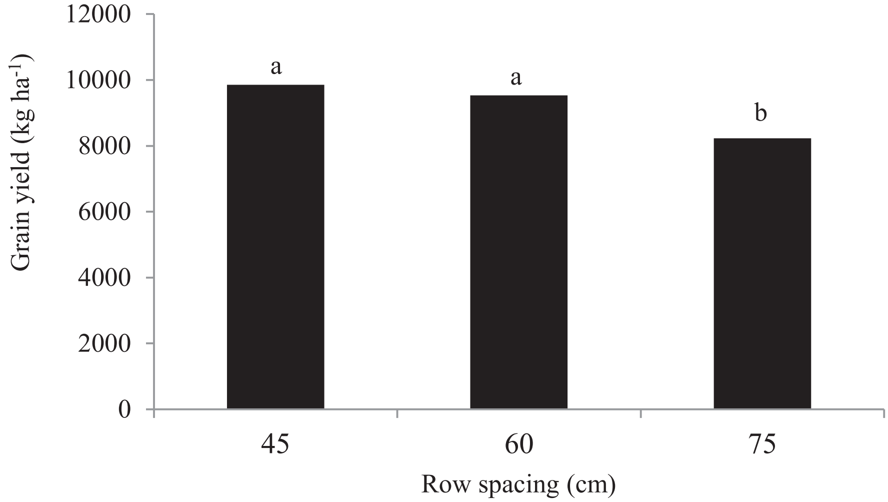



**Figure 8:** The effect of row spacing on corn grain yield. The same letters show non significant difference at the 0.05 level of probability

Weed interference decreased grain yield by 20 % (Fig. 9). Reduced crop yield in response to weed interference has been reported by other workers. Habibisavadkoohi et al. (2008) found a 60 % decrease in corn yield when weeds interfered with corn for a long period. In other studies the reductions of 30.7 and 41.1 % were reported for corn grain yield in full season weedy condition (Mohammadi, 2010; Mohammadi et al., 2012b). In general, weeds reduce crop yield via competition to obtain essential growth resources and releasing allelochemicals into the environment.

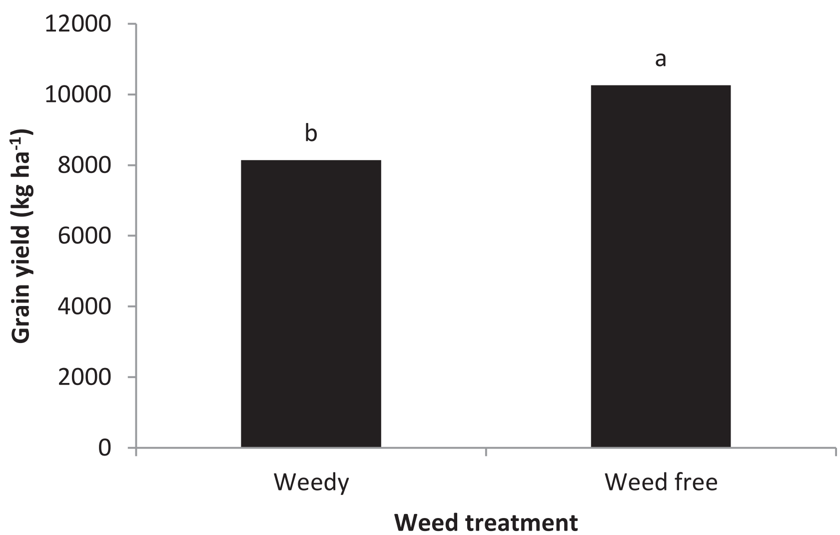



**Figure 9:** The effect of weed interference on corn grain yield. The same letters show non significant difference at the 0.05 level of probability

## 4 CONCLUSION

This study revealed that in comparison with the conventional plant row spacing (75 cm) narrower rows improved corn yield. These improvements were 16.5 and 13.6 % for the row spacings of 45 and 60 cm, respectively. This can be related to a more equidistant plant distribution pattern and reduced intra-specific competition between corn plants which consequently lead to a more efficient use of the environmental resources. The narrower rows also reduced weed biomass and density. This can be attributed to the improved growth of corn plants and their competitive ability against weeds probably due to an earlier canopy closure under this condition.

## 5 REFERENCES

Akbari, A., Zand E. and Mousavi, S. K. (2011). Evaluation the effect of row space and weed management approaches on biomass, chickpea (*Cicer arietinum* L.) yield, and yield components in Khorramabad dryland conditions. *Electronic Journal of Crop Production,3*, 1-21.

Andrade, F. H., Calvino, P., Cirilo, A. and Barbieri, P. (2002). Yield responsesto narrow rows depend on increased radiation interception. *Agronomy Journal*, *94*, 975-980. doi:10.2134/agronj2002.0975

Barbieri, P. A., Echeverría, H. E., SaínzRozas, H. R. and Andrade, F. H. (2008). Nitrogen use efficiency in maize as affected by nitrogen availability and row spacing. *Agronomy Journal,100*, 1094-1100. doi:10.2134/agronj2006.0057

Berkowitz, A. R. (1988). Competition for resources in weed-crop mixtures.Pages 89–119 *in* M. A. Altieri and M. Liebman, eds. *Weed management in agroecosystems: Ecological approaches*. CRC Press, Inc., Boca Raton, FL.

Bradley, K. W. (2006). A review of the effects of row spacing on weed management in corn and soybean.[Online], Available at www.plant management network.org/cm/. *Crop Management,* doi:10.1094/CM-2006-0227-02-RV

Bullock, D. G., Nielson, R. L. and Nyquist, W. E. (1988). A growth analysis comparison of corn grown in conventional and equidistant plant spacings. *Crop Science*, *28*, 254-258. doi:10.2135/cropsci1988.0011183X002800020015x

Chauhan B. S. and Johnson D. E. (2011). Row spacing and weed control timing affect yield of aerobic rice. *Field Crops Research*, *121,* 226-231. doi:10.1016/j.fcr.2010.12.008

Draper, S. R. (1985). International rules for seed testing. *Seed Science and Technology*, *13*, 342–343.

Drews S., Juroszek P., Neuhoff D., Kopke U. (2004). Optimizing shading ability of winter wheat stands as a method of weed control. *Journal of Plant Diseases and Protection*, *19*, 545–552.

Evans, S. P., Kenezevic, Z. S., Lindquist, J. L., Shapiro, C. A. and Blankenship, E. E. (2003). Nitrogen application influences the critical period for weed control in corn. *Weed Science*, *15*, 408-417. doi:10.1614/0043-1745(2003)051[0408:NAITCP]2.0.CO;2

Ford, G. T. and Mt Pleasant, J. (1994). Competitive abilities of six corn (*Zea mays* L.) hybrids with four weed control practices. *Weed Technology*, *8*, 124–128. doi:10.1017/S0890037X00039312

Ghadiri, H. and Bayat, M. L. (2004). Effect of row and plant spacing on weed competition with Pinto Beans (*Phaseolus vulgaris* L.). *Journal of Agricultural Science and Technology*, *6*, 1-9.

Habibisavadkoohi, M., Pirdashti, H., Amini, I., Abbasian, A. and Keramati, S. (2008). Effect of weeding time on the species composition, plant density, dry weight and physiological traits of weeds in corn (*Zea mays* L.). *Iranian Society of Weed Science*, *2*, 9-21.

Harker, K. N., O'Donovan J. T., Irvine R. B., Turkington T. K., and Clayton G. W. (2009). Integrating cropping systems with cultural techniques augments wild oat (*Avena fatua*) management in barley. *Weed Science*, *57*, 326–337. doi:10.1614/WS-08-165.1

Johnson, G. A. and Haverstad, T. R. (2002). Effect of row spacing and herbicide application timing on weed control and grain yield in corn (*Zea mays* L.). *Weed Technology*, *16*, 548-553. doi:10.1614/0890-037X(2002)016[0548:EORSAH]2.0.CO;2

Lemerle, D., Verbeek, B., Cousens, R. D., Coombes, N. E. (1996). The potential for selecting wheat varieties strongly competitive against weeds. *Weed Research*, *36*, 505–513. doi:10.1111/j.1365-3180.1996.tb01679.x

Lindquist, J. L. and Kropff M. J. (1996). Applications of an ecophysiological model for irrigated rice (*Oryza sativa*)—*Echinochloa*competition. *Weed Science*, *44*, 52–56.

Lindquist, J. L. and Mortensen, D. A. (1999). Tolerance and velvet leaf (*Abutilon theophrasti*) suppressive ability of two old and two modern corn (*Zea mays*) hybrids. *Weed Science*, *46*, 569-574.

McDonald, G. K. (2003). Competitiveness against grass weeds in field pea genotypes. *Weed Research*, *43*, 48–58. doi:10.1046/j.1365-3180.2003.00316.x

Mohammadi, G. R. (2007). Growth parameters enhancing the competitive ability of corn (*Zea mays* L.) against weeds. *Weed Biology and Management*, *7*, 232-236. doi:10.1111/j.1445-6664.2007.00261.x

Mohammadi, G. R. (2010). Weed control in irrigated corn by hairy vetch interseeded at different rates and times. *Weed Biology and Management*, *10*, 25–32. doi:10.1111/j.1445-6664.2010.00363.x

Mohammadi, G. R., Chatrnour, S., Jalali-honarmand, S. and Kahriz, D. (2015). The effects of planting arrangement and phosphate biofertilizer on soybean under different weed interference periods. *Acta Agriculturae Slovenica*, *105*, 313 – 322. doi:10.14720/aas.2015.105.2.14

Mohammadi, G. R., Ghobadi, M. E. and Sheikheh poor, S. (2012a). Phosphate biofertilizer, row spacing and plant density effects on corn (*Zea mays* L.) yield and weed growth. *American Journal of Plant Science*, *3*, 425-429. doi:10.4236/ajps.2012.34051

Mohammadi, G. R., Mozafari, S., Ghobadi, M. E. and Najaphy, A. (2012b). Interaction effects of weed interference and berseem clover (*Trifolium alexandrinum*) as a living mulch on corn (*Zea mays*). *Advances in Environmental Biology*, *6*, 763-767.

Mohler, C. L. (1996). Ecological bases for the cultural control of annual weeds. *Journal of Production Agriculture*, *9*, 468–474. doi:10.2134/jpa1996.0468

Porter, P. M. and Hicks, D. R., Lueschen, W. E., Ford, J. H., Warnes, D. D. and Hoverstad, T. R. (1997). Corn response to row width and plant population in the Northern Corn Belt. *Journal of Production Agriculture, 10*, 293-300. doi:10.2134/jpa1997.0293

Rajcan, I. and Swanton C. J. (2001). Understanding maize-weed competition:resource competition, light quality and the whole plant. *Field Crops Research*, *71*, 139–150. doi:10.1016/S0378-4290(01)00159-9

SAS Institute (2003). *SAS/STAT*. User's Guide.Version 9.1. SAS Inst., Inc., Cary, NC.

So, Y. F., Williams M. M., Pataky J. K., and Davis A. S. (2009). Principal canopy factors of sweet corn and relationships to competitive ability with wildproso millet (*Panicum miliaceum*). *Weed Science*, *57*, 296–303. doi:10.1614/WS-08-158.1

Teasdale, J. R. (1995). Influence of narrow row/high population corn (*Zea mays*) on weed control and light transmittance. *Weed Technology*, *9*, 113-118. doi:10.1017/S0890037X00023046

Turgut, I., Duman, A., Bilgili, U. and Acikgoz, E. (2005). Alternate row spacing and plant density effects on forage and dry matter yield of corn hybrids (*Zea mays* L.). *Journal of Agronomy and Crop Science*, *191*, 146-151. doi:10.1111/j.1439-037X.2004.00146.x

Vera C. L., Woods S. M. and Raney J. P. (2006). Seeding rate and row spacing effect onweed competition, yield and quality of hemp in the Parkland region of Saskatchewan. *Canadian Journal of Plant Science*, *86*, 911-915. doi:10.4141/P05-177

Watson, P. R., D. A. Derksen, and Van Acker R. C. (2006). The ability of 29 barley cultivars to compete and withstand competition. *Weed Science*, *54*, 783–792. doi:10.1614/WS-05-020R3.1

doi:10.14720/aas.2018.111.3.02 Original research article / izvirni znanstveni članek

# Behavioral and biological responses of black bean aphid (*Aphis fabae*, Scopoli, 1763) on seven Algerian local broad bean cultivars

Fouad MERADSI[1*] and Malik LAAMARI[1]

Received June 30, 2017; accepted November 04, 2018.
Delo je prispelo 30.junija 2017, sprejeto 04. novembra 2018.

**ABSTRACT**

We studied the behavioral and biological parameters of *Aphis fabae* (Homoptera: Aphididae, Scopoli, 1763) on seven local *Vicia faba* L. cultivars. The antixenosis was conducted under laboratory controlled conditions of the temperature, light regime and relative humidity (18 ± 0.25 °C; L16: D8; 37.5 ± 0.6 %) for test in light, and (19 °C; 42 % relative humidity) for test in dark. The least preferred host plants for attractivity was the cultivar 141 in both tests while the cultivar 145 was the most preferred in light test, and the cultivar 107 in dark test. The antibiotic experiment was conducted also under laboratory conditions (L16: D8 photoperiod, 17 ± 1 °C, and 43.5 ± 5 % r. h.). Antibiosis was determined by studying the pre-reproductive period, reproductive period, adult longevity, survival, daily and total fecundity. The analysis of variance indicated that no significant differences on pre-reproductive period and daily fecundity of the *A. fabae* among the cultivars. However, the longest times of reproductive period, adult longevity, and survival were recorded on cultivar 135 followed by cultivar 141. The highest (85.8) and the lowest (15.8) number of progeny were observed on 135 and 141 cultivars, respectively.

**Key words:** *Aphis fabae;* antibiosis; antixenosis; cultivar; resistance*;* *Vicia faba* L.

**IZVLEČEK**

**OBNAŠALNI IN BIOLOŠKI ODZIVI ČRNE FIŽOLOVE UŠI (*Aphis fabae* Scopoli, 1763) NA SEDEM ALŽIRSKIH SORT BOBA**

Preučevani so bili obnašalni in biološki parametri črne fižolove uši (*Aphis fabae* Scopoli, 1763, Homoptera: Aphididae) na sedem lokalnih sort boba (*Vicia faba* L.). Poskusi antiksenoze so bili izvedeni v nadzorovanih laboratorijskih temperaturnih, vlažnostnih in svetlobnih razmerah, 18 ± 0.25 °C; dan 16 ur, noč 8 ur in 37,5 ± 0,6 % relativni zračni vlagi za poskuse na svetlem in pri 19 °C in 42 % relativni zračni vlagi za poskuse v temi. Najmanj priljubljena gostiteljska rastlina glede privlačnosti je bila v obeh poskusih sorta 141, medtem, ko je bila pri poskusih na svetlem preferirana sorta 145 in pri poskusih v temi sorta 107. Tudi poskusi z antibiozo so bili izvedeni v laboratorijskih razmerah (dan 16 ur, noč 8 ur, pri temperaturi 17 ± 1 °C in pri 43,5 ± 5 % relativni zračni vlagi. Antibiotični učinki so bili določeni s preučevanjem predreproduktivnega obdobja, reproduktivnega obdobja, dolžine življenske dobe imagov, njihovim preživetjem in dnevno ter celokupno plodnostjo. Analiza variance je pokazala, da glede na sorto boba ni bilo značilnih razlik v dolžini predreprokcijskega obdobja in dnevne plodnosti uši. Najdaljše reproduktivno obdobje, največja življenska doba imagov in največje preživetje uši je bilo zabeleženo na sorti 135 in nato na sorti 141. Največje (85,8) in najmanjše (15,8) število potomcev je bilo opaženo na sortah 135 in 141.

**Ključne besede:** *Aphis fabae;* antibioza; antiksenoza; sorta; odpornost*;* *Vicia faba* L.

## 1 INTRODUCTION

Broad bean, *Vicia faba* L., is one of the oldest cultivated field crop. It constitutes a major protein source for human population in many countries (Laudadio et al., 2011). The black bean aphid, *Aphis fabae* Scopoli, 1763 (Homoptera: Aphididae) is the most destructive insect pest of broad bean in Algeria. In Algeria, broad bean production includes highly diversified local cultivars. About 68 local cultivars, have been identified by

[1] Laboratory of Improvement of the Phytosanitary Protection Techniques in Mountainous Agrosystems, Aureses Region. Agronomy Department, Institute of veterinary and Agricultural sciences, Batna 1 University (05000), Batna, Algeria; *corresponding author: fouadmeradsi@gmail.com or fouad.entomo@yahoo.fr

morphological and agronomic characterization (Meradsi, 2009).

The black bean aphid is one of the 14 aphid species of most agricultural importance in the worldwide (Blackman & Eastop, 2007). *A. fabae* is a very polyphagous species, but the actual host range of the aphid that colonizes beans and sugar beet is unclear, because it is a number of a bewildering complex of species, at least some of which also have wide host ranges (Blackman & Eastop, 2007). *A. fabae* has a wide distributional range. It occurs in Europe, Western Asia, Arab countries particularly Jordan (Mustafa & Qasem, 1984), Africa, and South America. It is a vector of more than 30 plant viruses, including non-persistent viruses of bean and peas, beets, crucifers, cucurbits, *Dahlia*, potato, tomato, and tulip, and the persistent beet yellow net virus and potato leaf roll virus (Blackman & Eastop, 2007).

Insects are usually controlled by insecticides. However, the excessive use of insecticides to control these pests can have a negative financial and environmental consequences, including the development of high levels of resistance to conventional insecticides (Ogendo et al., 2003), the high costs of synthetic pesticides and associated toxicity risks (Mihale et al., 2009), the destruction of beneficial insects (pollinators, parasitoids and predators), pesticide residue magnification in humans and wildlife and disruption of ecosystem (Ruchika & Kumar, 2012). Accordingly, the need to search for alternative insect control methods has been increased.

Host plant resistance has been used as a control measure for various agricultural pests for many years (Smith, 2005). Plant resistance may be the most effective, economical, and environmentally sound management tactic to control *A. fabae* in crops. Some progress has been archived in screening broad bean cultivars against black bean aphid and a number of cultivars have been identified as possible source of resistance for breeding programs (El-Dafrawi et al., 1991; Laamari et al., 2008; Meradsi & Laamari, 2016a, 2016b, 2016c; Meradsi, 2017).

Smith (2005) indicated three general categories of plant resistance to insects, which have become widely accepted by entomologists: non preference or, antixenosis, tolerance, and antibiosis. Antixenosis is one of the most important category of plant resistance to aphids on broad bean as well as antibiosis and tolerance of plants to insects. Antixenosis and non-preference denote the presence of morphological or chemical plant factors that adversely alter arthropod behavior, resulting in the selection of an alternate host plant. Physical barriers such as thickened plant epidermal layers, waxy deposits on leaves, stems, or fruits, or a change in the density of trichomes (plant hairs) on normally susceptible plants may force arthropods to abandon their efforts to consume, ingest or oviposit on an otherwise palatable plant (Smith, 2005). The antibiotic effects of a resistant plant range from mild to lethal, and may result from both chemical and morphological plant defensive factors. Lethal effects may be acute, in which case they often affect young larvae and eggs. The chronic effects of antibiosis often lead to mortality in older larvae and prepupae that fail to pupate, and in pupae and adults which fail to eclose. Individuals surviving the direct effects of antibiosis may also suffer the debilitating effects of reduced body size and mass, prolonged periods of development in the immature stages, and reduced fecundity (Smith, 2005).

In the present study, the modalities of the antixenotic and antibiotic resistance were used to determine the most resistant cultivars among seven local broad bean cultivars for the black bean aphid.

## 2 MATERIALS AND METHODS

### 2.1 Plant

Broad bean cultivars used in this study were selected on the basis of field evaluation for their resistance to *A. fabae* (Meradsi, 2009). They included seven cultivars: Six resistant cultivars (12, 111, 126, 135, 141, and 145) and one highly susceptible cultivar (107). Seeds of the broad bean cultivars were acquired from Plant Protection laboratory of Batna (in the east of Algeria).

The (Table 1) showed the agronomical characteristics of the seven broad bean cultivars used in this study.

**Table 1:** Agronomical characteristics of seven broad bean cultivars used to study of the resistance to the black bean aphid, *Aphis fabae*

| Cultivar | Origin | Agronomical characteristics | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 4 | 6 | 9 | 10 | 13 | 15 | 21 | 23 | 24 | 27 | 31 |
| 12 | Biskra | 25.84 | 2.60 | p | 4.53 | 2.34 | 3 | 3.28 | 4.17 | 13.12 | 2.5 | 2.83 | 1.11 |
| 111 | Biskra | 27.17 | 2.50 | p | 5.05 | 2.43 | 3.50 | 3.30 | 3.37 | 12.33 | 2.30 | 1.13 | 0.87 |
| 126 | Biskra | 28.08 | 2.75 | p | 4.98 | 2.29 | 2.50 | 2.71 | 5.43 | 13.33 | 2.27 | 1.50 | 0.85 |
| 135 | Khenchela | 20.08 | 2.50 | p | 4.75 | 2.22 | 2.40 | 3.06 | 3.33 | 10.63 | 2.58 | 3.80 | 0.86 |
| 141 | Biskra | 27.13 | 2.50 | p | 6.36 | 3.35 | 1.5 | 3.17 | 7 | 10.50 | 2.70 | 0.75 | 0.31 |
| 145 | Khenchela | 25.53 | 3.67 | p | 5.07 | 2.17 | 2 | 3.30 | 7.67 | 11 | 2.60 | 1.33 | 0.92 |
| 107 | Biskra | 33.16 | 4.80 | p | 5 | 2.83 | 3.80 | 3.46 | 11.6 | 11.4 | 3.4 | 0.8 | 1.53 |

*Notes*. Biskra: in the south of Algeria, Khenchela: in the east of Algeria, **3**: plant: height (cm), **4**: plant : number of stems (including tillers more than half the length of the main stem), **6**: stem: anthocyanin coloration (p: present), **9**: leaflet: length (basal pair of leaflet at secondary node, cm), **10**: leaflet width (basal pair of leaflet at secondary node, cm), **13**: raceme: number of flowers, **15**: flower: length (cm), **21**: truss: number of pods, **23**: pod: length (without beak, cm), **24**: pod: width (from suture to suture, cm), **27**: pod: number of ovules (including seeds), **31**: dry seed: mass (g).

### 2.2 Insects

The aphids used for this study were obtained from a single winged adult of *A. fabae*. The adult aphid was collected in early October 2008 from culture of the broad bean plants in field of the Batna region (in the east of Algeria). the single winged adult was reared on broad bean seedlings under greenhouse conditions [6 ± 4 °C, 93 ± 7 % relative humidity and a photoperiod of 14: 10 (L : D) h].

### 2.3 Antixenosis

Antixenosis against aphid was evaluated by allowing aphids a free choice between plants of similar growth stage (Castro et al., 2005), second leaf fully expanded, 12 growth stage (Mier, 2001), each planted in a pot (6.5cm diameter × 8cm height), one plant of every cultivar tested was randomly placed in a circle, with their leaves directed towards the center of the circle (Castro et al., 1999; 2001). Twenty-eight adult aphids, equivalent to four aphids per plant (Budak et al., 1999), were placed in the lid of a Petri dish (5.7 cm diameter). After 2 h the number of adult aphids on each plant was recorded, and this was repeated 24 h, 26 h, 48 h, and 72 h later.

Only the adult aphids were recorded because nymphs were not involved in the host selection process (Budak et al., 1999). This experiment was conducted under laboratory conditions of the temperature, light regime and humidity (18 ± 0.25 °C; L16 : D8; 37.5 ± 0.5 % relative rumidity).

The same experiment was repeated under the dark (Hesler & Tharp, 2005) with (19 °C; 42 % relative humidity). After 24 h the number of adults on each plant was recorded.

### 2.4 Antibiosis

The antibiotic test was conducted under laboratory controlled conditions at L16 : D8 photoperiod, 17 ± 1 °C, and 43.5 ± 5 % relative humidity. The test was assessed using eight plants for each cultivar (one plant per pot) and the experiment was conducted according to simple randomized block design. Two apterous adults were placed on each plant at 11 growth stage (Mier, 2001), and allowed to deposit nymphs. After 24 h, we removed the adults and all offspring but one nymph (first larval stage) from each plant. Pre-reproductive period, reproductive period, adult longevity, survival, daily and total fecundity were determined.

### 2.5 Statistical analysis

Data concerning all counts of antixenosis (in the tow experiments; light and dark), pre-reproductive period, reproductive period, adult longevity, survival, daily and total fecundity of the aphid on the broad bean cultivars were compared using analysis of variance (ANOVA). When the ANOVA demonstrated significant differences, the means were separated using the Tukey's test at $P \leq 0.05$. The experiments were arranged in the randomized complete block design (RCBD). All statistical analyses were performed with IBM SPSS statistical software (Version 23.0.0.0) (SPSS, 2015).

# 3 RESULTS AND DISCUSSION

## 3.1 Antixenosis

### 3.1.1 Test in light

Significant antixenosis effect (P < 0.05) against of *A. fabae* was found in the seven cultivars of broad bean for 24 h, 26 h, and 48 h (Table 2). Aphids on 145 were generally found to have the highest antixenosis level (6 adults per plant), while those on 141 were found to be (0.4 adults per plant) (Table 2).

### 3.1.2 Test in dark

The results indicated significant differences ($F_{2.57}$ = 3.017; P = 0.028) (Table 2) among cultivars. It was highest on cultivar 107 (5 adults per plant) and ranged from 0.75 to 2.25 adults per plant for the six other cultivars (Table 2).

**Table 2:** Number of *Aphis fabae* (Mean ± SE) on seven broad bean cultivars

| Cultivar | Light tests | | | | | Dark test |
|---|---|---|---|---|---|---|
| | 2 h | 24 h | 26 h | 48 h | 72 h | 24 h |
| 12 | 4 ± 1,87a | 4.6 ± 1.63ab | 4.6 ± 1.63ab | 4.8 ± 1.59b | 4.2 ± 1.24a | 0.75 ± 0.48a |
| 107 | 3.6 ± 2.06a | 4 ± 2.02ab | 3.8 ± 1.98ab | 38 ± 1.98ab | 3.8 ± 1.98a | 5 ± 1.22b |
| 111 | 0.6 ± 0.4a | 0.6 ± 0.4a | 0.6 ± 0.4a | 0.6 ± 0.4a | 0.6 ± 0.4a | 1.5 ± 0.29a |
| 126 | 1.4 ± 0.68a | 1.4 ± 0.68a | 1.4 ± 0.68a | 1.4 ± 0.68ab | 1.4 ± 0.68a | 0.75 ± 0.48a |
| 135 | 2.6 ± 1.54a | 2 ± 1.55ab | 2.2 ± 1.5ab | 2.2 ± 1.5ab | 2.4 ± 1.5a | 2.25 ± 1.31a |
| 141 | 0.4 ± 0.24a | 0.4 ± 0.24a | 0.4 ± 0.24a | 0.4 ± 0.24a | 0.4 ± 0.24a | 1.5 ± 0.96a |
| 145 | 5.2 ± 1.93a | 6 ± 1.67b | 5.4 ± 1.5b | 5.4 ± 1.5b | 5.4 ± 1.5a | 1.75 ± 0.48a |
| N | 5 | 5 | 5 | 5 | 5 | 4 |
| F | 1.60 | 2.59 | 2.454 | 2.455 | 2.42 | 3.01 |
| P | 0.183 (ns) | 0.040* | 0.049* | 0.049* | 0.052 (ns) | 0.028* |

Means within a column followed by the same letter do not differ significantly. N: number of repetitions, ns: not significant, (ANOVA: *P ≤ 0.05; Tukey's test).

Antixenotic resistance observed against black bean aphid have resulted from multiple factors such as plant morphology (shape and size), pigmentation of assay and chemical defenses.

During the first test (in light) the cultivar 145 was the favorite of the insect while the cultivar 141 was the last choice with 111 and 126. However, in second test (in obscurity) the susceptible control 107 was the first choice of adults than the other cultivars.

The comparison of both tests showed that the morphology or color of plant (assay) or the both had a high part in the selection of *A. fabae* for 145 and 12 cultivars, because the attraction of this cultivars was high in light (6 and 4.6 adults per plant in mean) and low in dark (1.75 and 0.75 adults per plant in mean) for the tow cultivars respectively. The susceptible control 107 was favorite in both tests (probably has a high concentration of attractive volatiles substances). The cultivars 126, 111 and 141 were least preferred by the apterous adults of *A. fabae* in both tests, probably they had likely a high content of repulsive volatiles substances. The cultivar 135 has an intermediate attraction for both tests. In the study of the preference of seven varieties of wheat by the RWA, Lage et al. (2004) had indicated that the resistant cultivar attract only 2.1 adults and the susceptible cultivar attract 5.2 adults. Sandanayaka et al. (2005) noted the mean percentage settlement of 25 first instar wolly apple aphid nymphs *Eriosoma lanigerum* (Hausmann, 1802) on 13 apple accessions, the results showed that the percentage settlement was the highest (41.08 %) on RG and the lowest (3.76 %) on G.

Several factors were responsible for the selection of the host plant such as volatile substances. Webster et al. (2008) identified fifteen electrophysiologically active compounds of broad bean ('Sutton Dwarf') to winged *A. fabae*. In the field, *A. fabae* lands preferentially on yellow leaves (Bernays & Chapman, 1994). Two other works about the pea aphid, *Acyrthosiphon pisum* Harris, 1776. In the first, on the alfalfa, *Medicago sativa* L., Golawska et al. (2008) indicated that the resistant cultivar Radius had a higher level of saponins and a lower level of flavonoids than the susceptible cultivgar Sapko. In the second work, on European lupine, *Lupinus angustifolius* L., Kordan et al. (2008) noted that also the resistance cultivar ('Juno') contained a higher concentration of lupanine (0.59 µg $g^{-1}$ dry matter) than the susceptible cultivar (Markiz) (0.51 µg $g^{-1}$ dry matter). Cai et al. (2004) reported that the resistant cultivar of wheat KOK1679 to the grain aphid *Sitobion avenae* Fabricius, 1794 had high indole alkaloids content during vegetative growth. Several studies at the order of Lepidoptera were determined the substances responsible on the oviposition of females. For examples, the presence of n-alkanes on corn, *Zea mays* L. (Udayagiri & Mason, 1997), the 2-tridecanone on *Lycopersicon* spp. (Maluf et al., 1997), the malic acid with a concentration from 0.1 to 0.7 µmol $cm^{-2}$ on chickpea, *Cicer arietinum* L. (Yoshida et al., 1997) and rutin and genistin on soy bean (Piubelli et al., 2005).

Morris et al. (2009) isolated three diterpinoid acids, grandifloric acid (1), 15β-hydroxy-ent-trachyloban-19-oic acid (2), and 17- hydroxy-16 α-ent-kauran-19-oic acid (3), from polar fractions of pre-bloom sunflower head extracts, as oviposition stimulants for the banded sunflower moth *Cochylis hospes* (Walsingham, 1884) Johnson et al. (2008) indicated that the resistant cultivar Nadine of potato has a high content of glucoalkaloids (309.33 mg $kg^{-1}$ dry mass) than the susceptible cultivar Marfona (96.90 mg $kg^{-1}$ dry mass). Chemical analysis of the host plant leaves of *Gonioctena linnaeana* Schrank, 1781, *Salix triandra* L. showed that quantities but not quality of the phenolic compounds influenced the feeding of the *G. linnaeana* larva. The two most important compounds were gallocatechin and salidroside (Neimi et al., 2005). Leiss et al. (2009) recorded that the thrips-resistant in *Senecio* hybrids contained higher amounts of the pyrrolizidine alkaloids (PA), jacobine and jaconine, especially in younger leaves.

### 3.2 Antibiosis

Cultivars showed no significant effects on pre-reproductive period (F = 1.41; df = 2.45; P = 0.243) or daily fecundity per female (F = 1.20; df = 2.45; P = 0.331) (Table 3). The statistical analysis of reproductive period, adult longevity, survival, and total fecundity showed a high significant difference (P < 0.001) (Table 3). Reproductive period, adult longevity, and survival were the longest on 135 (19; 21.2; and 27.6 days respectively), and the lowest on 141 (3.2; 3.4; and 11 days respectively) (Table 3). However, number of total offspring per female of *A. fabae* was the highest on 135 (85.8 nymphs) and the smallest on 141 (15.8) (Table 3).

**Table 3:** Biological parameters (Mean ± SE) of *Aphis fabae* on seven broad bean cultivars (n = 5)

| Cultivar | Pre-reproductive period | reproductive period | Adult longevity | Survival | Daily fecundity | Total fecundity |
|---|---|---|---|---|---|---|
| 126 | 7.4±0.24a | 3.4±1.17ab | 4.2±1.24a | 10.8±1.11a | 5.67±0.43a | 19.6±7.03a |
| 12 | 8.4±0.4a | 3.8±1.32ab | 4.2±1.5a | 12.2±1.24a | 5.26±0.83a | 17.0±3.17a |
| 107 | 7.4±0.24a | 6.6±2.13ab | 6.8±2,39a | 13.8±2.39a | 6.01±0.85a | 38.8±12.49ab |
| 135 | 7.6±0.24a | 19.0±2.14c | 21.2±2.73b | 27.6±2.67b | 4.62±0.27a | 85.8±5.91c |
| 141 | 8.0±0.0a | 3.2±0.2a | 3.4±0.24a | 11.0±0.0a | 4.9±0.41a | 15.8±1.93a |
| 145 | 7.6±0.4a | 8.2±1.91b | 8.8±1.8a | 15.4±1.83a | 6.18±0.78a | 47.6±10.45b |
| 111 | 7.8±0.37a | 4.2±0.49 | 4.2±0.5a | 11.8±0.86a | 6.37±0.34a | 26.8±3.35ab |
| P | 0.243 | 0.000* | 0.000* | 0.000* | 0.331 | 0.000* |
| F | 1.417 | 13.774 | 13.621 | 12.460 | 1.209 | 11.652 |
| df | 2.45 | 2.45 | 2.45 | 2.45 | 2.45 | 2.45 |

Means within a column followed by the same letter do not differ significantly (Duncan's test, P < 0.05). *P < 0.001.

In the present study, the effect of cultivars on the pre-reproductive period was absent. In contrast, several works showed that this effect was present, for example, the green peach aphid *Myzus persicae* (Sulzer, 1776) on spinach, *Spinacia oleracea* L. (Mcleod et al., 1991), the cotton aphid, *Aphis gossypii* Glover, 1877on chrysanthemum and cucumber (Storer & van Emden, 1995; van Steenis & El-Khawass, 1995), the bird cherry-oat aphid *Rhopalosiphum padi* (Linnaeus, 1758) on wheat and triticale (Hesler & Tharp, 2005), the greenbug *Schizaphis graminum* (Rondani, 1852) on wheat (Castro et al., 2001), and the pea aphid *Acyrthosiphon pisum* on alfalfa, *Medicago sativa* (Golawska et al., 2008). Also, Guillaume & Boissot (2001) observed that the resistant cultivar of melon was prolonged the larval development of the melonworm *Diaphania hyalinata* (Linnaeus, 1767) to 17.08 days that the susceptible cultivar (12.57 days). Other study showed that the days to adult emergence of bruchid *Zabrotes subfasciatus* (Boheman, 1833) was 50.3 on resistant cultivar of common bean *Phaseolus vulgaris* 'SMARC1-PN1' and 33 on susceptible cultivar 'SMARC4N-PN' (Hartweck et al., 1997).

The present experiment demonstrated the greater effect of the cultivar 135 on reproductive period and adult longevity of *A. fabae*. However, these parameters were six times longer than the cultivar 141. The total fecundity on the cultivar 135 was 5.4 times higher than the cultivar 141. Indeed, Klinger et al. (1998) reported that the reproductive period of *A. gossypii* was 21.5 days on susceptible cotton cultivar PMR5 and 13.5 days on the resistant cultivar AR5. The total fecundity was twice on susceptible cultivar in comparison with the resistant cultivar (Formusoh et al., 1994). Wearing et al. (2003b) indicated that the percentage of the larval survival of the brownheaded leafroller, *Ctenopseustis obliquana* (Walker, 1863) after three weeks on the resistant apple cultivar was 6.4 % and 81.5 % on the susceptible cultivar. Wearing et al. (2003a) noted that the survival of larvae to pupation was 64 % on the resistant apple cultivar Nevis 1 and 100 % on the susceptible cultivar Mother to lightbrown apple moth, *Epiphyas postvittana* (Walker, 1863) at 20 °C.

The secondary metabolites were important for the life of the insects. Wilkinson & Douglas (2003) reported that one or more of the eight clones of *A. fabae* tested displayed depressed larval survival, larval growth rate, on diets lacking histidine, methionine, threonine, and valine. Other study noted by Bastide et al. (1988) indicated that the phenolic extracts of young peach leaves influenced the survival and the mass of the nymphs of *M. persicae*. Dried alfalfa (*Medicago sativa*) leaf tissue incorporated in artificial diet to give a final concentration of 0.5 or 1.6 mg $g^{-1}$ fresh mass of saponins significantly inhibited growth and development of larvae of the European corn borer, *Ostrinia nubilalis* (Hubner, 1796) (Nozzolillo et al., 1997). Ulmer & Dosdall (2006) indicated that high levels of specific glucosinolates such as *p*-hydroxybenzyl and 3-butenyl glucosinolate were associated with increased developmental time or reduced mass of the cabbage speedpod weevil, *Ceutorhynchus obstrictus* (Marshham, 1802) in Brassicaceae species.

The constitutive activity of phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO), and peroxidase (POD) in resistant and susceptible wheat cultivars against cereal aphid *Sitobion avenae* at various developmental stages, tillering, stem elongation, flag leaf, and ear was analyzed by Han et al. (2009), the results showed that the PAL and POD in resistant cultivars exhibited greater activity than susceptible ones at the tillering, stem elongation, and flag leaf stages. The PPO had a higher activity in the resistant cultivars at all developmental stages.

## 4 CONCLUSIONS

These preliminary data suggest that among the seven cultivars tested in this study, the mediocre performance of the black bean aphid was noted on the cultivars 141. But, the best performances of *A. fabae* were found on the cultivar 135. The present study revealed that cultivar 141 had an insecticidal propriety against *A. fabae* and could be employed as alternative for chemical pesticides.

## 5 ACKNOWLEDGEMENTS

The authors thank Salim LEBBAL for excellent help.

## 6 REFERENCES

Bastide, P., Massonie, G., & Macheix, J. J. (1988). Influence in vitro des composés phénoliques des jeunes feuilles du pêcher, *Prunus persica* (L.) Batsch, sur le puceron vert du pêcher, *Myzus persicae* Sulzer. *Agronomie, 8*(9), 787-792. doi:10.1051/agro:19880905

Bernays, E. A., & Chapman, R. F. (1994). *Host-Plant Selection by Phytophagous Insects*. New York, Chapman & Hall. doi:10.1007/b102508

Blackman, R. L., Eastop, V. F. (2007). Taxonomic issues. In H. F. van Emden & R. Harrington (Eds.), *Aphids as Crop Pests* (pp. 1-29). U. K., CABI Millennium. doi:10.1079/9780851998190.0001

Budak, S., Quisenberry, S.S., & Ni, X. (1999). Comparison of *Diuraphis noxia* resistance in wheat isolines and plant introduction lines. *Entomologia Experimentalis et Applicata, 92*, 157-164. doi:10.1046/j.1570-7458.1999.00535.x

Cai, Q. N., Zhang, Q. W., & Cheo, M. (2004). Contribution of indole alkaloids to *Sitobion avenae* (F.) resistance in wheat. *Journal of Entomology and Nematology, 128*(8), 517-521.

Castro, A. M., Ramos, S., Vasicek, A., Worland, A., Giménez, D., Clúa, A. A., & Suárez, E. (2001). Identification of wheat chromosomes involved with different types of resistance against greenbug (*Schizaphis graminum*, Rond.) and the Russian wheat aphid (*Diuraphis noxia*, Mordvilko). *Euphytica, 118*, 321-330. doi:10.1023/A:1017503828952

Castro, A. M., Vasicek, A., Manifiesto, M., Giménez, D. O., Tacaliti, M. S., Dobrovolskaya, O., Röder, M. S., Snape, J. W., & Börner, A. (2005). Mapping antixenosis genes on chromosome 6A of wheat to greenbug and to a new biotype of Russian wheat aphid. *Plant Breeding, 124*, 229-233. doi:10.1111/j.1439-0523.2005.01082.x

Castro, A. M., Vasicek, A., Ramos, S., Worland, A., Suárez, E., Muňoz, M., Giménez, D., & Clúa, A. A. (1999). Different types of resistance against greenbug, *Schizaphis graminum* Rond, and the Russian wheat aphid, *Diuraphis noxia* Mordvilko, in wheat. *Plant breeding, 118*, 131-137. doi:10.1046/j.1439-0523.1999.118002131.x

El-Defrawi, G, El-Gantiry, A. M., Weigand, S., Khalil, S. A. (1991). Screening of faba bean (*Vicia faba* L.) for resistance to *Aphis craccivora* Koch. *Arab Journal of Plant Protection, 9*(2), 138-141.

Formusoh, E. S., Wilde, G. E., Hatchett, J. H., & Collins, R. D. (1994). Resistance to the Russian Wheat Aphid (Homoptera: Aphididae) in Wheat-Related Hybrids. *Journal of Economic Entomology, 87*(1), 241-244. doi:10.1093/jee/87.1.241

Golawska, S., Lukasik, L., & Leszczynski, B. (2008). Effect of alfalfa saponins and flavonoids on pea aphid. *Entomologia Experimentalis et Applicata, 128*, 147-153. doi:10.1111/j.1570-7458.2008.00709.x

Guillaume, R., & Boissot, N. (2001). Resistance to *Diaphania hyalinata* (Lepidoptera: Crambidae) in *Cucumis* Species. *Journal of Economic Entomology, 94*(3), 719-723. doi:10.1603/0022-0493-94.3.719

Han, Y., Wang, Y., Bi, J. L., Yang, X. Q., Huang, Y., Zhao, X., … Cai, Q. N. (2009). Constitutive and Induced Activities of Defense-Related Enzymes in Aphid-Resistant and Aphid-Susceptible Cultivars of Wheat. *Journal of Chemical Ecology, 35*(2), 176-182. doi:10.1007/s10886-009-9589-5

Hartweck, L. M., Cardona, C., & Osborn, T. C. (1997). Bruchid resistance of common bean lines having an altered seed protein composition. *Theoretical and Applied Genetics, 95*, 1018-1023. doi:10.1007/s001220050656

Hesler, L. S., & Tharp, C. I. (2005). Antibiosis and antixenosis to *Rhopalosiphum padi* among triticale accessions. *Euphytica, 143*, 153-160. doi:10.1007/s10681-005-3060-7

Johnson, S. N., Anderson, E. A., Dawson, G., & Griffiths, D. W. (2008). Varietal susceptibility of potatoes to wireworm herbivory. *Agricultural and Forest Entomology, 10*, 167-174. doi:10.1111/j.1461-9563.2008.00372.x

Klingler, J., Powell, G., Thompson, G. A., & Isaacs, R. (1998). Phloem specific aphid resistance in *Cucumis melo* line AR5: effects on feeding behaviour and performance of *Aphis gossy*pii. *Entomologia Experimentalis et Applicata, 86*, 79-88. doi:10.1046/j.1570-7458.1998.00267.x

Kordan, B., Gabryś, B., Dancewicz, K., Lahuta, L. B., Piotrowicz-Cieślak, A., & Rowińska, E. (2008). European yellow lupine, *Lupinus luteus*, and narrow-leaf lupine, *Lupinus angustifolius*, as hosts for the pea aphid, *Acyrthosiphon pisum*. *Entomologia Experimentalis et Applicata, 128*, 139-146. doi:10.1111/j.1570-7458.2008.00702.x

Laamari, M., Khelfa, L., & Coeur d'Acier, A. (2008). Resistance source to cowpea aphid (*Aphis craccivora* Koch) in broad bean (*Vicia faba* L.) Algerian landrace collection. *African Journal of biotechnology, 7*(14), 2486-2490.

Lage, J., Skovmand, B., & Andersen, S. B. (2004). Resistance categories of synthetic hexaploid wheats resistant to the Russian wheat aphid (*Diuraphis noxia*). *Euphytica, 136*, 291-296. doi:10.1023/B:EUPH.0000032732.53350.93

Laudadio V., Ceci E., & Tufarelli V. (2011). Productive traits and meat fatty acid profile of broiler chicken fed diets containing micronized fava beans (*Vicia faba* L. var. minor) as the main protein source. *Journal of Applied Poultry Research*, *20*, 12-20. doi:10.3382/japr.2010-00173

Leiss, K. A., Choi, Y. H., Abdel-Farid, I. B., Verpoorte, R., & Klinkhamer, P. G. L. (2009). NMR Metabolomics of Thrips (Frankliniella occidentalis) Resistance in Senecio Hybrids. *Journal of Chemical Ecology, 35*(2), 219-229. doi:10.1007/s10886-008-9586-0

Maluf, W. R., Barbosa, L. V., & Costa Santa-Cecília, L. V. (1997). 2-Tridecanone-mediatedmechanisms of resistance to the South American tomato pinworm *Scrobipalpuloides absoluta* (Meyrick, 1917) (Lepidoptera-Gelechiidae) in *Lycopersicon* spp. *Euphytica, 93*, 189-194. doi:10.1023/A:1002963623325

McLeod, P., Morelock, T. E., & Goode, M. J. (1991). Prefernce, Developmental Time, Adulte Longivity and Fecundity of Green Peach Aphid (Homoptera: Aphididae) on Spinach. *Journal of Entomological Science, 26*(1), 95-98. doi:10.18474/0749-8004-26.1.95

Meradsi, F. (2009). *Contribution à l'étude de la résistance naturelle de la fève Vicia faba L. au puceron noir Aphis fabae Scopoli, 1763 (Homoptera: Aphididae)*. Mémoire de Magister. Protection des végétaux. Université Batna 1.

Meradsi, F. (2017). *Natural resistance of broad bean against the Black bean aphid. Origin and* *catogories of the resistance. Germany*, LAP: Lambert Academic Publishing. ICS Morebooks.

Meradsi, F., & Laamari, M. (2016a). Genetic resource of the resistance of *Vicia faba* L. against the black bean aphid, *Aphis fabae* Scopoli. *Journal of Agricultural Studies*, *4*(2), 107-114. doi:10.5296/jas.v4i2.9333

Meradsi, F., & Laamari, M. (2016b). Population dynamics and biological parameters of *Aphis fabae* Scopoli on five broad bean cultivars. *International Journal of Biosciences, 9*(2), 58-68. doi:10.12692/ijb/9.2.58-68

Meradsi, F., & Laamari M. (2016c). Aphicidal and behavioral effects of *Vicia faba* L. (Fabales: Fabaceae) leaf extracts against *Aphis fabae* Scopoli (Hom., Aphididae). *Jordan Journal of Agricultural Sciences, 12*(4), 1085-1094. doi:10.12816/0035070

Mier, U. (2001). Growth stages of mono-and dicotyledonous plants. *BBCH Monograph*. Germany: Federal Biological Research Centre for Agriculture and Forestry.

Mihale, M. J., Deng, A. L., Selemani, H. O., Mugisha-Kamatenesi, M., Kidukuli, A. W., & Ogendo, J. O. (2009). Use of Indigenous knowledge in the management of field and storage pests around Lake Victoria basin in Tanzania. *African Journal of Environmental Science and Technology, 3*(9), 251-259.

Morris, B. D., Charlet, L. D., & Foster, S. P. (2009). Isolation of three Diterpenoid Acids from Sunflowers, as Oviposition Stimulants for the Banded Sunflowers Moth, *Cochylis hospes*. *Journal of Chemical Ecology, 35*(1), 50-57. doi:10.1007/s10886-008-9567-3

Mustafa, T. M., & Qasem, J. R. (1984). Host ranges of the bean aphid *Aphis fabae* in Jordan. *Entomologica Basiliensia, 9*, 48-53.

Niemi, L., Wennström, A., & Ericson, L. (2005). Insect feeding preferences and plant phenolic glucosides in the system *Gonioctena linnaeana - Salix triandra*. *Entomologia Experimentalis et Applicata, 115*, 61-66. doi:10.1111/j.1570-7458.2005.00269.x

Nozzolillo, C., Arnason, J. T., Campos, F., Donskov, N., & Jurzysta M. (1997). Alfalfa leaf saponins and insect resistance. *Journal of Chemical Ecology, 23*(4), 995-1002. doi:10.1023/B:JOEC.0000006384.60488.94

Ogendo, J. O., Belmain, S. R., Deng, A. L., & Walker, D. J. (2003). Comparison of toxic and repellent effects of *Lantana camara* L. with *Tephrosia vogelii* hook and a synthetic pesticide against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) in stored maize grain. *Insect science and its Application, 23*(2), 127-135. doi:10.1017/S1742758400020348

Piubelli, G. C., Hoffmann-Campo, C. B., Moscardi, F., Miyakubo, S. H., & Neves de Oliveira, M. C. (2005). Are chemical compounds important for soybean resistance to *Anticarsia gemmatalis*? *Journal of Chemical Ecology, 31*, 1509-1525. doi:10.1007/s10886-005-5794-z

Ruchika, K., & Kumar D. (2012). Occurrence and infestation level of sucking pests: Aphids on various host plants in agricultural fields of Vadora, Gujarat (India). *International Journal of Scientific and Research Publications, 2*(7), 1-6.

Sandanayaka, W. R. M., Bus, V. G. M., & Connolly, P. (2005). Mechanisms of woolly aphid [*Eriosoma lanigerum* (Hausm.)] resistance in apple. *Journal of Applied Entomology, 129*, 534-541. doi:10.1111/j.1439-0418.2005.01004.x

Smith, C. M. (2005). *Plant resistance to arthropods: Molecular and conventional approaches*. Berlin Heidelberg New York: Springer. doi:10.1007/1-4020-3702-3

Storer, J. R., & van Emden, H. F. (1995). Antibiosis and antixenosis of chrysanthemum cultivars to the aphid *Aphis gossypii*. *Entomologia Experimentalis et Applicata, 77*, 307-314. doi:10.1111/j.1570-7458.1995.tb02328.x

SPSS, Inc. 2015. *SPSS for Windows*. Version 23.0.0.0. Chicago, Illinois.

Udayagiri, S., & Mason, C. E. (1997). Epicuticular wax chemicals in *Zea mays* influence oviposition in *Ostrinia nubilalis*. *Journal of Chemical Ecology, 23*, 1675-1687. doi:10.1023/B:JOEC.0000006443.72203.f7

Ulmer, B. J., & Dosdall, L. M. (2006). Glucosinolate profile and oviposition behavior in relation to the susceptibilities of Brassicaceae to the cabbage seedpod weevil. *Entomologia Experimentalis et Applicata, 121*, 203-213. doi:10.1111/j.1570-8703.2006.00480.x

van Steenis, M. J., & E1-Khawass, K. A. M. H. (1995). Life history of *Aphis gossypii* on cucumber: influence of temperature, host plant and parasitism. *Entomologia Experimentalis et Applicata, 76*, 121-131. doi:10.1111/j.1570-7458.1995.tb01954.x

Wearing, C. H., Colhoun, K., Attfield, B., Marshall, R.R., & McLaren, G. F. (2003a). Screening for resistance in apple cultivars to lightbrown apple moth, *Epiphyas postvittana*, and greenheaded leafroller, *Planotortrix octo*, and its relationship to field damage. *Entomologia Experimentalis et*

*Applicata, 109*, 39-53. doi:10.1046/j.1570-7458.2003.00091.x

Wearing, C. H., Colhoun, K., McLaren, G. F., Attfield, B., & Bus, V. G. M. (2003b). Evidence for single gene resistance in apple to brownheaded leafroller, *Ctenopseustis obliquana*, and implications for resistance to other New Zealand leafrollers. *Entomologia Experimentalis et Applicata, 108*, 1-10. doi:10.1046/j.1570-7458.2003.00062.x

Webster, B., Bruce, T., Dufour, S., Birkemeyer, C., Birkett, M., Hardie, J., & Pickett, J. (2008). Identification of Volatile Compounds Used in Host Location by the Black Bean Aphid, *Aphis fabae*. *Journal of Chemical Ecology, 34*(9), 1153-1161. doi:10.1007/s10886-008-9510-7

Wilkinson, T. L., & Douglas, A. E. (2003). Phloem amino acids and the host plant range of the polyphagous aphid, *Aphis fabae*. *Entomologia Experimentalis et Applicata, 106*, 103-113. doi:10.1046/j.1570-7458.2003.00014.x

Yoshida, M., Cowgill, S. E., & Wightman, J.A. (1997). Roles of oxalic and malic acids in chickpea trichome exudate in host-plant resistance. *Journal of Chemical Ecology, 23*, 1195-1210. doi:10.1023/B:JOEC.0000006395.45516.e8

doi:10.14720/aas.2018.111.3.03 Original research article / izvirni znanstveni članek

# Protein pattern analysis in tolerant and susceptible wheat cultivars under salinity stress conditions

Marouf KHALILI[1], Mohammad Reza NAGHAVI[2*], Said YOUSEFZADEH[2]

Received July 27, 2017; accepted November 19, 2018.
Delo je prispelo 27. julija 2017, sprejeto 19. novembra 2018.


## ABSTRACT

To investigate proteome pattern of wheat cultivars, young leaves were collected from tillering stage of seedlings two weeks after development of the salinity stress. The extraction of proteins from leaf tissue was done and two dimensional electrophoresis using IPG strips and SDS-PAGE in the control and salinity treatments were performed. In total, 198 and 203 protein spots were identified in tolerant ('Moghan3') and susceptible ('Pishtaz') cultivars respectively. Also, among these, spots number 21 and 22 were detected with significant IF in 'Moghan3' and 'Pishtaz' respectively. Two-stage mass spectrometry (MS/MS) was used to identify protein spots. Common identified proteins, including proteins involved in removal of oxidants, Calvin cycle proteins, proteins involved in light reaction of photosynthesis and proton transfer, and heat shock protein were identified on basis of the functional groups and their frequency. In total, 'Moghan3' maintained the stability of the structure and performance of carbon metabolism under stress better than susceptible cultivar. In addition, defense against oxidative stress induced by salinity stress was performed by 2-cys peroxiredoxin BAS1 and Cu-Zn SOD proteins that tolerant cultivar defended against oxidative stress better than the susceptible cultivar. The greatest strength of 'Moghan3' and major weakness in 'Pishtaz' are relying on the unique proteins formed under salinity stress for the removal of oxidants and to maintain the activity of the photosynthetic light reactions, respectively.


**Key words:** proteomics analysis; salt tolerance; stress response proteins; two-dimensional electrophoresis; wheat


## IZVLEČEK

### ANLIZA VZORCA BELJAKOVIN V ODPORNI IN OBČUTLJIVI SORTI PŠENICE V RAZMERAH SLANOSTNEGA STRESA

Za analizo proteomskega vzorca v dveh sortah pšenice so bili vzorčeni mladi listi v fazi bilčenja dva tedna po izpostavitvi slanostnemu stresu. Izvleček beljakovin iz listnih tkiv je bil narejen z dvodimenzionalno elektroforezo z uporabo IPG trakov in SDS-PAGE, za rastline iz kontrole in tiste v slanostnem stresu. Celokupno je bilo evidentiranih 198 beljakovinskih točk za odporno sorto ('Moghan3') in 203 beljakovinskih točk za občutljivo sorto ('Pishtaz'). Med temi sta bili ugotovljeni beljakovinski točki št. 21 in 22 z značilnimi vrednostmi IF za 'Moghan3' in 'Pishtaz'. Za določitev beljakovin v točkah je bila uporabljena dvofazna masna spektrometrija (MS/MS). Določene beljakovine so obsegale encime, ki so vključeni pri odstranjevanju oksidantov, encime Kalvinovega cikla, beljakovine, ki so udeležene v svetlobnih reakcijah fotosinteze in v protonskem transportu ter beljakovine vročinskega udara. Beljakovine so bile določene na osnovi funkcionalnih skupin in njihove frekvence. V splošnem je v stresnih razmerah odporna sorta 'Moghan3' ohranjala stabilnost zgradbe in poteka presnove ogljika bolje kot občutljiva sorta. Dodatno sta se za obrambo proti oksidacijskem stresu v razmerah slanosti inducirala dva proteina, 2-cis peroksiredoksin BAS1 in Cu-Zn SOD protein, ki sta odporno vrsto ščitila bolje kot občutljivo. Odpornost sorte 'Moghan3' in občutljivost sorte 'Pishtaz' na slanostni stres temelji na edinstvenem vorcu beljakovin, ki se tvorijo v razmerah slanosti za odpravljanje oksidantov in vzdrževanje aktivnosti svetlobnih rekacij fotosinteze.


**Ključne besede:** proteomska analiza; odpornost na sol; stresni proteini; dvodimenzionalna elektroforeza; pšenica

[1] Associate Professor, Department of Agriculture, Payame Noor University, PO BOX 19395-3697 Tehran, Iran

[2] Assistant Professor, Department of Agriculture, Payame Noor University, PO BOX 19395-3697 Tehran, Iran; corresponding author: mr_naghavi@ymail.com

## 1 INTRODUCTION

Salinity stress tolerance in plants is a complex phenomenon and it is communicated with the physiological, biochemical and molecular mechanism. In this regard, proteomic approaches are identified as one of the most important methods for understanding the molecular basis of salt stress tolerance at the protein levels (Thiellement et al., 2002). Abiotic stresses such as salinity, before any effects are detected at the production level cause the change in cytoplasmic calcium concentration and pH which is understood as the main plant response mechanism under these condition. Osmotic stress and ion toxicity (sodium and chloride) derived from salinity stress act in both the inner and outer plasma membrane by trans-membrane proteins or enzymes inside cytosol. Many osmotics raised under drought stress are understood as stress sensors (Abdul Kader and Lindberg, 2010). Salt interferes with plant growth and can lead to physiological drought and ionic toxicity. Thus, salinity and drought stresses often affects the physiological aspects of plant metabolism, creating tension (hyperionic and hyper osmotic), and eventually plant will die. Salinity and drought stresses overlap on physiological level because salt in soil decreases the amount of available water and leads to reduced water absorption (Tuteja, 2007).

Salinity stress causes ion stress through the changes in potassium and sodium ion ratios. External sodium ions can have a negative effect on the absorption of calcium ions. Salinity resulted increases in the concentration of sodium and chloride ions in cytosol could be detrimental to the cells. Sodium ion can eliminate membrane potential, thus facilitates the absorption of chloride. High concentration of sodium ions (up to 100 mM), is toxic for the cell metabolism and can prevent activity of many essential enzymes, cell division and expansion, causing membrane damage and osmotic imbalance and thus stops the growth. High concentration of sodium ions can lead to the production of reactive oxygen species and reduction of photosynthesis. Potassium is one of the most essential elements required for plant growth. The concentration of potassium ions (due to severe salinity stress) causes osmotic imbalance problem in stomata functions and action of enzymes. Salinity damages cells, reduces leaf transpiration, resulting in the prevention of growth and causing cell intoxication. Salts can accumulate in older leaves and causing cell death (Tuteja, 2007).

Several studies involved in identification of proteins' response to salinity stress have used proteomics approaches. Most of the proteins affected under stress were involved in process of photosynthesis, photorespiration, transduction, metabolism, defense against oxidative stress, ion channels control and folding of proteins (Joseph and Jini, 2010). For example, changes in wheat proteome 30 days after exposure to 125 mM NaCl in the culture chamber were evaluated and a significant negative correlation between tolerance to salt and sodium concentration in wheat stems were observed. Protein expression change was more than 5 %, but the difference between the different groups of protein modifications (over expression, knockdown, disappearance and appearance) was variable from 1 to 8 % under salinity stress (Saqib et al., 2006). In order to better understand the development of wheat roots, Song et al. (2007) established a reference map of the major soluble proteins using a combination of 2-DE and MALDI TOF MS and a total of 450 protein spots were detected with silver staining in a pH ranges of 4 - 7, in which 282 protein spots were identified. These identified proteins grouped into diverse functional categories. In comparison with wheat leave proteome, in root, proteins involved in metabolism and transport increased expression, whereas proteins involved in energy, disease and defense, transcription, and signal transduction were of reduced expression. They also showed that hybridization between two parental lines could be different in protein expression in the offspring in comparison with their parents. Proteomic approach was used to identify the salt stress-responsive proteins in an elite Chinese wheat cultivar, 'Zhengmai 9023', which exhibits a high yield, superior gluten quality and better biotic resistance. Three-week-old seedlings were treated with NaCl of four different concentrations (1.0 %, 1.5 %, 2.0 %, and 2.5 %). The total proteins from the leaves of untreated and NaCl-treated plants were extracted and separated by two-dimensional gel electrophoresis (2D-DIGE). A total of 2358 protein spots were detected on the gels, among which 125 spots showed a significant change in protein abundance, and 83 differentially expressed spots were localised on preparative gels. A total, 52 salt-responsive spots were identified, which were classified into six functional categories that included transport-associated proteins, detoxifying enzymes, ATP synthase, carbon metabolism proteins, protein folding proteins, and proteins with unknown biological functions. Of the 52 differentially expressed proteins, 26 were upregulated, 21 were downregulated, and five spots showed multi-expression patterns. In particular, some important proteins for salt tolerance were found to be upregulated in this cultivar under salt stress, such as $H^+$-ATPases, glutathione S-transferase, ferritin and triose phosphate isomerase (Gao et al., 2011). On the other hands, proteomic investigation have been conducted to further understand the mechanism of plant responses to salinity in a salt-tolerant ('Afzal') and a salt-sensitive ('Line 527') genotype of barley. At the 4-leaf stage, plants were exposed to 0 (control) or 300 mM NaCl. Salt treatment was maintained for 3 weeks. Total proteins of leaf were extracted and separated by two-dimensional gel electrophoresis. More than 500 protein spots were reproducibly detected. Of these, 44 spots showed significant changes to salt treatment compared to the control: 43 spots were upregulated and 1 spot was downregulated. Using MALDI-TOF-TOF MS, 44 cellular proteins were identified , which represented 18 different proteins and were classified into seven categories and a group with unknown biological function. These proteins were involved in various many cellular functions. Upregulation of proteins which were involved in reactive oxygen species scavenging, signal transduction, protein processing and cell wall may increase plant adaptation to salt stress (Fatehi et al., 2012).

This study compares two-dimensional electrophoresis pattern of salinity susceptible and tolerant wheat cultivars and ultimately, identification of expression changes and evaluation role of identified proteins under salinity stress.

## 2 MATERIALS AND METHODS

### 2.1 Plant material and experimental design

In this study, two cultivars of spring wheat namely 'Moghan3' (tolerant) and 'Pishtaz' (susceptible) at seedling stage in hydroponic culture system under salinity stress were evaluated in the greenhouse at University of Mahabad, Iran in 2015. Hoagland solution specifications used in wheat culture was mixture of $NO_3$ = 15 mM, K = 6 mM, Mg = 2 mM, Zn = 8 mM, B = 100 mM, Mn = 8 mM, Cu = 2 mM, Mo = 2 mM, Ca = 5 mM and Fe as Fe-EDTA = 4 mg $l^{-1}$. Experimental plots were tubes which were filled with sandy loam soil and were connected with nutrition source. The plant material was evaluated with factorial experiment based on Randomized Complete Block Design (RCBD) with four replications. The first factor included two levels of salinity exposed with chloride sodium such as control (non-stress) and 250 mM (NaCl) (as severe stress) and the second factor was wheat, above mentioned cultivars. Salinity started from tillering stage for two weeks and then treatments were sampled for proteome analysis. The rest of leaf samples were used for measuring of leaf water potential with pressure chamber, leaf relative water content by Morant-Manceau et al. (2004) method, osmotic potential by osmometer. Also, chlorophyll content (SPAD) and chlorophyll fluorescence was identified by chlorophyll meter and fluorometer respectively. In final, plant height and plant dry mass were measured.

### 2.2 Proteome analysis

#### 2.2.1 Protein extraction

Total protein were extracted from 0.5 g frozen leaf for each biological replicate and it suspended as fine powder in cold acetone containing 10 % TCA and 0.07 % 2-mercapthoetanol. The resultant powder was dissolved in lysis buffer containing 7 M urea, 2 M thiourea, 2 % CHAPS, 60 mM DDT and 1 % ampholyte (pH:3 - 10). In addition, protein concentration was determined by Bradford assay (Bradford, 1976).

#### 2.2.2 The first and second dimension electrophoresis

The first dimension electrophoresis was performed using IPG strips. For the first dimension of PROTEAN IEF focusing tray (Bio Rad) and the PROTEAN IEF cell (Bio Rad) was used. Then balancing of strips (equilibration) was carried out ((Herbert, 1999). Also, the second dimension gels as two pieces (including separator gel (separating gel) and holder gel (stacking gel)) were prepared. The separation gel, 8.5 ml, was prepared from the combination of acrylamide , 6.3 ml separating gel buffer (pH = 8.8), 2 ml distilled water, 120 µl 10 % APS and 20 µl TEMED. While stacking gel was prepared from combining of acrylamide for 1 ml stacking gel, l. 3 ml stacking gel buffer (pH = 6.8), 2 ml distilled water, 30 µl 10 % APS, and 20 µl TEMED). Then first dimension strips were put on the second dimension gel using agarose 1 %. Finally protein loading in second dimension with a current of 35 mA for each gel was conducted. After the second dimension electrophoresis, gel staining was performed using a solution of Coomassie blue (Herbert, 1999).

#### 2.2.3 Gel imaging and protein spots analysis

Gels were scanned using BioRad GS-800 scanner. Images analyses were performed with PDQuest™ software (BioRad). After determining the protein spots with significant expression and data normalization, a one-way ANOVA model was used to identify the differentially expressed protein spots between normal and stress conditions. Also it was used IF (Induction Factor) measurement for selection among significantly different spots for detecting spots with more expression change during salinity stress. Then the two-stage mass spectrometry (MS/MS) and liquid chromatography combined with bioinformatics tools were used to identify target spots. One microliter of digested peptides was injected into the C18 column of PepMap nano-chromatography. The peptides were then diluted with 0.1 % formic acid in acetonitrile and separated in C18 columns by inverting phase movement. Subsequently, peptides were sprayed into mass spectrometers. The range of ratio of mass to load in peptides was considered to be between 100 and 2000. The data obtained from the spectrophotometer with Bioworks software (ver. 3.3.1, Thermo Fisher) were converted into a usable format by Mascot search engine.

## 3 RESULTS AND DISCUSSION

### 3.1 Analysis of variance and Mean comparisons

Variance analysis is shown in Table 1. According to the results, between stress levels and cultivars were significant differences for all studied traits. The cultivar × stress interaction was not significant for any studied traits. The minimum and maximum coefficients of variation were related to SPAD (6.67) and chlorophyll fluorescence (10.18), respectively. Table 2 shows the comparison of the mean of stress levels and cultivars. According to results, quantitative mean of 'Moghan3' cultivar was better than 'Pishtaz' cultivar for all studied traits under salinity stress.

**Table 1:** Analysis of variance for morphological and physiological traits in wheat under salt stress

| S.O.V | degree of freedom | Mean of squares | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Plant Dry Mass | Plant Height | Fluorescence | SPAD | Osmotic Potential | RWC | LWP |
| Replication | 3 | $0.01^{ns}$ | $36.65^{ns}$ | $0.003^{ns}$ | $1.33^{ns}$ | $0.008^{ns}$ | $3.67^{ns}$ | $0.005^{ns}$ |
| Stress (S) | 1 | $0.98^{**}$ | $95.56^{**}$ | $0.010^{**}$ | $19.25^{**}$ | $0.34^{**}$ | $153.01^{**}$ | $0.38^{*}$ |
| Cultivar (C) | 1 | $0.96^{**}$ | $98.88^{**}$ | $0.011^{**}$ | $20.28^{**}$ | $0.43^{**}$ | $169.44^{**}$ | $0.42^{**}$ |
| C × S | 1 | $0.04^{ns}$ | $33.70^{ns}$ | $0.001^{ns}$ | $3.88^{ns}$ | $0.005^{ns}$ | $2.65^{ns}$ | $0.003^{ns}$ |
| Error | 9 | 0.05 | 45.40 | 0.005 | 5.87 | 0.009 | 3.97 | 0.22 |
| CV (%) | | 7.34 | 9.66 | 10.18 | 6.67 | 10.08 | 8.50 | 7.53 |

ns ,* and ** are non-significant and significantly in 5 % and 1 % probability levels respectively.

**Table 2:** Comparison of of the means for the stress levels and cultivars for studied traits in wheat

| | Plant Dry Mass (g) | Plant Height (cm) | Fluorescence | SPAD | Osmotic Potential (MPa) | RWC (%) | LWP (MPa) |
|---|---|---|---|---|---|---|---|
| Control | 1.73±0.02 | 29.3±0.7 | 0.8325±0.0023 | 42.1±0.3 | -0.65±0.02 | 75.33±1.40 | -1.36±0.03 |
| Salt stress | 1.08±0.04 | 21.1±0.9 | 0.7812±0.0034 | 39.8±0.5 | -1.02±0.02 | 64.25±1.23 | -1.89±0.01 |
| Difference | $\pm 0.65^{**}$ | $\pm 8.2^{**}$ | $\pm 0.0513^{**}$ | $\pm 2.3^{**}$ | $\pm 0.37^{**}$ | $\pm 11.08^{**}$ | $\pm 0.53^{*}$ |
| Moghan3 | 1.92±0.02 | 31.4±1.0 | 0.8992±0.0020 | 45.3±0.4 | -1.14±0.03 | 79.69±1.95 | -1.06±0.02 |
| Pishtaz | 0.88±0.01 | 19.5±0.8 | 0.7761±0.0009 | 38.9±0.9 | -0.53±0.01 | 61.04±0.89 | -1.92±0.03 |
| Difference | $\pm 1.04^{**}$ | $\pm 11.9^{**}$ | $\pm 0.1231^{**}$ | $\pm 6.4^{**}$ | $\pm 0.61^{**}$ | $\pm 18.65^{**}$ | $\pm 0.86^{**}$ |

*, ** are significantly in 5 % and 1 % probability levels, respectively

### 3.2 Proteomics results

3.2.1 2Dimensional Gel Electrophoresis

Protein analysis discovered 198 and 203 protein spots in tolerant (‘’Moghan3) and susceptible (‘Pishtaz’) cultivars, respectively. Following, among significant spots, protein spots with significantly larger IF than 2 or smaller IF than 0.5 were picked (IF more than 1 points the increased protein expression under salt stress). According to IF value, out of 198 and 203 protein spots, 21 and 22 protein spots in ‘Moghan’ and ‘Pishtaz’ cultivars were detected, respectively. Out of these protein spots, 16 protein spots between the two cultivars were in common while five and six protein spots were unique for the ‘Maghan3’ and ‘Pishtaz’ cultivars, respectively. In other words, a total of 27 responsive protein spots under stress in both cultivars were identified. Gel image for both cultivars,‘Moghan3’ and ‘Pishtaz’ are shown in Figures 1 and 2.

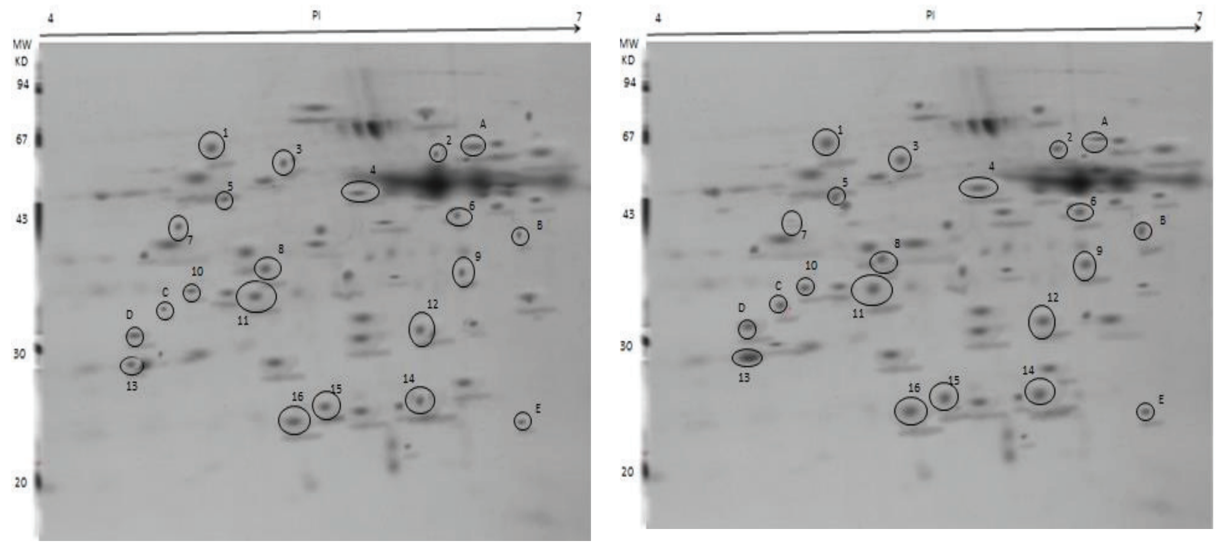



**Figure 1:** Comparison of 2D gel electrophoresis of ‘Moghan3’ under control (left) and salinity stress (right). Responsive common protein spots for salt stress are shown with numbers and uncommon protein with letters

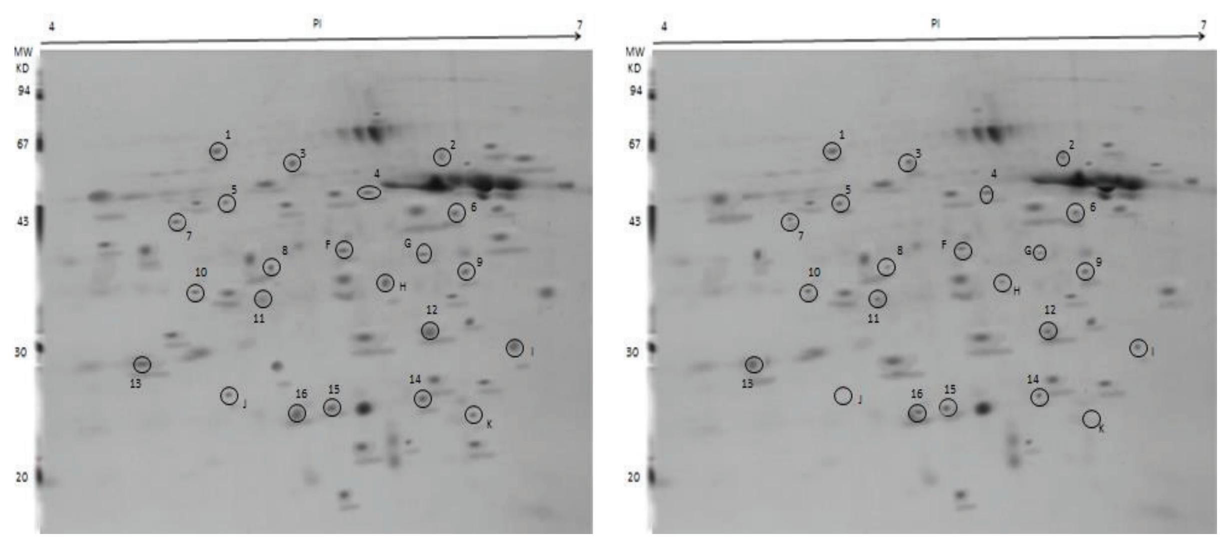



**Figure 2:** Comparison of 2D gel electrophoresis of ‘Pishtaz’ under control (left) and salinity stress (right). Responsive common protein spots for salt stress are shown with numbers and uncommon protein with letters

Based on the results it can be stated that increased expression of proteins in tolerant wheat cultivar under salinity stress was bigger than in susceptible cultivar (Tables 3 and 4). Also tolerant cultivar reaction to maintain its growth was better than in susceptible cultivar under salinity stress. These results are similar to results reported by Hosseini Salekdeh et al. (2002) and Naghavi (2014).

#### 3.2.2 Protein identification by mass spectrometry

The responsive proteins to salinity stress on the staining gel were isolated and were identified using mass spectrometry. 16 common proteins and 11 uncommon proteins such as 5 spots in 'Moghan3' and 6 spots in 'Pishtaz' were detected (Tables 3 and 4). Also in Figure 3 numbers of downregulated, upregulated and absent/present proteins in two cultivars are shown The majority of responsive proteins in 'Moghan3' were upregulated while the majority of them in 'Pishtaz' were downregulated by salinity.

**Table 3:** Characteristics of 16 known common protein spots from all of significantly different accessions of both wheat cultivar under salinity stress

| Functional group of protein | Spot number | Experimental MW | Experimental pI | Theoretical MW | Theoretical pI | name of protein | Accession number | Expression in 'Moghan3' | Expression in 'Pishtaz' |
|---|---|---|---|---|---|---|---|---|---|
| proton transport | 1 | 65.1 | 4.9 | 53.88 | 5.06 | ATP synthase CF1 beta subunit | gi\|14017579 | upregulated | downregulate |
| proton transport | 2 | 64.2 | 6.1 | 53.88 | 5.06 | ATP synthase CF1 beta subunit | gi\|14017579 | upregulated | downregulated |
| heat shock protein | 3 | 63.4 | 5.4 | 73.72 | 4.9 | 70 kDa heat shock protein | gi\|254211611 | upregulated | downregulated |
| Calvin cycle | 4 | 50.3 | 5.75 | 53.4 | 6.2 | Ribulose-1,5-bisphosphate carboxylase/oxygenase, large subunit | gi\|61378609 | upregulated | downregulate |
| Calvin cycle | 5 | 45.1 | 5.1 | 47.34 | 8.62 | ribulose 1,5-bisphosphate carboxylase activase isoform | gi\|167096 | further increase | increased less |
| protein synthesis/degradation | 6 | 44.4 | 6.1 | 39.9 | 6.5 | Triticain gamma | gi\|111073719 | upregulated | downregulated |
| photoreaction of photosynthesis | 7 | 42.9 | 4.7 | 37.01 | 5.4 | photosystem II stability/assembly factor HCF136, chloroplastic-like | gi\|357117071 | decrease less | further decrease |
| removal of oxidants | 8 | 38.3 | 5.3 | 27.9 | 5.6 | acidic endochitinase | gi\|116346 | upregulated | downregulated |
| Calvin cycle | 9 | 38.1 | 6.15 | 18.80 | 8.83 | ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit | gi\|4038719 | upregulated | downregulated |
| removal of oxidants | 10 | 37.3 | 4.8 | 29.5 | 10.2 | HrPB1 | gi\|38679331 | further increase | increased less |
| removal of oxidants | 11 | 36.5 | 5.2 | 20.35 | 5.3 | Cu/Zn superoxide dismutase | gi\|1572627 | upregulated | downregulated |
| Calvin cycle | 12 | 33.1 | 5.95 | 42.21 | 5.9 | chloroplast fructose-bisphosphate aldolase | gi\|223018643 | upregulated | downregulated |
| removal of ioxidants | 13 | 28.9 | 4.6 | 23.39 | 5.4 | 2-cys peroxiredoxin BAS1, chloroplastic | gi\|2499477 | further increase | increased less |
| removal of oxidants | 14 | 26.0 | 5.9 | 23.39 | 5.4 | 2-cys peroxiredoxin BAS1, chloroplastic | gi\|2499477 | upregulated | downregulated |
| photoreaction of photosynthesis | 15 | 25.2 | 5.6 | 27.42 | 8.84 | oxygen-evolving enhancer protein 2, (OEE2) chloroplastic | gi\|131394 | upregulated | downregulated |
| Remove of antioxidant | 16 | 24.1 | 5.5 | 23.39 | 5.4 | 2-cys peroxiredoxin BAS1, chloroplastic | gi\|2499477 | upregulated | downregulated |

**Table 4:** Characteristics of five uncommon protein spots in 'Moghan3' and six uncommon spots in 'Pishtaz' under salinity stress

| Functional group of protein | Spot code | Experimental | | Theoretical | | name of protein | Accession number | Expression in'Moghan3'' | Expression in 'Pishtaz' |
|---|---|---|---|---|---|---|---|---|---|
| | | MW | pI | MW | pI | | | | |
| heat shock protein | A | 65.2 | 6.18 | 73.72 | 4.9 | 70 kDa heat shock protein | gi\|254211611 | downregulated | - |
| protein synthesis/degradation | B | 39.9 | 6.3 | 24.4 | 10.1 | 50S ribosomal protein L10 | gi\|218192573 | upregulated | - |
| removal of oxidants | C | 34.1 | 4.68 | 29.52 | 9.51 | HrPB1 | gi\|38679331 | upregulated | - |
| removal of oxidants | D | 32.0 | 4.61 | 17 | 5.37 | Type 2 peroxiredoxin | gi\|473787383 | upregulated | - |
| removal of oxidants | E | 24.3 | 6.35 | 20.35 | 5.3 | Cu/Zn superoxide dismutase | gi\|1572627 | upregulated | - |
| photoreaction of photosynthesis | F | 39.9 | 5.7 | 24.44 | 8.69 | light-harvesting complex I, partial (chloroplast) | gi\|544700 | - | downregulated |
| photoreaction of photosynthesis | G | 39.5 | 5.9 | 28.6 | 7.7 | thylakoid lumenal 29.8 kDa protein | gi\|195656049 | - | downregulated |
| removal of oxidants | H | 37.2 | 5.85 | 23.6 | 5.8 | glutathione S-transferase | gi\|5923877 | - | downregulated |
| removal of oxidants | I | 31.1 | 6.28 | 27.96 | 5.0 | ascorbate peroxidase | gi\|15808779 | - | downregulated |
| proton transport | J | 27.0 | 5.1 | 17.72 | 4.49 | ATP synthase delta chain, chloroplastic | gi\|475627717 | - | absent under stress |
| photoreaction of photosynthesis | K | 23.5 | 6.2 | 27.42 | 8.84 | oxygen-evolving enhancer protein 2, chloroplastic | gi\|131394 | - | absent under stress |

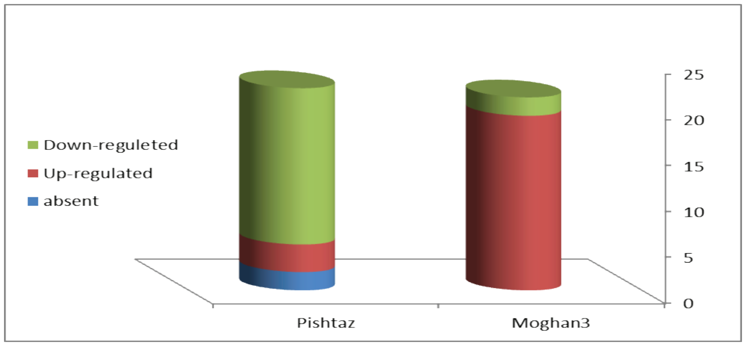



**Figure 3:** Number of protein spots in two cultivars of wheat under salinity stress

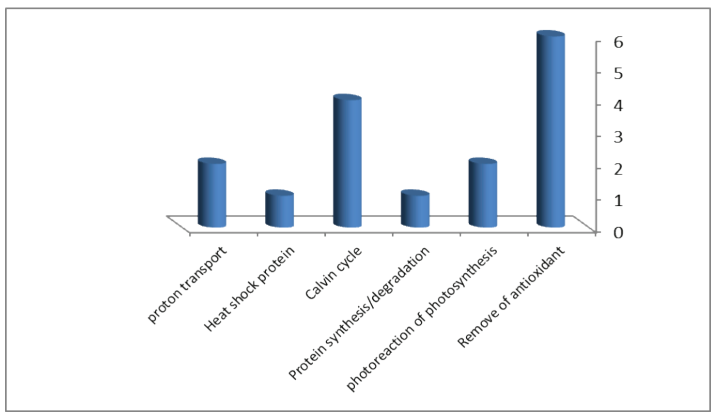



**Figure 4:** Number of common specific proteins in two cultivars of wheat under salinity stress

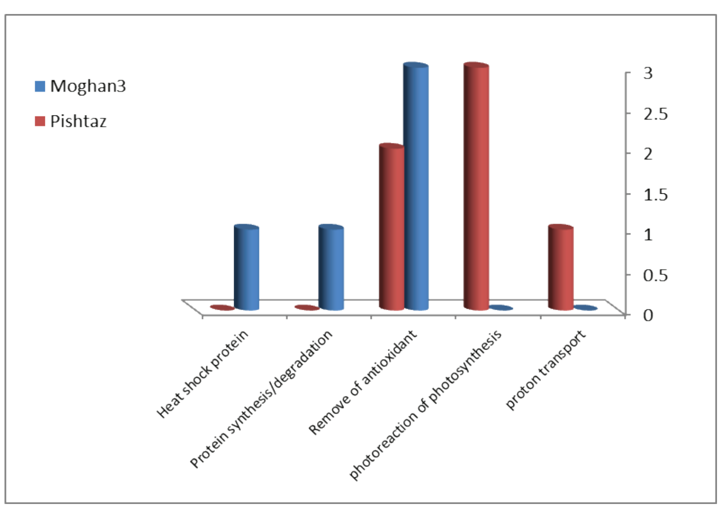



**Figure 5:** Number of uncommon specific proteins in two cultivars of wheat under salinity stress

3.2.3 Classification responsive proteins in two cultivars of wheat

A total 16 common protein spots were identified between tolerant and susceptible cultivars under salinity stress with difference expression (Table 3). According to Figure 4 the majority of these proteins are inside the cell, involved in removal of antioxidants (6 proteins), the Calvin cycle (4 proteins), light reaction of photosynthesis (2 proteins), proton transport (2 proteins), heat shock proteins (1 proteins) and protein involved in protein synthesis/degradation (1 protein), respectively. These results are similar to results reported by Mittler (2002) and Noreen and Ashraf (2008). A correlation between the antioxidant enzyme activities and salinity tolerance was demonstrated by comparison of tolerant cultivar with sensitive cultivar. These activities were ascribed to increased protein expression under salinity stress and are closely related to salt tolerance in many plants (Athar et al., 2008). In addition, five protein spots were unique to the tolerant cultivar (‘Moghan3’) and had more to do with the removal of antioxidants. Due to expression increase of these proteins, this cultivar has stronger cellular detoxification system (Table 4, Figure 5). Six protein spots were seen uniquely in the sensitive cultivar (‘Pishtaz’) wherer the majority of proteins were related to photosynthetic light reaction (Table 4, Figure 5). According to results their decreased expression could be a cause for the reduction in the performance of photosynthesis under salinity stress in susceptible cultivar.

On the whole, the protein expression pattern in control (non-stress) and stress conditions were inserted into 4 groups (state) such as: a - proteins with reduced expression in both susceptible and tolerant cultivars (like protein 7) .b - proteins with increased expression in tolerant and reduce expression in susceptible cultivar (like protein 1), c - proteins present uniquely in the tolerant cultivar (such as A code protein), d - proteins only in the susceptible cultivar (such as H code protein) (Twyman, 2004).

**Defense against oxidative stress**

From total of common proteins 6 of them were related to removal of oxidants. The presence of 2-cys peroxiredoxin BAS1, chloroplastic (spots no. 13, 14 and 16), acidic endochitinase (spots no. 8), Harpin binding protein 1 (HrPB1) (spots No. 10) and Cu/Zn superoxide dismutase (spots No. 11), were induced under salinity stress in both cultivars (Table 3, Figure 1, 2, 4). On the other hands, 3 unique proteins (uncommon proteins) such as HrPB1 (C code), type 2 peroxiredoxin (D code) and Cu/Zn superoxide dismutase (E code) were identified related to remove antioxidant were upregulated in ‘Moghan3’ under salinity stress (Figure 3, Table 4). While, 2 unique proteins such as glutathione S-transferase (H code) and ascorbate peroxidase (I code) were identified related to remove of oxidants were downregulated in ‘Pishtaz’ (Figure 3, Table 4). Superoxide dismutase (SOD) proteins are in fact, the first defense line against ROS, which convert superoxide into hydrogen peroxide which is less toxic. In the absence of sufficient carbon dioxide as the final receptor of electrons, electrons migrate from the photosynthetic membrane to oxygen molecules via the Mehler reaction and generate superoxide ions (Cakmak, 2005). In response to salinity stress, one protein spot named chloroplast Cu-Zn SOD (No. 11 spot) in susceptible cultivar (‘Pishtaz’) showed decreased expression, but besides that in ‘Moghan 3’, protein spot with E code increases expression, as showed in Tables 3, 4. In line with these results, SOD accumulation in rice tolerant cultivar and its reduction in susceptible cultivars have been reported in response to salinity stress (Komatsu and Tanaka, 2004). Lower amount of this protein in stressed leaves of ‘Pishtaz’ caused a high level of hydroxyl radicals in the chloroplasts of this cultivar. In other words, under salinity stress conditions when increased production of ROS takes place, the disturbed balance between their production and elimination, especially in the tolerant genotypes is ameliorated by altering the expression of proteins associated with cellular homeostasis, the cell's balance is reestablished (Sun et al., 2006). On the other hand, peroxiredoxin proteins are expressed extensively in the tissues and are found in mitochondria and cytosols. The cell's location of these proteins, expresses the crucial role of antioxidants in the cellular organells that are the main source of ROS. These proteins, in addition to antioxidant activity, are also active in controlling signal transduction. The N-terminal of this protein, which contains cysteine, is oxidized to sulfenic acid, which acts as a bridge to react with peroxides. In fact, copper/zinc superoxide dismutase enzyme activity has been transformed ROS to $H_2O_2$, and the type 2 peroxiredoxin enzyme recovers $H_2O_2$ molecule, and in many studies, peroxirdoxin protein has been suggested as a protein responding to stress (Hashimoto et al ., 2009). The presence and activation of this protein in the ‘Moghan 3’ (spot No. 13, 14 and 16) shows the role of this protein in tolerance to salinity stress (Figure 1, 2 and Table 3). In general, due to the change of these proteins under salinity stress, reaction of ‘Moghan3’ was better than ‘Pishtaz’ as removal of antioxidant proteins.

**Photosynthesis and carbon metabolism**

Splitting of water by light takes place in the OEC (Oxygen-evolving complex) reaction center of photosystem II (Heide et al., 2004). The subunit of the PSII complex is the protein involved in the photosynthetic water splitting system known as OEC proteins and contributes to the stability of the PSII complex (Ifuku et al., 2008) and disruption of these proteins causes light damage to photosystem II (Takahashi and Murata, 2008). Therefore, due to reduced expression or lack of expression of this protein in the ‘Pishtaz’ (susceptible cultivar) (spot No. 15 and spot code K, Table 3, 4), the activity of photosystem II and eventually the efficiency of photosynthetic light reaction be reduced in this cultivar while this protein (Spot No. 15) showed increased expression (upregulated) in tolerant cultivar (‘Moghan3’). These results indicate that under salinity stress, one of the important components of the photosynthesis machine, namely the oxygen swirling and the photosystem II complex has strongly affected, thus contributing to the aging of the leaves and possibly the gradual death of the cells. Komatsu and Tanaka (2004) made proteome analysis of leaf sheath in rice under salinity stress caused by sodium chloride. The frequency of this group of proteins in response to salinity stress was increased. It indicated the protective role of this protein against of salinity stress. On the other hands, HCF136 protein is a basic protein for repair, construction and stability of photosystem II complex (Plucken et al., 2002). In this experiment was found decreased expression of this protein in the 'Moghan3' (spot No.7) and further decreased expression in the sensitive 'Pishtaz'(Table 3). These results are similar to proteome analysis reported by Ford et al. (2011). In order to avoid light damage to photosynthetic apparatus, several mechanisms including adjusting absorbing antenna to light (LHC proteins) (spot code F in the 'Pishtaz') and reducing the size of antenna to reduce the absorption of light could be carried out (Eberhard et al., 2008). Our results showed that 'Pishtaz' for preventing of further damage to photosystem machine changed the expression in F code protein and these results are similar to results reported by Liu et al. (2014). Also, TL29 (thylakoid lumenal 29.8 kDa protein) (spot code G in the 'Pishtaz' with downregulated under stress) is a 29 kDa protein and is located in the thylakoid lumen (Kieselbach et al., 2000). Based on high homology with ascorbate peroxidase (APX) it was previously called also APX4 and was thought to plays a role in protecting cells against reactive oxygen species (Panchuk et al., 2005). Recently, based on testing Granlund et al. (2009) reported that this protein is associated with photosystem II and involved in prevention of photo damage to the photosystem II. So, according to reduced expression of this protein in susceptible cultivar, it could be the reason for decrease of photosystem II performance under salinity stress. In this regards, Zadraznik et al., (2013) reported that accumulation of these proteins and their isomerases in the tolerant genotypes is higher than in susceptible.

RuBisCO is a key enzyme for fixation of carbon dioxide in photosynthesis. It is formed from several catalyzing large subunits (catalytic large subunits) (spot No. 4) and several regulatory smaller subunits (regulative small subunits) (spot No. 9) (Spreitzer and Salvucci, 2002). In this experiment, both spots of 4 and 9 in 'Maghan3' showed increase expression under stress while these protein spots showed reduced expression in 'Pishtaz' (Table 3). Wan and Liu (2008), Naghavi (2010) and Naghavi (2014), found similar results in leaves of rice, canola and wheat under hydrogen peroxide, osmotic and drought stresses, respectively. Also, Ye et al. (2013) reported that 72 hours after stress imposed by PEG, the increased expression of RuBisCO took place in wheat leaves. This increase helped plants during drought stress with the increase of assimilation and better efficiency of photosynthesis in using of carbon dioxide and enables plants to overcome the stress. On the other hands, Calvin cycle consists of three phases. The third phase of the cycle is regeneration of RuBP molecules and Calvin cycle starts from the beginning. This phase is known by a series of enzymatic reactions in which triose-phosphate is converted to RuBP. Enzymes intermediary or mediatory in this phase include sedoheptulose-1,7-biphosphate and fructose 1,6-biphosphate aldolase (spot No. 12). Together these two enzymes catalyze a reaction that eventually results in the formation of ribulose-5-phosphate. Then ribulose-5-phosphate is phosphorylated and forms RuBP (Tamoi et al., 2005). Thereby reducing the mediator enzyme in this process (spots 12) in susceptible cultivar ('Pishtaz') causes reduction of efficiency of Calvin cycle and reduces sugar production. On the other hand, it is reported that photosynthesis-related proteins such as RuBisCO activaze (spot no. 5) (Table 3) showed decreased expression in susceptible cultivar of barley under stress condition (Kausar et al., 2013).

**Other protein groups**

A total two common spots such as 1 and 2 (with up-regulated in 'Moghan3' and down-regulated in 'Pishtaz') and one spots in the 'Pishtaz' (spot code J with absent expression under stress condition) were detected as different subunits of ATP synthase complex (Tables 3, 4). In previous experiments different subunit components of this complex in canola (Albertin et al., 2009) and leaves of corn (Porubleva et al., 2001) have been identified. Structurally, ATP synthase in chloroplast has two main components which include extrinsic CF1 and CF0.With their help protons are transmitted over the thylakoid membrane. CF1 has five subunits, alpha, beta (common spots No. 1 and 2), gamma, delta (spot code J in the 'Pishtaz') and epsilon while CF0 has three subunits a, b and c (von Ballmoos and Dimroth, 2007). One of these subunits is the β subunit, which is a catalytic and ADP-binding unit.It plays an important role in energy conversion by converting ADP to ATP when there is a proton-slope between the membranes (Ye et al., 2013). Increased expression of ATP synthesis-associated proteins under abiotic stress conditions, including drought and salinity, have been reported in previous studies (Guo et al., 2012).

Heat shock proteins (spot No. 3 and spot code A) (Tables 3 and 4) are molecular chaperones. These proteins help to stabilize and regenerate the proteins that have been opened and decomposed during various stresses. These proteins play a decisive role in plant protection against stress to restore proteins to their original natural form and are involved in the establishment of cell homeostasis (Wang et al., 2004). Toorchi et al. (2009) reported on reduced expression of these proteins in soybean under osmotic stress. While, Naghavi (2010) working on canola tolerant cultivar under drought stress noticed increased expression of these proteins. In other study Naghavi (2014) reported that these proteins showed decreased expression in wheat under the drought stress, in susceptible cultivar a decreased expression was bigger than in tolerant one.

## 4 CONCLUSIONS

According to the results, 198 and 203 protein spots in tolerant and susceptible cultivars were identified, among them 21 and 22 protein spots with significantly different IF were identified, respectively. A total of 16 protein spots were identified common for both, tolerant and susceptible cultivars under salinity stress. The majority of these proteins were involved in removal of oxidants where the highest activity had 2-cys peroxiredoxin BAS1, chloroplast protein and the Calvin cycle proteins (among them were the most common subunits of Rubisco). In addition, five protein spots were present only in the tolerant cultivar. Majority of them were involved in the removal of oxidants. Due to increased expression of these proteins in tolerant cultivar it had better performance of cellular detoxification. Six protein spots were found uniquely in the sensitive cultivar. Majority of these proteins were related to light reactions of photosynthesis. According to the decreased expression of these proteins a reduction of photosynthesis performance under salinity stress appeared in susceptible cultivar.

## 5 ACKNOWLEDGMENTS

The financial support of this experiment was performed by the research grant of Payame Noor University under support of Ministry of Science, Research and Technology's information of Iran. The authors also thank to all of unknown reviewers for their most useful comments on the manuscript.

## 6 REFERENCES

Abdul Kader, M. D. and Lindberg, S. (2010). Cytosolic calcium and pH signaling in plants under salinity stress. *Plant Signaling and Behavior, 5,* 233-238. doi:10.4161/psb.5.3.10740

Albertin, W., Langella, O. Joets, J. Negroni, L. Zivy, M. Damerval C. and Thiellement, H. (2009). Comparative proteomics of leaf, stem, and root tissues of synthetic *Brassica napus*. *Proteomics*, *9,* 793-799. doi:10.1002/pmic.200800479

Athar, H. U. R. Khan, A. and Ashraf, M. (2008). Exogenously applied ascorbic acid alleviates Salt-induced oxidative stress in wheat. *Environmental and Experimental Botany, 63*, 224-231. doi:10.1016/j.envexpbot.2007.10.018

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

Cakmak, I. (2005). The role of potassium in alleviating detrimental effects of abiotic stresses in plants. *Journal of Plant Nutrition and Soil Science, 168,* 521-530. doi:10.1002/jpln.200420485

Eberhard, S., Finazzi, G. and Wollman, F. A. (2008). The dynamics of photosynthesis. *Annual Review of Genetics, 42,* 463-515. doi:10.1146/annurev.genet.42.110807.091452

Fatehi, F., Hosseinzadeh, A. Alizadeh, H. Brimavandi T. and Struik, P. C. (2012). The proteome response of salt-resistant and salt-sensitive barley genotypes to long-term salinity stress. *Molecular Biology Report*s*, 39,* 6387-6397. doi:10.1007/s11033-012-1460-z

Ford, K. L., A. Cassin, and Bacic, A. (2011). Quantitative proteomic analysis of wheat cultivars with differing drought stress tolerance. *Plant Science, 2*(44), 1-11.

Gao, L., Yan, X. Li, X. Guo, G. Hu, Y. Ma W. and Yan, Y. (2011). Proteome analysis of wheat leaf under salt stress by two-dimensional difference gel electrophoresis (2D-DIGE). *Photochemistry, 72*(10), 1180-1191. doi:10.1016/j.phytochem.2010.12.008

Granlund, I., Storm, P. Schubert, M. Garcia-Cerdi, J. G. Funk C. and Wolfgang, P. S. (2009). The TL29 Protein is Lumen Located, Associated with PSII and Not an Ascorbate Peroxidase. *Plant and Cell Physiology, 50*(11), 1898-1910. doi:10.1093/pcp/pcp134

Guo, G., Ge, P. Ma, C. Li, X. Lv, D. Wang, S. Ma W. and Yan, Y. (2012). Comparative proteomic analysis of salt response proteins in seedling roots of two wheat varieties. *Journal of Proteomics, 75*(6), 1867-1885. doi:10.1016/j.jprot.2011.12.032

Hashimoto, M., Toorchi, M. Matsushita, K. Iwasaki Y. and Komatsu, S. (2009). Proteome analysis of rice root plasma membrane and detection of cold stress responsive proteins. *Protein & Peptide Letters, 16,* 685-697. doi:10.2174/092986609788490140

Heide, H., Kalisz H. M. and Follmann, H. (2004). The oxygen evolving enhancer protein 1 (OEE) of photosystem II in green algae exhibits thioredoxin activity. *Journal of Plant Physiology, 161,* 139-149. doi:10.1078/0176-1617-01033

Herbert, B. (1999). Advances in protein solubilisation for two-dimensional electrophoresis. *Electrophoresis, 20*(4-5), 660-663. doi:10.1002/(SICI)1522-2683(19990101)20:4/5<660::AID-ELPS660>3.0.CO;2-Q

Hosseini Salekdeh, Gh., Siopongco, J. Wade, L. J. Ghareyazie B. and Bennett, J. (2002). Proteomics analysis of rice leaves during drought stress and recovery. *Proteomics, 2,* 1131-1145. doi:10.1002/1615-9861(200209)2:9<1131::AID-PROT1131>3.0.CO;2-1

Ifuku, K., Ishihara, S. Shimamoto, S. Ido K. and Sato, F. (2008). Structure, function, and evolution of the PsbP protein family in higher plants. *Photosynthesis Research, 98,* 427-437. doi:10.1007/s11120-008-9359-1

Joseph, B. and Jini, D. (2010). Proteomic analysis of salinity stress-responsive proteins in plants. *Asian Journal of Plant Sciences, 9,* 307-313. doi:10.3923/ajps.2010.307.313

Kausar, R., Arshad. M. Shahzad A. and Komatsu, S. (2013). Proteomics analysis of sensitive and tolerant barley genotypes under drought stress. *Amino Acids, 44,* 345-359. doi:10.1007/s00726-012-1338-3

Kieselbach, T. Bystedt M. and Zentgraf, U. (2000). A peroxidase homologue and novel plastocyanin located by proteomics to the Arabidopsis chloroplast thylakoid lumen. *Febs Letters, 480*(2-3), 271-276. doi:10.1016/S0014-5793(00)01890-1

Komatsu, S. and Tanaka, N. (2004). Rice proteome analysis: A step toward functional analysis of the rice genome. *Proteomics, 4*, 938-949.

Liu, S., Liu, S. Wang, M. Wei, T. Meng, C. Wang, M. and Xia, G. (2014). A wheat SIMILAR TO RCD-ONE gene enhances seedling growth and abiotic stress resistance by modulating redox homeostasis and maintaining genomic integrity. *The Plant Cell, 26,* 164-180. doi:10.1105/tpc.113.118687

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science, 7,* 405-410. doi:10.1016/S1360-1385(02)02312-9

Morant-Manceau, A., Pradier E. and Tremblin, G. (2004). Osmotic adjustment, gas exchanges and chlorophyll fluorescence of a hexaploid triticale and its parental species salt stress. *Journal of Plant Physiology, 169,* 25-33. doi:10.1078/0176-1617-00963

Naghavi, M. R. (2014). *Evaluation of spring wheat cultivars under drought stress and proteome analysis for the most tolerant and sensitive ones*. PhD Thesis in Plant Breeding (Biometrical Genetics). Department of Plant Breeding and Biotechnology. Faculty of Agriculture. University of Tabriz, Iran. (In Persian).

Naghavi, M. R. (2010). *Response and 2-Dimensional electrophoresis pattern of spring rapeseed genotypes under osmotic stress*. Master Science Dissertation in Plant Breeding. Department of Agronomy and Plant Breeding. Faculty of Agriculture, University of Tabriz, Iran. (In Persian).

Noreen, S. and Ashraf, M. (2008). Alleviation of adverse effects of salt stress on sunflower (*Helianthus annus* L.) by exogenous application of salicylic acid: Growth and photosynthesis. *Pakistan Journal of Botany, 40,* 1657-1663.

Panchuk, I. I., Zentgraf U. and Volkov, R. A. (2005). Expression of the Apx gene family during leaf senescence of *Arabidopsis thaliana*. *Planta, 222*(5), 926-932. doi:10.1007/s00425-005-0028-8

Plucken, H., Muller, B. Grohmann, D. Westhoff P. and Eichacker, L. A. (2002). The HCF136 proteinis essential for assembly of the photosystem II reaction center in *Arabidopsis thaliana*. *FEBS Letters, 532,* 85-90. doi:10.1016/S0014-5793(02)03634-7

Porubleva, L., Vander Velden, K. Kothari, S. Oliver D. J. and Chitnis, P. R. (2001). The proteome of maize leaves: use of gene sequences and expressed sequence tag data for identification of proteins with peptide mass fingerprints. *Electrophoresis, 22,* 1724-1738. doi:10.1002/1522-

2683(200105)22:9<1724::AID-ELPS1724>3.0.CO;2-2

Saqib, M., Zorb C. and Schubert, S. (2006). Salt-resistant and salt-sensitive wheat genotypes show similar biochemical reaction at protein level in the first phase of salt stress. *Journal of Plant Nutrition and Soil Science, 169,* 542-548. doi:10.1002/jpln.200520557

Song, X. Ni, Z. Yao, Y. Xie, C. Li, Z. Wu, H. Zhang, Y. and Sun, Q. (2007). Wheat (*Triticum aestivum* L.) root proteome and differentially expressed root proteins between hybrid and parents. *Proteomics, 7*(19), 3538-3557. doi:10.1002/pmic.200700147

Spreitzer, R. J. and Salvucci, M. E. (2002). Rubisco: structure, regulatory interactions, and possibilities for a better enzyme. *Annual Review of Plant Biology, 53,* 449-475. doi:10.1146/annurev.arplant.53.100301.135233

Sun, Y., Ahokas, R. A. Bhattacharya, S. K. Gerling, I. C. Carbone L. D. and Weber, K. T. (2006). Oxidative stress in aldosteronism. *Cardiovascular Research, 71,* 300-309. doi:10.1016/j.cardiores.2006.03.007

Takahashi, S. and Murata, N. (2008). How do environmental stresses accelerate photo inhibition? *Trends in Plant Science, 13,* 178-182. doi:10.1016/j.tplants.2008.01.005

Tamoi, M., Nagaoka, M. Yabuta Y. and Shigeoka, S. (2005). Carbon metabolism in the Calvin cycle. *Plant Biotechnology, 22,* 355-360. doi:10.5511/plantbiotechnology.22.355

Thiellement, H., Zivy M. and Plomion, C. (2002). Combining proteomic and genetic studies in plants. *Chromatography B, 782,* 137-149. doi:10.1016/S1570-0232(02)00553-6

Toorchi, M., Yukawa, K. Nouri M. Z. and Komatsu, S. (2009). Proteomics approach for identifying osmotic-stress-related proteins in soybeans roots. *Peptides, 30,* 2108-2117. doi:10.1016/j.peptides.2009.09.006

Tuteja, N. (2007). Mechanisms of high salinity tolerance in plants. *Methods in Enzymology, 428,* 419-438. doi:10.1016/S0076-6879(07)28024-3

Twyman, R. M. (2004). Principles of proteomics. BIOS Scientific Publishers. doi:10.4324/9780203507391

von Ballmoos, C. and Dimroth, P. (2007). Two distinct proton binding sites in the ATP synthase family. *Biochemistr*y, *46,* 11800-11809. doi:10.1021/bi701083v

Wan, X. Y. and Liu, J. Y. (2008). Comparative proteomics analysis reveals an intimate protein network provoked by hydrogen peroxide stress in rice seedling leaves. *Molecular & Cellular Proteomics, 7,* 1469-1488. doi:10.1074/mcp.M700488-MCP200

Wang, W., Vinocur, B. Soseyov O. and Altman, A. (2004). Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. *Trends in Plant Science, 9,* 244-52. doi:10.1016/j.tplants.2004.03.006

Xue, G. P., Mcintyre, C. L. Glassop D. and Shorter, R. (2008). Use of expression analysis to dissect alterations in carbohydrate metabolism in wheat leaves during drought stress. *Plant Molecular Biology, 67,* 197-214. doi:10.1007/s11103-008-9311-y

Ye, J., Wang, S. Zhang, F. Xie D. and Yao, Y. (2013). Proteomic analysis of leaves of different wheat genotypes subjected to $PEG_{6000}$ stress and rewatering. *Plant Omics Journal, 6*(4), 286-294.

Zadraznik, T. Hollung, K. Egge-Jacobsen, W. Meglic, V. and Sustar-Vozlic, J. (2013). Differential proteomic analysis of drought stress response in leaves of common bean (*Phaseolus vulgaris* L.). *Journal of Proteome, 78,* 254-272. doi:10.1016/j.jprot.2012.09.021

Zhu, M., Simons, B. Zhu, N. David, G. Oppenheimer M. and Chen, S. (2010). Analysis of abscisic acid responsive proteins in *Brassica napus* guard cells by multiplexed isobaric tagging. *Journal of Proteomics, 73,* 790-805. doi:10.1016/j.jprot.2009.11.002

doi:10.14720/aas.2018.111.3.04 Original research article / izvirni znanstveni članek

# Risk and risk management strategies of smallholder onion farmers in Sokoto state, Nigeria

Tohib Oyeyode OBALOLA[1*] and Opeyemi Eyitayo AYINDE[2]

Received November 23, 2017; accepted October 08, 2018.
Delo je prispelo 23. novembra 2017, sprejeto 08. oktobra 2018.

**ABSTRACT**

The study examines risk and its management strategies among smallholder onion farmers in Sokoto State. Data were collected with the use of structured questionnaire designed to pull together information on the socioeconomic characteristics of the farmers in the area such as age, level of education, experience, family size, membership of farmer association, extension contact, risk preference of the farmers etc. Data was also collected on risk sources and risk management strategies. The primary data used were obtained from structured questionnaire administered to 120 randomly selected farmers. The analytical techniques that were used in the analysis of data were descriptive statistical tools such as means and percentages, Equally Likely Certainty Equivalent with a Purely Hypothetical Risky prospect (ELCEPH) technique and the 5-point Likert scale. The result showed that majority of the farmers are risk averse having a positive Arrow-Pratt absolute risk aversion coefficient.

**Key words:** risk; risk management; onion; smallholder farmers; strategies; ELCEPH

**IZVLEČEK**

**TVEGANJA IN STRATEGIJE UPRAVLJANJA S TVEGANJI MAJHNIH PRIDELOVALCEV ČEBULE V DRŽAVI SOKOTO, NIGERIJA**

Raziskava preučuje tveganja in strategije upravljanja s tveganji majhnih pridelovalcev čebule v državi Sokoto, v Nigeriji. Podatki so bili zbrani z vprašalnikom, ki je bil zasnovan tako, da je zbral podatke o socioekonomskih lastnostih kmetov na območju kot so starost, raven izobrazbe, izkušenost, velikost družin, članstvo v kmečkih združenjih, povezava s svetovalno službo, prednostna tveganja kmetov, itd. Podatki so bili izbrani tudi glede na vire tveganja in strategije upravljanja z njimi. Primarni podatki so bili pridobljeni z vprašalnikom, ki ga je izpolnilo 120 naključno izbranih kmetov. Pri obdelavi podatkov so bila uporabljena orodja opisne statistike kot so poprečja in odstotki. Uporabljena sta bila ekvivalent enako verjetne gotovosti in tehnika popolnega hipotetičnega predvidevanja tveganja (ELCEPH) in pettočkovna Likertova skala. Rezultati so pokazali, da se večina kmetov izogiba tveganju, saj imajo pozitiven Arrow-Prattov koeficient absolutnega odklanjanja tveganj.

**Ključne besede:** tveganje; upravljanje s tveganji; čebula; majhni pridelovalci; strategije; ELCEPH

## 1 INTRODUCTION

Agricultural production is highly characterized by risks, which range from adverse weather, pests to diseases, which in turn lead to price uncertainty (Ayinde et al., 2008). For these reasons, farmers' attitude towards risk is imperative in understanding their behavior towards the adoption of new technology and managerial decisions. For example, the more risk-averse a farmer is, the more likely the farmer is to make managerial decisions that emphasize the goal of reducing variation in income, rather than the goal of maximizing income; the converse is also true (Binici et al., 2003).

Production, which is considered as risky investment activity, takes place under either a perfect or an imperfect knowledge situation. A perfect knowledge occurs when the cause (action) and results are known

[1] Department of Agricultural Economics, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto, Nigeria; *Corresponding author: oyeyodeobalola@yahoo.com

[2] Department of Agricultural Economics and Farm Management, University of Ilorin, P.M.B. 1515, Ilorin, Kwara State, Nigeria

with certainty. Most economic analyses assume a perfect knowledge which is more theoretical than real. An imperfect knowledge situation occurs when the decision-maker (farmer) is not very sure of the result(s) of the action to be undertaken. A situation of imperfect knowledge is more common in agricultural enterprises than non-agricultural enterprises. However, there are two variants of imperfect knowledge situations. One of them is a situation of uncertainty, in which either all the possible outcomes of an event/action or the probabilities associated with each outcome or both are not known. The other is a situation of risk, which occurs when all possible outcomes for a given management decision (action) and the probability associated with each possible outcome are known (Kay, 1981).

In Nigeria, onion is produced through commercial as well as smallholder farmers both as a source of income and food. However, due to perishable nature and biological nature of production process, onion productions are risky investment activities. The behavior of farmers under risk has been studied using two approaches. The expected utility model (EUM) which is an extension of the consumer behavior theory in which consumer behave like they have a utility function and make choices that maximize it. The second approach, been a situation in which risk is defined as the likelihood that income will fall below a predetermined disaster level thus, giving rise to the safety first models (SFM). Riskiness of onion production may be atributed to several factors that are beyond the control of the farmers. Sokoto state is endowed with resources for onion production but smallholder onion farmers in the state are faced with many risks in their farming activities. In the past, the state has recorded flood, drought, crop and animal diseases and pests as well as fluctuations in prices of both farm produce (outputs) and inputs. As a result, there has been variability in farmer's household income. The lack of clear understanding of farmers' attitudes towards risks remains an important factor inhibiting increased agricultural productivity. It is not in any way difficult to find out that the observed resource use of the farmers reveals their underlying degrees of risk preferences (Olarinde et al., 2008).

Researches on risk analysis in Sokoto State of Nigeria are relatively scanty. However, there is no real evidence to prove the expectations of the behavior of farmers in the production environment. There is a need to have a better understanding of the risk and the coping strategies among onion farmers in order to ascertain the decision-making behaviors of the farmers, to develop appropriate risk-coping strategies for the farmers, and to add to the existing knowledge in the field of agricultural risk in the study area. These are key issues central to this study and which investigation can be useful for the formulation of policies to strengthen and improve the farmers' productivity.

## 2 MATERIAL AND METHODS

### 2.1 Study area

Sokoto state is situated in the north-western part of Nigeria, close to Sokoto and Rima rivers confluence. It is situated between Latitudes 10°40' and 13°55' N and longitudes 3°30' and 7°06' E (Singh, 2000). It is one of the hottest region in the world. The maximum daytime temperature generally is under 40 $^0$C (104.0 $^0$F). The state falls within the semi-arid region where rainfall range from (400 – 700 mm per annum) which is erratic and poorly distributed (Singh, 1995). The main source of livelihood of the dwellers is farming and the crops cultivated include both food and cash crops such as millet, sorghum, rice, groundnut, cotton, cowpea, cassava and sweet potatoes. In addition, vegetable crops like onion, tomato, as well as sweet and hot peppers are grown during dry season under irrigation.

### 2.2 Sampling procedure

A multi-stage sampling technique was used to select 120 farmers. In the first stage, two local government areas were purposively selected. The reason for the purposive selection was on the preponderance of smallscale onion farmers in these LGAs. The second stage involved a random selection of two villages from each LGA. In the third stage, there was a random selection of respondents each from the LGA and this form the sample size for the study. Since the population of the LGAs is not homogeneous, the number of farmers selected from each of the selected LGAs was calculated using the formula:

$$P = \frac{S}{N} \text{ x n} \quad (1)$$

Where, P = Proportion, S = Desired sample size, N = Total population, n = Population of the villages in LGA in question. The LGAs and the number of respondents are shown in Table 1 below.

**Table 1**: The local government areas and the number of farmers.

| Local Government Area | Village | Sample |
|---|---|---|
| Wamakko | Kwalkwalawa | 23 |
| | Kalambaina | 26 |
| Kware | RuggarLiman | 40 |
| | More | 31 |
| Total | | 120 |

Source: Authors computation

### 2.3 Analytical technique

Descriptive statistical tools such as means, percentages etc., Equally Likely Certainty Equavilent with a Purely Hypothetical technique and the 5-point Likert scale type were used.

#### 2.3.1 ELCE-PH

This process begins by assigning the expected utility (EU) at two end point outcomes. Considering a low income of N 50, 000 and a high income of N 100, 000. This was followed by assigning utility value at each end point (low and high income) such that:
U (50,000) = 0
U (100,000) = 1, respectively for the low and high income end point outcome.

The researcher then asked the farmers how much they would be willing to take i.e. its certainty equivalent (CE) for a gamble paying of N50, 000 and N100, 000 with equal probability of 0.5 each. The CE was then used for utility function elicitation. The figures resulting from the elicitation sequence was then fit using the quadratic utility specification to yield:

$$U(Y) = a + bY + cY^2 \qquad (2)$$

Where Y represents the unknown, and a, b, and c represent known numbers such that: ‘c’ is not equal to 0. If c = 0, then the equation is linear and not quadratic.

The coefficients gotten from the fitted equation were used to estimate absolute risk-aversion coefficient. The coefficient was computed using equation below.

$$r_a = - \frac{U''(Y)}{U'(Y)} \qquad (3)$$

Where $r_a$= coefficient of absolute risk aversion;
U’’ = second differential of the function;
U’ = first differential of the function

The Arrow-pratt coefficient is positive if the individual is averse to risk, zero in the case of an individual that is indifferent to risk, and negative if the individual prefers to take risk (Korir, 2011).

## 3 RESULTS AND DISCUSSION

### 3.1 Socioeconomic characteristics of the farmers

The results (Table 2) show that 21.7 % of the farmers are within the age group of 20 – 29 years, while 26.7 % of them fall within the age group 30 – 39 years old. 40.8 % and 10.8 % are observed for the 40 – 49 and 50 years above, respectively. The indication is that, most of the farmers are still very young, agriculturally active and energetic and the implication is their likeliness to have prospects for improvement upon their efficiency in onion production by better harnessing available production resources. Majority of the onion farmers (64.2 %) are married. The unmarried farmers constitute the minority (35.8 %). The implication of this is that those with children are assumed to have cheap agricultural family labour which will aid in the timely accomplishment of farm operations and in turn increases output at reduced rate.

Education provides a base of understanding changes within agriculture, which may improve welfare and as such education is essential in any activity. The level of education determines the quality of skills of farmer, his allocative abilities and shows how informed they are of the new innovations and technology around him. In Table 2, majority of the farmer (63.3 %) had no formal education. Farmers with completed primary education constitute 17.5 %. Secondary education is achieved by 19.2 % of the farmers. The outcome is not a surprising one as the area falls within educationally deprived state of Nigeria. It corroborate with the finding of Tsoho and Salau (2012). Experience in farming is an essential factor affecting the farmer’s level of production. Experienced farmers are able to combine factors of production (land, labour and capital) better to maximize output. However, 41.7 % of the farmers sampled have been into onion farming for between 1 – 10 years. Also, 42.5 % and 15.8 % of the farmers were within 11 – 20 and 21-30 years respectively. The experience years will significantly increase the farmers' attitude towards decision making.

A household usually comprise of the man, his wife, children and other dependents if any. Majority of farmers (50.0 %) have 3-10 persons in the household. Another reasonable percentage (42.5 %) had 18 and above household member. All the above representations may be found important as it reduces the costs of production likely to be incurred by farmers with fewer household members. The polygamous nature as well as the family pattern of the area probably will explain the large family size recorded in the area. It is against the findings of Okoruwa, et al. (2009) which showed that 64.4 % of the farmers had less than 6 family members while 35.6 % had 6 and above. Also majority of the farmers (68.3 %) have no extension visit in the last cropping season. However, it was revealed that 21.7 % of the farmers have an extension visit of between 1 – 2 times, with 10.0 % between 3 – 4 times in the last growing season. It corroborates with the finding of Ojo et al. (2009) who reported that 60.9 % of the farmers do not have extension contact.

Table 2 also shows the responses of the onion farmers as regards to their level of income obtained from onion production. It was observed that 40.0 % of the farmers are of income level between N51, 000.00 - N 150, 000.00. Another 35.0 % indicates farmers that fall between N 151,000 – N 250,000.

The size of the farm is concerned with the land size. Land is a very important factor of production alongside with labour, capital and management. It is a true statement to say that without land, there is no agriculture. The size of the farm is vital to a farmer and the production of output. In view of this importance, questions are set about their farm sizes, since the size of the farm to some degrees determine the input to be used and responses shows that 54.2 % farmers have farm sizes between 0.7 – 1.1 hectare. Only a few of them have about 1.7 hectare and above. However, conclusion can be inferred that the farmers are smallholder onion farmers that limit their production on small hectares of land due to one reason or the other. It is in contrary to the work of Tsoho and Salau (2012), whose analysis although revealed that farm size ranged from 0.13 to 1.7 ha with the mean of 0.5 ha.

**Table 2:** Socioeconomic characteristics of the farmers

| Parameter | Option | Frequency | Percentage |
|---|---|---|---|
| Age (years) | 20-29 | 26 | 21.7 |
| | 30-39 | 32 | 26.7 |
| | 40-49 | 49 | 40.8 |
| | 50 and ABOVE | 13 | 10.8 |
| Marital status | Single | 43 | 35.8 |
| | Married | 77 | 64.2 |
| Level of education (years) | No formal education | 76 | 63.3 |
| | Primary education | 21 | 17.5 |
| | Secondary education | 23 | 19.2 |
| Years of experience | 1-10 | 50 | 41.7 |
| | 11-20 | 51 | 42.5 |
| | 21-30 | 19 | 15.8 |
| Household size (no of persons) | 3-10 | 60 | 50.0 |
| | 11-17 | 51 | 42.5 |
| | 18 and ABOVE | 9 | 7.5 |
| Extension contacts (no of times) | 1-2 | 26 | 21.7 |
| | 3-4 | 12 | 10.0 |
| | No extension contact | 82 | 68.3 |
| Membership of cooperative | Yes | 54 | 45.0 |
| | No | 66 | 55.0 |
| Annual income (naira) | 51,000 – 150,000 | 48 | 40.0 |
| | 151,000 – 250,000 | 42 | 35.0 |
| | 251,000 – 350,000 | 26 | 21.7 |
| | 351,000 – 450,000 | 4 | 3.3 |
| Farm size (hectares) | 0.2-0.6 | 28 | 23.3 |
| | 0.7-1.1 | 65 | 54.2 |
| | 1.2-1.6 | 23 | 19.2 |
| | 1.7 and ABOVE | 4 | 3.3 |

Source: Field Survey, 2016

### 3.4 Risk attitude of the farmers

Following the procedure outlined in the methodology; the farmers risk aversion coefficient were estimated and presented in Table 3 and were subsequently grouped into risk averters and risk takers and as such presented in Table 4.

**Table 3**: Absolute risk aversion coefficient of the farmers

| Farmer number | Absolute risk aversion coefficient | Farmer number | Absolute risk aversion coefficient | Farmer number | Absolute risk aversion coefficient | Farmer number | Absolute risk aversion coefficient |
|---|---|---|---|---|---|---|---|
| 1 | 0.000004954 | 31 | 0.000009770 | 61 | 0.00001091 | 91 | 0.000003688 |
| 2 | 0.000006130 | 32 | 0.000009770 | 62 | 0.00001055 | 92 | -0.000001245 |
| 3 | 0.000002640 | 33 | -0.000009590 | 63 | 0.00001112 | 93 | 0.0000009171 |
| 4 | -0.00003615 | 34 | -0.000009590 | 64 | 0.000009770 | 94 | 0.00001014 |
| 5 | -0.00005389 | 35 | -0.00002409 | 65 | -.000009590 | 95 | -0.000001124 |
| 6 | 0.00001558 | 36 | 0.0000009171 | 66 | 0.000001608 | 96 | 0.00001608 |
| 7 | 0.00001561 | 37 | 0.000004746 | 67 | -0.000001245 | 97 | 0.0000009171 |
| 8 | 0.00001166 | 38 | 0.0000009171 | 68 | 0.00005554 | 98 | 0.00001608 |
| 9 | 0.00000117 | 39 | -0.000009590 | 69 | 0.000009770 | 99 | -0.000009590 |
| 10 | 0.000006327 | 40 | 0.0000009171 | 70 | -0.000009590 | 100 | 0.00005240 |
| 11 | 0.000003184 | 41 | -0.000009590 | 71 | 0.00001608 | 101 | 0.00001608 |
| 12 | 0.000003184 | 42 | -0.000009590 | 72 | 0.000009769 | 102 | 0.00001073 |
| 13 | 0.00005956 | 43 | 0.0001198 | 73 | 0.00001608 | 103 | 0.000009769 |
| 14 | 0.00001668 | 44 | -0.000009590 | 74 | -0.000009590 | 104 | 0.00001608 |
| 15 | 0.00001668 | 45 | 0.000004813 | 75 | 0.00005554 | 105 | 0.000009769 |
| 16 | 0.000002770 | 46 | 0.0000007041 | 76 | 0.000009715 | 106 | 0.000009769 |
| 17 | 0.000003379 | 47 | 0.000004813 | 77 | 0.0000336 | 107 | -0.000009590 |
| 18 | 0.000002770 | 48 | 0.0000009171 | 78 | -0.000009590 | 108 | -0.000009525 |
| 19 | 0.000003184 | 49 | -0.000009590 | 79 | 0.00001604 | 109 | 0.0000009171 |
| 20 | 0.00004254 | 50 | 0.000009770 | 80 | 0.00005554 | 110 | -0.000009575 |
| 21 | 0.00001069 | 51 | 0.000002234 | 81 | 0.000009769 | 111 | -0.000009590 |
| 22 | 0.000007467 | 52 | 0.00001041 | 82 | 0.000009769 | 112 | 0.000009229 |
| 23 | 0.000003891 | 53 | 0.000005042 | 83 | 0.000009769 | 113 | 0.000009769 |
| 24 | 0.0001 | 54 | 0.0000009171 | 84 | 0.000009769 | 114 | -0.000009590 |
| 25 | 0.0001485 | 55 | 0.00001075 | 85 | -0.000009590 | 115 | 0.0000009171 |
| 26 | 0.0001485 | 56 | 0.0000009171 | 86 | 0.000009769 | 116 | -0.000009590 |
| 27 | 0.0001 | 57 | 0.000008466 | 87 | 0.00001704 | 117 | 0.000009769 |
| 28 | 0.00001113 | 58 | -0.00002514 | 88 | 0.000009769 | 118 | 0.000009769 |
| 29 | 0.00005680 | 59 | -0.000009590 | 89 | 0.00001608 | 119 | -0.000009575 |
| 30 | -0.000001245 | 60 | -0.000009590 | 90 | 0.00001608 | 120 | 0.000009731 |

Source: Authors Computation, 2016

**Table 4:** Distribution of the risk attitude of the farmers

| Risk attitude | Frequency | Percentage |
|---|---|---|
| Risk averse | 90 | 75.0 |
| Risk neutral | 0 | 0.00 |
| Risk loving | 30 | 25.0 |
| Total | 120 | 100.0 |

Source: Field Survey, 2016

Tables 4 revealed that 75.0 % of the farmers in the study area have positive Arrow-Pratt absolute risk aversion coefficients and were therefore categorized as risk averters. The remaining 25.0 % of them have negative Arrow-Pratt absolute risk aversion coefficients and were grouped as risk seekers. However, none of the farmers has zero risk coefficients; an indication of risk indifference, hence none of the farmers was risk indifferent or neutral. The result of the study is a confirmation of the general assumption in the world of agriculture that farmers are risk averse and it is in line with empirical results of various studies (Sekar and Ramasamy, 2001; Korir, 2011).

### 3.3 Sources of risk

The unpredictability nature of the outcome of production with certainty is believed to emanate from several sources and as such this study help looked into the various sources of risk and it is presented in Table 5.

**Table 5:** Risk sources associated to the farmers in the study area

| Source of risk | VI | I | NS | NI | NVI | WS | MS | MPS | RANK |
|---|---|---|---|---|---|---|---|---|---|
| Pests | 74 (61.7) | 46 (38.3) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 554 | 4.62 | 92.34 | 1st |
| Diseases | 73 (60.8) | 45 (37.5) | 2 (1.7) | 0 (0.00) | 0 (0.00) | 551 | 4.59 | 91.82 | 2nd |
| Price fluctuation | 44 (36.7) | 72 (60.0) | 3 (2.5) | 1 (0.8) | 0 (0.00) | 519 | 4.33 | 86.52 | 3rd |
| Flood | 36 (30.0) | 83 (69.2) | 1 (0.8) | 0 (0.00) | 0 (0.00) | 515 | 4.29 | 85.84 | 4th |
| Drought | 48 (40.0) | 60 (50.0) | 11 (9.2) | 1 (0.8) | 0 (0.00) | 515 | 4.29 | 85.84 | 4th |
| Change in climate condition | 34 (28.3) | 84 (70.0) | 2 (1.7) | 0 (0.00) | 0 (0.00) | 512 | 4.27 | 85.32 | 6th |
| Fertilizer | 37 (30.8) | 77 (64.2) | 6 (5.0) | 0 (0.00) | 0 (0.00) | 511 | 4.26 | 85.16 | 7th |
| Erratic rainfall | 23 (19.2) | 83 (69.2) | 11 (9.2) | 3 (2.5) | 0 (0.00) | 486 | 4.05 | 81.08 | 8th |
| Illness of household member | 65 (54.2) | 17 (14.2) | 12 (10.0) | 22 (18.3) | 4 (3.3) | 477 | 3.98 | 79.54 | 9th |
| Excessive rainfall | 18 (15.0) | 80 (66.7) | 19 (15.8) | 3 (2.5) | 0 (0.00) | 473 | 3.94 | 78.84 | 10th |
| Market failure | 25 (20.8) | 54 (45.0) | 37 (30.8) | 4 (3.3) | 0 (0.00) | 460 | 3.83 | 76.60 | 11th |
| Insufficient family labour | 22 (18.3) | 25 (20.8) | 16 (13.3) | 40 (33.3) | 17 (14.2) | 409 | 3.41 | 68.08 | 12th |
| Change in govt. & agricultural policy | 27 (22.5) | 19 (15.8) | 3 (2.5) | 48 (40.0) | 23 (19.2) | 339 | 2.83 | 56.48 | 13th |
| Difficulties of finding labour | 6 (5.0) | 26 (21.7) | 20 (16.7) | 51 (42.5) | 17 (14.2) | 313 | 2.61 | 52.22 | 14th |
| Fire outbreak | 13 (10.8) | 13 10.8 | 21 (17.5) | 44 (36.5) | 29 (24.2) | 297 | 2.48 | 49.38 | 15th |
| Theft | 10 (8.3) | 18 (15.0) | 14 (11.7) | 18 (15.0) | 60 (50.0) | 260 | 2.17 | 43.32 | 16th |

VI = Very important; I = Important; NS = Not sure; NI = Not important; NVI = Not very important; WS = Weighted score; MS = Mean score; MPS = Mean percent score. Figures in parenthesis are in percentages;Source: Field Survey, 2016

Pests and diseases were recorded as the most important source of risk to the farmers as they were ranked first and second respectively. This corroborates with the findings of Obalola et al. (2017).

However, an insight into the price movement during the irrigation season indicates that the prices of onion fluctuate widely and as such is an important source of risk. It is generally the highest at the beginning of the season but falls rapidly until it reaches its lowest values at the peak of harvest period and the farmers are forced to sell their produce at low prices after which the prices begins to rise again.

Table 5 also reveals that drought, flood and change in climatic condition are important sources of risk to the farmers as it was ranked 4th and 6th respectively. This is in line with the findings of Ayinde et al. (2008) who reflected production risk in terms of weather to variation in yield of the crops over years and crop failures due to bad weather (drought or too much rain). Difficulty in finding labour was not seen as a bottleneck in their production and as such could pose little or no threat to the farmers. This was proven by a 42.5 % response who considers difficulties in finding labour not important and it was ranked 14th. It was observed that the respondents do not consider theft as a factor as it was recorded that 50.0 % of them indicated it as not very important.

### 3.4 Coping strategies

The strategies that can help in coping or minimizing the source of risk faced by the farmers in the study area are captured and presented in Table 6.

**Table 6:** Risk management strategies

| Risk management strategies | VI | I | NS | NI | NVI | WS | MS | MPS | RANK |
|---|---|---|---|---|---|---|---|---|---|
| Investing off-farm | 84 (70.0) | 34 (28.3) | 2 (1.7) | 0 (0.00) | 0 (0.00) | 562 | 4.68 | 93.66 | 1st |
| Spraying for diseases & pests | 75 (62.5) | 44 (36.7) | 1 (0.8) | 0 (0.00) | 0 (0.00) | 554 | 4.62 | 92.34 | 2nd |
| Adashe (Cash contribution) | 69 (57.5) | 51 (42.5) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 549 | 4.58 | 91.50 | 3rd |
| Gathering market information | 49 (40.8) | 68 (56.7) | 3 (2.5) | 0 (0.00) | 0 (0.00) | 526 | 4.38 | 87.66 | 4th |
| Training & education | 48 (40.0) | 70 (58.3) | 2 (1.7) | 0 (0.00) | 0 (0.00) | 526 | 4.38 | 87.66 | 4th |
| Borrowing | 44 (36.7) | 45 (37.5) | 20 (16.7) | 9 (7.5) | 2 (1.7) | 480 | 4.00 | 80.06 | 6th |
| Cooperative societies | 39 (32.5) | 31 (25.8) | 32 (26.7) | 18 (15.0) | 0 (0.00) | 451 | 3.76 | 75.16 | 7th |
| Selling of assets | 12 (10.0) | 12 (10.0) | 31 (25.8) | 55 (45.8) | 10 (8.3) | 331 | 2.76 | 53.46 | 8th |

VI = Very important; I = Important; NS = Not sure; NI = Not important; NVI = Not very important; WS = Weighted score; MS = Mean score; MPS = Mean percent score. Figures in parenthesis are in percentages

Source: Field Survey, 2016

Investing off-farm was ranked as first as a very important strategy in managing risk. Ayinde et al. (2008) have shown the importance of diversification (investment in more than one portfolio) as important risk management strategies for agricultural enterprises.

Spraying for diseases and pests was ranked second. This is not surprising considering the fact that pest and diseases were identified as a very important source of risk. Therefore, spraying for pests as well as diseases could help manage the riskiness attributed to it and as such help improve farmer's production and at the same time their productivity. This is in conformity with the finding of Obalola et al. (2017) who revealed that incidence of pests and diseases are the major problem limiting farmers output.

In addition, cash contribution was ranked third as management strategy to help manage risk in the study area. Training and education was recorded as an important factor that helps to minimize risk. This was proven by 58.3 % of the farmers highlighting it as important and as such ranked 4th. If the farmers are educated and trained, it could go a long way in helping improve the awareness level of the farmers with regards to a better perception of themselves and their problems. It is important to note that most of the farmers use more than one coping strategy in the face of risks.

Other risk management strategies recorded in increasing order of importance are borrowing (37.5 %) and cooperative societies (32.5 %). It was however revealed that selling of assets is not an important factor in risk management as 45.8 % of the farmers attest to it and thus, ranked 9th.

## 4 CONCLUSION

The majority of onion farmers were found to be risk averse. However, it should be noted that most of the sources of risk highlighted by the farmers could be analyzed within the context of the farmers operational level and can be managed by the farmers, if motivated in one way or another either by training and education, diversification of the enterprise (off-farm investment), crop insurance, spraying for pests and diseases etc.

The study therefore recommends programmes towards education and diversification. The farmers were found to be risk averse implying that they were not fully insured by their self-insurance strategies. In order to improve this, policies that enhance access to insuring farm activities should be put in place.

This can however be achieved by improving and intensifying extension services to impact technical and economic knowledge on farmers especially the farmers with few years of experience.

## 5 REFERENCES

Ayinde, O.A., Omotesho, A.O., & Adewunmi, M.O. (2008). Risk attitudes and management strategies of small-scale crop producers in Kwara State, Nigeria: A ranking approach. *African Journal of Business Management, 2*(12), 217-221.

Binici, T., Koc, A.A., Zulauf, C.R., & Bayaner, A. (2003). Risk attitudes of farmers in terms of risk aversion: A case study of lower seyhan plain farmers in adana province, Turkey. *Turkish Journal of Agriculture, 27*, 305-312.

Kay, R.D. (1981). *Farm management, planning, control and implementation*: 334-353. McGraw Hill Book Company.

Korir, L.K. (2011). *Risk Management among Agricultural Households and the Role of Off-Farm Investments in Uasin Gishu County, Kenya*. Thesis submitted to the Graduate School in Partial fulfillment for the Requirements of Master of science Degree in Agricultural and Applied Economics of Egerton University: 1-74

Obalola, T. O., Agboola, B. O., & Odum, E. B. E. (2017). Profitability and Constraints to Irrigated Onion Production in Wamakko and Kware Local Government Areas, Sokoto State, Nigeria. *Journal of Global Agriculture and Ecology, 7*(3), 106-110.

Ojo, M.A., Muhammed, U.S., Adeniji, B., & Ojo, A.O. (2009). Profitability and Technical Efficiency in irrigated onion production under middle rima valley irrigation project in Goronyo, Sokoto State. *Nigeria. Journal of Agricultural Science, 3,* 7-14.

Okoruwa, V.O., Akindeinde, A.O., & Salimonu, K.K. (2009). Relative economic efficiency of farms in rice production: A profit function Approach in North Central Nigeria Tropical and Sub-tropical. *Agroecosystem, 10*, 279-286.

Olarinde, L.O., Manyong, V.M., & Okoruwa, V.O. (2008). Analysing optimum and alternative farm plans for risk averse grain crop farmers in Kaduna State, Northern Nigeria. *World Journal of Agricultural Sciences, 4*(1), 28-35.

Sekar, I., & Ramasamy, C. (2001). Risk and resource analysis of rainfed tanks in South India. *Journal of Social and Economic Development, 3*(2), 208-215.

Singh, B. R. (1995). Soil management strategies for the semi-arid ecosystem in Nigeria: The case of Sokoto and Kebbi States. *African Soils, 28*, 317-320.

Singh, B. R. (2000). Quality of irrigation water in *fadama* lands of north-western Nigeria II. Tube-well waters in Zamfara State. *Nigeria Journal of Basic and Applied Sciences, 9*, 191-193.

Tsoho, B.A., & Salau, S.A. (2012). Profitability and Constraints to dry season Vegetable production under fadama in sudan savannah ecological zone of Sokoto State, Nigeria. *Journal of Development and Agricultural Economics, 4*(7), 214-222.

doi:10.14720/aas.2018.111.3.05 Original research article / izvirni znanstveni članek

# Responses of young cucumber plants to a diurnal temperature drop at different times of day and night

Tatjana G. SHIBAEVA[1*], Elena G. SHERUDILO[1], Elena N. IKKONEN[1], Alexander F. TITOV[1]

Received January 24, 2018; accepted October 25, 2018.
Delo je prispelo 24. januarja 2018, sprejeto 25. oktobra 2018.

**ABSTRACT**

In greenhouse production of a number of vegetable and ornamental plant species, a short diurnal temperature drop in the end of the night or in the morning is commonly used to reduce stem elongation as an alternative to chemical growth retardants. Experiments were carried out to quantify the effects of a temperature drop at different times of the day and night on growth and photosynthetic activity of young cucumber plants. During 6 days plants were exposed daily to a temperature of 10 °C for 2 h at the beginning, in the middle and at the end of the night and day periods. The results have shown that plant response to drop may be qualitatively different in the light and darkness. While strongest effects of drop are observed when it is given in the daytime, for practical application in greenhouses it is more appropriate to reduce temperature at night. However, it may not be strictly necessary for cucumber seedlings to apply drop at the end of the night as it was stated in the literature. Thus, our results may cast doubt on the following statements: (a) temperature drops are not effective when delivered at other times of the day or night (except before sunrise), (b) optimal time for drop effects depends on the daily dynamics of stem and petiole elongation rate. It is rather drop itself is capable of modifying the dynamics of plant growth in the daily cycle.

**Key words:** *Cucumis sativus* L.; growth; daily rhythms

**IZVLEČEK**

**ODZIV MLADIH KUMAR NA DIURNALNI UPAD TEMPERATURE V RAZLIČNIH OBDOBJIH DNEVA IN NOČI**

Pri pridelavi različne zelenjave in okrasnih rastlin v rastlinjakih se pogosto uporablja kratkotrajno znižanje temperature proti koncu noči ali proti jutru za zmanjšanje podaljševanja stebla kot alternativa kemijskim rastnim zaviralcem. Izvedeni so bili poskusi za ovrednotenje učinkov znižanja temperature na rast in fotosintetsko aktivnost mladih kumar v različnih obdobjih dneva in noči. V obdobju šestih dni so bile rastline izpostavljene temperaturi 10 °C za 2 h na začetku, v sredini in ob koncu noči, ter podnevi. Rezultati so pokazali, da je odziv na znižanje temperature kvalitativno različen v svetlobi in v temi. Čeprav je bil največji učinek znižanja zabeležen ob tretmanu podnevi, je iz praktičnih razlogov za uporabo v rastlinjakih primernejše znižanje ponoči. Kljub temu, pa pri sadikah kumar ni nujno izvajati znižanje proti koncu noči kot navaja literatura. Na osnovi naših rezulatov lahko dvomimo o naslednjih trditvah: (a) znižanja temperature so neučinkovita, če so izvedena v drugih obdobjih dneva ali noči, razen pred sončnim vzhodom, (b) optimalni čas za učinke znižanja je odvisen od dnevne dinamike dolžinske rasti stebla in listnih pecljev. Obratno, že samo znižanje temperature je sposobno spremeniti dinamiko rasti rastlin v dnevnem ciklu.

**Ključne besede:** *Cucumis sativus* L.; rast; dnevni ritmi

## 1 INTRODUCTION

Plant growth retardants are widely used as tools for height control in order to obtain more compact plants. However, the use of growth retardants on vegetables is prohibited in many countries because of the potential danger of chemical residues to the environment and the consumer. At the same time, it has long been known that stem elongation can be controlled by temperature treatments rather than by the use of chemical growth retardants (Moe & Heins, 1990). In particular, a daily temperature drop or dip for 2-4 h is the practice of lowering the temperature in order to obtain compact transplants of vegetable crops, bedding plants and ornamentals (Myster & Moe, 1995; Berghage, 1998; Runcle, 2009). It is believed that the time during 24-h

[1] Institute of Biology, Karelian Resarch Center, Russian academy of Sciences; Petrozavodsk, Karelia, Russia; [*]Corresponding author: shibaeva@krc.karelia.ru

period when the temperature decreases, has a certain effect on the degree of stem elongation inhibition (Myster & Moe, 1995; Grindal & Moe, 1995; Grimstad, 1995). In the earlier studies on *Lilium longiflorium* Wiebe, *Begonia x hiemalis* Fortsch and *Solanum lycopersicum* L., it was shown that the temperature drop effect is the greatest in the first period of the day (Myster & Moe, 1995). Later, it was found that the temperature drop in the last part of the night is even more effective in reducing plant height and internode length in *Begonia x chiemantha* Everett (Moe & Mortensen, 1992; Grindal & Moe, 1994, 1995; Bakken & Moe, 1995), *Euphorbia pulcherrima* Willd. (Moe et al., 1992), tomato and cucumber (Grimstad, 1995). This turned out to be very convenient for practical application of this technique as the lowering temperature in the end of the night is far more energy efficient than at the start of the day, especially if the lights are switched on in the morning. The required decrease in temperature can be obtained by turning off heaters for a short time. If the greenhouse does not cool rapidly enough, venting may be necessary to achieve the desired low-temperature setpoint. It is generally believed that temperature drops are not effective when delivered at other times of the day or night (except before sunrise) (Runcle, 2009). However, the information about the effects of a temperature drop given at other times of the day or night (except for the end of the night and the beginning of the day) is extremely scarce. There is evidence that a temperature drop in the middle of the night had small effect on plant height and petiole length in *Euphorbia pulcherrima* (Moe et al., 1992) and *Begonia x hiemalis* (Moe & Mortensen, 1992; Grindal & Moe, 1994). In our previous studies we have shown that a temperature drop at the beginning, middle or end of the night was equally effective in reducing plant height and petiole length in cucumber (Sysoeva et al., 1997, 1999). It is also suggested that a temperature drop at the beginning of the day is much more effective than later during the day in *Euphorbia pulcherrima* (Ueber & Hendriks, 1992) and *Pelargonium* (Ueber & Hendriks, 1995), and that a temperature decrease at any time during photoperiod may be equally effective in inhibiting stem elongation as a temperature decrease at the beginning of the day in *Fuchsia, Antirrhinum majus* L.*, Petunia* and *Salvia splendens* F. (Erwin & Heins, 1995).

Data on the effects of a temperature drop at different times of the day on plant characteristics other than stem elongation rate are even more fragmentary and contradictory. It was shown that plant dry mass was decreased by low temperature pulse treatment in cucumber and tomato and the largest effect was achieved at the end of the night in cucumber, but at the beginning of the day in tomato (Grimstad, 1995). Begonia plants affected by a temperature drop at the end of the night or in the morning had smaller dry mass compared to the control plants, but a temperature drop at the beginning of the night increased plant dry mass. Moreover, a temperature drop given in the night increased leaf weigh ratio (LWR), while a temperature drop given in the morning did not affect LWR (Bakken & Moe, 1995). There are also data on the effect of a temperature drop on the chlorophyll content. In experiments with *Ocimum basilicum* L., there was a tendency that plants treated by a temperature drop at the beginning and in the end of the day had lower concentrations of chlorophyll *a* and *b*, but in the cold-resistant species *Viola* x *wittrockiana* Gams., a temperature drop in the early morning increased total chlorophyll content (Vågen et al., 2003).

The objective of the present experiment was to quantify the effects of a temperature drop at different times of the day and night on growth and photosynthetic activity of young cucumber plants.

## 2 MATERIALS AND METHODS

Experimental facilities for this study were offered by the Shared Equipment Center of the Institute of Biology, Karelian Research Center, Russian Academy of Sciences. Imbibed seeds of cucumber (*Cucumis sativus* 'Zozulya F1') were sown in 7 cm pots containing sand and seedlings were watered daily with a complete nutrient solution (based on 1 g $l^{-1}$ $Ca(NO_3)_2$, 0.25 g $l^{-1}$ $KH_2PO_4$, 0.25 g $l^{-1}$ $MgSO_4$ $7H_2O$, 0.25 g $l^{-1}$ $KNO_3$, trace quantity of $FeSO_4$ and pH 6.2-6.4, EC 2.0 mS $cm^{-1}$). Plants were grown under air temperature 23 °C, air humidity 70 %, a photoperiod of 12 h (from 9:00 to 21:00), under controlled environmental conditions (*Vötsch* growth chamber, Germany) with a photosynthetic photon flux density of 200 µmol $m^{-2}$ $s^{-1}$.

Starting from the 7th day from seed soaking, different groups of plants were exposed daily for 6 days to a temperature of 10 °C for 2 hours at the beginning (21:00-23:00, N1), in the middle (2:00-4:00, N2) and at the end of the night period (7:00-9:00, N3), as well as at the beginning (9:00-11:00, D1), in the middle (14:00-16:00, D2) and at the end of the day period (19:00-21:00, D3). Control plants were not treated by a temperature drop (DROP). At the end of DROP treatments all plants were grown under air temperature of 23 °C. All measurements were carried out on the next day after the last DROP treatment (on the 14th day). Plant height, petiole length of the first true leaf, leaf area ($n \geq 10$), dry mass of leaves, stems and roots ($n = 5$) were recorded. The total chlorophyll content was determined non-destructively by using a SPAD 502 Plus chlorophyll meter (Konica Minolta, Japan) ($n \geq 10$). The chlorophyll fluorescence was measured using a portable chlorophyll fluorometer MINI-PAM (Walz, Germany) on the 1st true leaf. The maximal quantum yield of PSII photochemistry was calculated as $F_v/F_m = (F_m - F_0)/F_m$ after 20 min of dark adaptation of leaves ($n = 5$) (Maxwell & Johnson, 2000).

All results are presented as means ± SE ($n \geq 5$). Data were tested for normality and homogeneity of variance using Chi-Square test and Levene's test in Statistica (v.8.0.550.0, StatSoft, Inc). Differences between the treatment means were tested with one-way ANOVA followed the Bonferroni test with $P < 0.05$ level of significance. Two similar trials were run.

## 3 RESULTS

The obtained results showed that in general DROP treatments reduced the plant height by 14-16 %, compared with the control (Fig. a). The leaf petiole length in all plants treated by DROP was also less than in the control (Fig. b). The greatest retardation of the leaf petiole growth was noted when DROP had been given in the middle and at the end of the day (by 46-47 %) (treatments D2 and D3), while the smallest inhibition (by 20 %) occurred when it had been given in the middle and at the end of the night (N2 and N3).

The leaf area was reduced by all DROP treatments compared with the control. In case of DROP given in the daytime the reduction was by 47-49 %, but in the night – by 25-29 % (Fig. c). There were no significant differences between leaf area of plants treated by DROP at the beginning, in the middle or at the end of the day as well as between treatments at different times in the night. The similar effect was observed for the plant dry mass, which was decreased by 51-53 % compared with the control by DROP given in the daytime and by 21-30 % by DROP given in the night (Fig. d). However, no effect of DROP on biomass allocation to leaves, stems and root (data not shown) was observed.

The chlorophyll content in the leaves of plants exposed to DROP-treatments did not differ from the control, except for the plants treated by DROP at the beginning of the day (D1), when the chlorophyll content was reduced by 23 % (Fig. e).

The values of $F_v/F_m$ in leaves treated by DROP in the night time were not significantly different from the control ones, but DROP given in the daytime lowered the values of $F_v/F_m$ (Fig. f).

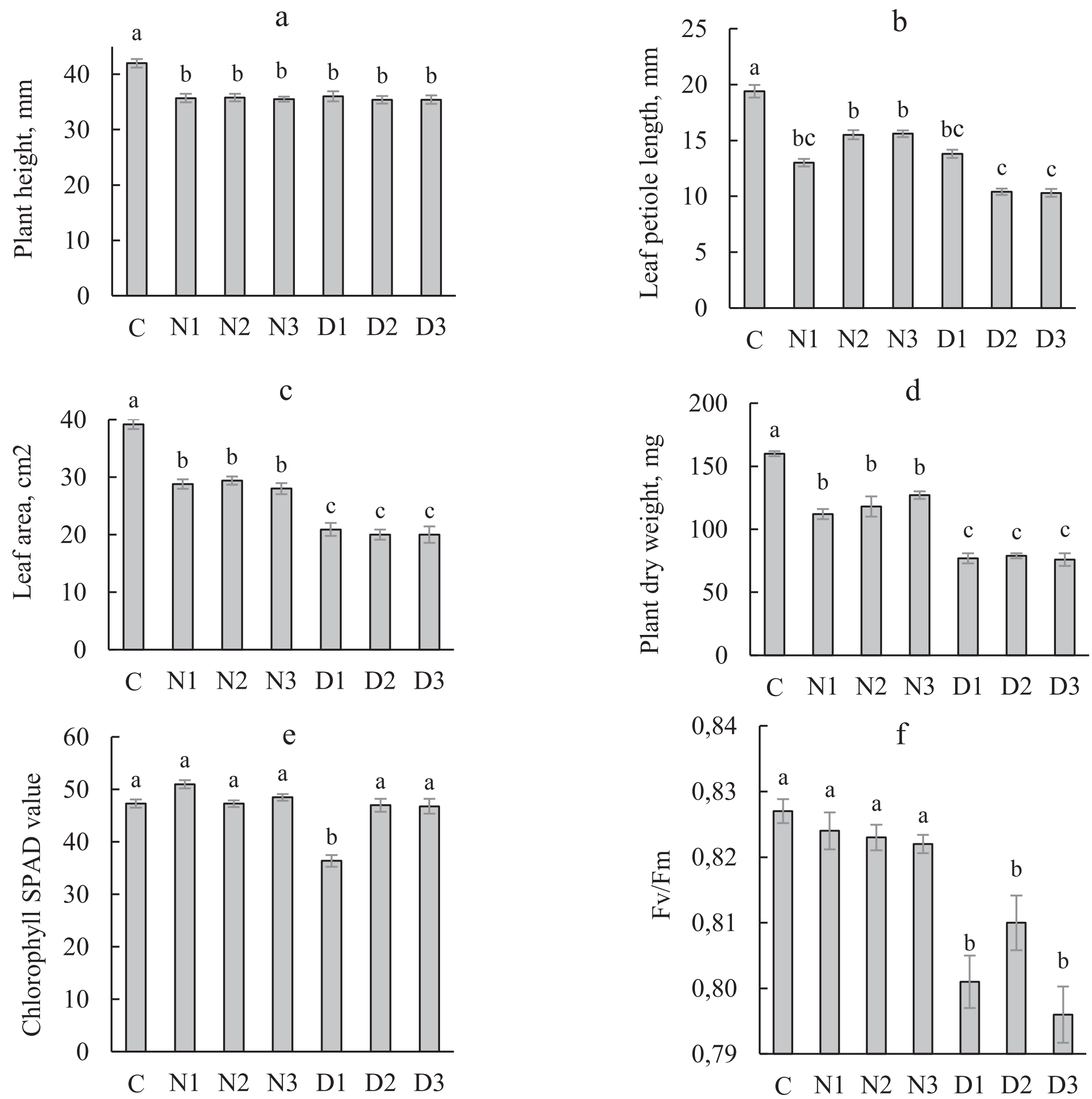



**Figure 1**: Effect of a temperature drop (DROP) given at the beginning (N1), in the middle (N2), in the end (N3) of the night and in the beginning (D1), in the middle (D2) or in the end (D3) of the day

## 4 DISCUSSION

The results of this work showed that the DROP treatments applied at any time of the day or night results in growth retardation (reduced plant height, leaf petiole length) of cucumber plants, which unequivocally indicates that there are no periods in the daily cycle when DROP treatments are absolutely ineffective for obtaining compact plants. The strongest morphogenetic responses to DROP treatments were observed when DROP had been given in the middle or at the end of the day, which from the practical point of view is of little use. DROP given at the end of the night or at the beginning of the day similarly reduced stem and petiole elongation, as it was earlier reported by Grimstad (1995) for young cucumber plants. Unlike in case with *Euphorbia pulcherrima* (Moe et al., 1992) and *Begonia x hiemalis* (Moe & Mortensen, 1992; Grindal & Moe, 1994), when DROP given in the middle of the night had only slight effect on plant height and leaf petiole length, in our work with cucumber plants there were no significant differences revealed between plant morphogenetic response to DROP given at different times during the night period. The same results we reported earlier (Sysoeva et al., 1997, 1999). Already in the early studies on the potential of fluctuating temperatures as alternative to growth retardants it was pointed out that plant responses are very much crop dependent (Cuijpers & Vogelezang, 1992). Therefore, it is not surprising that the results obtained in experiments with different species may differ significantly.

Pronounced morphogenetic effect of DROP given in the daytime also indicates that although it is known that stem elongation is not constant during a 24-h period and that the major stem extension occurs during the night (Lecharny et al., 1985; Sweeny, 1987; Erwin & Heins, 1988; Bertram & Karlsen, 1994; Tutty et al., 1994; Luna-Maldonado et al., 2017), however, the optimal time for DROP treatments in order to obtain compact plants may not coincide with the periods of the greatest stem elongation rate. This has been also shown in several experiments with tomato (Gertsson, 1992; Grimstad, 1995). There are data that an increase in a temperature drop duration from 2 to 4 h (Grimstad, 1995; Sysoeva et al., 2008) or from 1.5 to 3 h (Mortensen & Moe, 1992) decreased or did not change the effect of DROP on stem length. These data are also formally in contradiction with the opinion that the optimal time for DROP effects depends on the daily dynamics of stem and petiole elongation rate. In this case it would seem logical to expect stronger DROP effects. Explanations for this fact have not yet been found, but there are some interesting results worth taking into consideration. In experiments with *Chenopodium rubrum* L., DROP stimulated stem elongation within 10 h after the termination of DROP treatment (Lecharny et al., 1985). In experiments with *Dendranthema grandiflorum* (Ramat.) Kitam., short-term temperature changes did not rephase the rhythm of stem growth, but significantly affected the amplitude for the remainder of the diurnal cycle (Tutty et al., 1994). These results let us to suggest that the DROP itself is capable of modifying the dynamics of plant growth in the daily cycle. Perhaps, sometimes this may be the reason of mismatch between the results obtained and those expected.

Our results demonstrate significant decrease in the leaf area of plants treated by DROP in the daytime (by 47-49 %) and in the night (by 25-29 %). Previously it was reported that DROP does not affect leaf length or width in cucumber, tomato (Grimstad, 1995), poinsettia (Moe et al., 1992). We believe that the reason for this disagreement may be related to the fact that in our experiments the temperature during DROP treatments was lowered to 10 °C, but in the experiments carried by Grimstad (1995) and Moe et al. (1992) the setpoint for low temperature was 12 ° and 13 °C, correspondingly. It is well established that at the temperature critical for chilling injury (10 °C for species of tropical and subtropical origin) cellular membranes in sensitive plants undergo a physical-phase transition from a normal flexible liquid-crystalline to a solid gel structure and other changes occur in cells that lead to numerous physiological dysfunctions (Theocharis et al., 2012).

Differences in plant response to DROP in the daytime or in the night, recorded in our experiments, can partly be explained by the involvement of a mechanism of the DROP effect on gibberellins (GA) metabolism, which is different in light and dark. It was shown in pea that DROP in the light increases expression of GA-deactivation gene *PsGA2ox2* (Stavang et al., 2007). By contrast, DROP in darkness does not affect steady-state expression of this gene, but instead slightly stimulate the GA-biosynthesis genes *PsGA20ox1, PsGA3ox1,* and *NA*. Therefore, plant responses to DROP in light and darkness can differ not only quantitatively, but also qualitatively. The fact that DROP given in the dark also reduces petiole length and leaf area (although to a lesser extent) indicates that this also involves other, not GA-dependent mechanisms. The reduced leaf area in DROP-treated plants can be considered as very important in practical terms, since undesirable elongation of stems when growing seedlings of vegetable or ornamental plants occurs mainly at the time when the leaves of neighboring plants begin to overlap and shade each other, leading to competition for light in dense monoculture crops.

Data on the plant dry mass also indicate a significant difference between plants treated by DROP in the light and in the dark (DROP given in the daytime decreased the plant dry mass the most) and no differences related to the timing of DROP within the dark or light periods. Earlier, we found that DROP in the light period results in greater decrease in the apparent quantum yield of photosynthesis in cucumber plants than DROP in the dark (Ikkonen et al., 2016). At the same time, DROP in the light reduces not only light use efficiency, but also the rate of photosynthesis, which does not occur in plants treated by DROP in darkness. It is to presume that a greater decrease of biomass in plants treated by DROP in the light is associated with temporal photoinhibition, which is likely to occur, as indicated by lower values of the intrinsic photochemical efficiency ($F_v/F_m$).

In our experiments DROP had no effect on the chlorophyll content, except for its decrease in the leaves of plants treated by DROP at the beginning of the day, which was also observed in the experiments with *Ocimum basilicum* (Vågen et al., 2003). The loss of chlorophyll in the leaves chilled in the light, as opposite to the leaves chilled in the dark, may occur as a result of the degradation of PSII and PSI and during photoinhibition (Hetherington et al., 1989). PSI, which had been believed to be tolerant to environmental stresses, was shown to be photoinhibited when chilling-sensitive plants were exposed to a chilling temperature under moderate light (Kudoh, Sonoike, 2002; Gururani et al., 2015). Why this does not happen when the leaves are short-term chilled in the middle or at the end of the day, remains unclear so far. It is worth noting that we observed very quick (within 24 h under optimal temperature conditions) increase in chlorophyll content to the control level in cucumber leaves after the cessation of DROP treatments (12 °C) (Shibaeva et al., 2018).

The results of our experiments with cucumber plants treated by short-term temperature drop at different times of the day and night have shown that light conditions during chilling treatment (light or darkness) rather than diurnal variability in growth rate are responsible for plant response to short-term temperature changes. Plant response to a temperature drop may be not only quantitatively, but also qualitatively different in light and darkness. While the strongest effects of a temperature drop are observed when it is given in the daytime, for practical application in greenhouses it is more appropriate to reduce temperature at night. However, it may not be strictly necessary for cucumber seedlings to apply a temperature drop at the end of the night, as it was stated earlier. In fact, the same effect may be obtained by a temperature drop given at any time within the night period as the timing of a temperature drop within the dark or light periods has little effect on plant response. It might be of use in plant factories, where the interaction with the exterior climate is minimized (Graamans et al., 2018). Thus, our results may cast doubt on the following statements: (a) temperature drops are not effective when delivered at other times of the day or night (except before sunrise), (b) optimal time for a temperature drop effects depends on the daily dynamics of stem and petiole elongation rate. It is rather DROP itself is capable of modifying the dynamics of plant growth in the daily cycle.

## 5 ACKNOWLEDGEMENTS

The reported study was partially supported by Russian Foundation for Basic Research, research project № 14-04-00840a and Russian Federal Agency for Scientific Organizations (№ 0221-2017-0051).

## 6 REFERENCES

Bakken A.K. & Moe, R. (1995). Height and quality control in Christmas begonia by growth-retarding temperature regimes. *Acta Agriculturae Scandinavica, Section B – Soil and Plant Science, 45,* 283-291.

Berghage, R. (1998). Controlling height with temperature. *HortTechnology, 8*(4).

Bertram, L. & Karlsen, P. (1994) Patterns in stem elongation rate in chrysanthemum and tomato plants in relation to irradiance and day/night temperature. *Scientia Horticulturae, 58,* 139-150. doi:10.1016/0304-4238(94)90134-1

Cuijpers, L.H.M. & Vogelezang, J.V.M. (1992). DIF and temperature drop for short-day pot plants. *Acta Horticulturae, 327,* 25-32. doi:10.17660/ActaHortic.1992.327.3

Erwin, J.E., Heins, R.D. (1988). Effect of diurnal temperature fluctuations on stem elongation circadian rhythms. *HortScience, 23,* 164.

Erwin, J.E. & Heins, R.D. (1995). Thermomorphogenic responses in stem and leaf development. *HortScience, 30,* 940-949.

Gertsson, U. (1992). Influence of temperature on shoot elongation in young tomato plants. *Acta Horticulturae, 327,* 71-76. doi:10.17660/ActaHortic.1992.327.8

Grimstad, S.O. (1995). Low-temperature pulse affects growth and development of young cucumber and tomato plants. *Journal of Horticultural Science, 70,* 75-80. doi:10.1080/14620316.1995.11515275

Grindal, G. & Moe, R. (1994). Effects of temperature-drop and a short dark interruption on stem elongation and flowering in *Begonia x hiemalis* Fotsch. *Sci Hortic, 57*, 123-132. doi:10.1016/0304-4238(94)90040-X

Grindal, G. & Moe, R. (1995). Growth rhythm and temperature DROP. *Acta Horticulturae, 378*, 47-52. doi:10.17660/ActaHortic.1995.378.6

Graamans, L., Baeza, E., Dobbelsteen, A.D., Tsafaras, I., & Stanghellini, C. (2018). Plant factories versus greenhouses: Comparison of resource use efficiency. *Agricultural Systems, 160,* 31-43. doi:10.1016/j.agsy.2017.11.003

Gururani, M.A., Venkatesh, J & Tran L.-S.P (2015) Regulation of photosynthesis during abiotic stress-induced photoinhibition. *Molecular Plant, 8,* 1304-1320. doi:10.1016/j.molp.2015.05.005

Hetherington, S.E., He, J. & Smillie, R.M. (1989). Photoinhibition at low temperature in chilling-sensitive and -resistant plants. *Plant Physiology, 90,* 1609-1615. doi:10.1104/pp.90.4.1609

Ikkonen, E.N., Shibaeva, T.G., Sherudilo, E.G. & Titov, A.F. (2016). Effect of a temperature drop on the apparent quantum yield of photosynthesis in cucumber plants. *Transactions of Karelian Research Centre of the Russian Academy of Sciences. Experimental Biology Series, 6*, 49-55.

Kudoh, H. & Sonoike, K. (2002) Irreversible damage to photosystem I by chilling in the light: cause of the degradation of chlorophyll after returning to normal growth temperature. *Planta, 215,* 541-548. doi:10.1007/s00425-002-0790-9

Lecharny, A., Schwall, M. & Wagner, E. (1985). Stem extension rate in light-grown plants. *Plant Physiology, 79*, 625-629. doi:10.1104/pp.79.3.625

Luna-Maldonado, A.I., Vidales-Contreras, J.A. & Rodrigues-Fuentes, H. (2017) *Advances and trends in development of plant factories.* Frontiers Media SA. doi:10.3389/978-2-88945-139-5

Maxwell, K. & Johnson, G.N. (2000). Chlorophyll fluorescence – a practical guide. *Journal of Experimental Botany, 51,* 659-668. doi:10.1093/jexbot/51.345.659

Moe, R. & Heins, R.D. (1990). Control of plant morphogenesis and flowering by light quality and temperature. *Acta Horticulturae, 272,* 81-90. doi:10.17660/ActaHortic.1990.272.11

Moe, R., Gromsrud, N., Bratberg, I. & Valsø, S. (1992). Control of plant height in poinsettia by temperature drop and graphical tracking. *Acta Horticulturae, 327,* 41-48. doi:10.17660/ActaHortic.1992.327.5

Moe, R. & Mortensen, L.M. (1992). Thermomorphogenesis in pot plants. *Acta Horticulturae, 305,* 19-25. doi:10.17660/ActaHortic.1992.305.2

Mortensen, L.M. & Moe, R. (1992). Effects of various day and night temperature treatments on the morphogenesis and growth of some greenhouse and bedding plant species. *Acta Horticulturae, 327,* 77-86. doi:10.17660/ActaHortic.1992.327.9

Myster, J. & Moe, R. (1995). Effect of diurnal temperature alternation on plant morphology in some greenhouse crops – a mini review. *Scientia Horticulturae, 62,* 205-215. doi:10.1016/0304-4238(95)00783-P

Runcle, E. (2009). Controlling height with temperature drops. *Greenhouse Product News, 4,* 50.

Shibaeva, T.G., Sherudilo, E.G. & Titov, A.F. (2018) Cucumber plant response to constant long-term and daily short-term low temperature treatments. *Russian Journal of Plant Physiology, 2*(65), 68-77.

Stavang, J.A., Junttila, O., Moe, R. & Olsen, J.E. (2007). Differential temperature regulation of GA metabolism in light and darkness in pea. *Journal of Experimental Botany, 58*, 3061-3069. doi:10.1093/jxb/erm163

Sysoeva, M., Markovskaya. E. & Kharkina, T. (1977). Optimal temperature drop for the growth and development of young cucumber plants. *Plant Growth Regulation, 23,* 135-139. doi:10.1023/A:1005834702680

Sysoeva, M., Markovskaya, E., Kharkina, T. & Sherudilo, E. (1999). Temperature drop, dry matter accumulation and cold resistance of young cucumber plants. *Plant Growth Regulation, 28,* 89-94. doi:10.1023/A:1006243230411

Sysoeva, M.I., Grindal Patil, G., Sherudilo, E.G., Torre, S., Markovskaya, E.F. & Moe, R. (2008). Effect of temperature drop and photoperiod on cold resistance in young cucumber plants – involvement of phytochrome B. *Plant Stress, 2*, 84-88.

Sweeny, B.M. (1987). *Rhythmic phenomena in plants*. Academic Press, San Diego, USA.

Theocharis, A., Clement, Ch. & Barka, E.A. (2012). Physiological and molecular changes in plants grown at low temperatures. *Planta, 235,* 1091-1105. doi:10.1007/s00425-012-1641-y

Tutty, J.R., Hicklenton, P.R., Kristie, D.N. & McRae, K.B. (1994). The influence of photoperiod and temperature on the kinetics of stem elongation in *Dendranthema grandiflorum. Journal of the American Society for Horticultural Science, 119,* 138-143.

Ueber, E. & Hendriks, L. (1992). Effects of intensity, duration and timing of a temperature drop on the growth and flowering of *Euphorbia pulcherrima* Willd. ex Klotzsch. *Acta Horticulturae, 327,* 33-40. doi:10.17660/ActaHortic.1992.327.4

Ueber, E. & Hendriks, L. (1995). Intensity effects of a temperature drop on pelargoniums. *Acta Horticulturae, 378,* 34. doi:10.17660/ActaHortic.1995.378.3

Vågen, I.M., Moe, R. & Ronglan, E. (2003). Diurnal temperature alternations (DIF/drop) affect chlorophyll content and chlorophyll *a*/chlorophyll *b* ratio in *Melissa officinalis* L. and *Ocimum basilicum* L., but not in *Viola x wittrockiana* Gams. *Scientia Horticulturae, 97,* 153-162. doi:10.1016/S0304-4238(02)00141-3

doi:10.14720/aas.2018.111.3.06 Original research article / izvirni znanstveni članek

# Fluctuations of aphid populations on grapefruit (*Citrus x paradisi* Macfad.)

Salim LEBBAL[1,2]

Received February 02, 2018; accepted October 30, 2018.
Delo je prispelo 02. februarja 2018, sprejeto 30. oktobra 2018.

**ABSTRACT**

Very few studies were carried out to investigate the aphids attacking grapefruit. These pests cause considerable damages on citrus trees and other crops. This paper reports on the fluctuations of aphids on grapefruit in the region of Skikda (Algeria). From January 2012 to December 2013, monthly surveys were performed to measure the abundance of aphids recorded on 16 leaves of grapefruit. Through this study, five aphid species were identified, among them *Aphis spiraecola* Patch, 1914 was the most numerous. Besides, we noticed that the populations of aphids reached high levels many times within the year. However, the most important densities were recorded in spring and autumn.

**Key words:** *Aphis spiraecola*; *Aphis gossypii;* inter-annual variations of populations; intra-annual variations; population dynamics

**IZVLEČEK**

**FLUKTUACIJE POPULACIJ LISTNIH UŠI NA GRENIVKI (*Citrus x paradisi* Macfad.)**

Zelo malo raziskav je bilo narejeno na listnih ušeh, ki napadajo grenivko. Ti škodljivci povzročajo znantne poškodbe na citrusih in drugih kulturah. Pripevek poroča o nihanju pojavljanja listnih uši na grenivki v območju Skikda (Alžirija). Od januarja 2012 do decembra 2013 so bili opravljeni mesečni pregledi za ovrednotenje pogostosti listnih uši, na osnovi ocene pojavljanja na 16 listih grenivke. V raziskavi je bilo najdenih pet vrst listnih uši, med katerimi je bila vrsta *Aphis spiraecola* Patch, 1914 najštevilčnejša. Opaženo je bilo, da so bile populacije listnih uši številčne večkrat v letu, vendar so bile najpomembnejše gostote zabeležene spomladi in v jeseni.

**Ključne besede:** *Aphis spiraecola; Aphis gossypii;* medletna spremenljivost populacij; letna variabilnost; populacijska dinamika

## 1 INTRODUCTION

Citrus fruits represent one of the most important fruit productions worldwide, with 109 million tonnes produced annually in the world (Maserti et al., 2011). In the Mediterranean region, the citrus fruits play a very important role in the nutrition, human health, food processing industry and economic incomes (Biche, 2012). The genus *Citrus* includes several species of economic importance such as grapefruits (*Citrus x paradisi* Macfad.) (Hanke & Flachowsky, 2010), which constitutes the only major citrus varieties having a level of processed utilization comparable to oranges (Lacirignola & D'Onghia, 2009). It is the largest citrus fruit grown commercially in many countries (Skaria, 2004). The production of grapefruit was estimated at about 8,550100 tonnes in 2015, including 2300 tonnes in Algeria (FAO, 2017). Besides, grapefruit or grapefruit juice is often recommended as a healthy dietary constituent, particularly in some weight reducing diets (Xiao & Hu, 2014). Furthermore, other authors reported many healthy benefits of grapefruit (Xu et al., 2007; Yin et al., 2012). In addition, its zest is exploited in the production of pectin and essential oils (Kimball, 1999).

Several pests and diseases may attack grapefruit and reduce its yield. Among these plant enemies, aphids have a big importance. They comprise about 4000 described species, most of which are found only in temperate regions (Dixon, 1987). They cause direct (sap-feeding, deformation of their hosts) and indirect damage (transmission of plant diseases, deposition of honeydew on the leaves) (Cœur d'acier et al., 2010). For

[1] Department of Agronomy, Faculty of Natural and Life Sciences, Abbas Laghrour University, Khenchela, Algeria; *Corresponding author: salim-leb@hotmail.com

[2] Department of Agronomy, Hadj Lakhdar University, Batna, Algeria

instance, *Citrus tristeza virus* (CTV) which is considered to be the most destructive virus of citrus crops (Rehman et al., 2016), is present in most of the countries in the Mediterranean region and is transmitted by different aphid species (Lacirignola & D'Onghia, 2009). Thus, knowledge of the biology of aphids is an important basis for successful management of the aphids themselves and of the diseases they transmit (Hales et al., 1997).

In Algeria, practically there is no specific studies on the aphids attacking grapefruit, although the importance of this aspect to obtain a good production qualitatively and quantitatively. Thus, our paper reports on the diversity and fluctuations of aphids on grapefruit in Skikda region (northeast Algeria), based on a two years investigation

## 2 MATERIAL AND METHODS

A citrus orchard at the Technical Institute of Fruit Arboriculture in Emjez Djich (6° 47’ E and 36° 42’ N, 200 m above the sea level), province of Skikda situated in northeast of Algeria, was used for this study. The trees were arranged in 5 m separated rows.

From January 2012 to December 2013, monthly surveys measured the abundance of aphids (adults and larvae) on grapefruit trees (‘Shambar’) grafted on Troyer citrange (*C. sinensis* L. × *Poncirus trifoliata* Raf.). Four young leaves from the four cardinal points per tree and four trees of grapefruit were selected randomly on each sampling date. Similarly, Yoldaş et al. (2011) and Mostefaoui et al. (2012) have sampled leaves to study citrus aphids.

Identification of collected aphids was carried out using identification keys especially those of Blackman & Eastop (2000) and Stoetzel (1994).

ANOVA analysis and Student-Newman-Keuls test were performed, by means of SPSS for Windows 10 software (SPSS Inc.), to compare the mean number of aphids between months and to classify homogeneous groups.

## 3 RESULTS AND DISCUSSION

Through 2012 and 2013, five aphid species were identified in total (Table 1). They are already reported on other citrus species in Algeria (Aroun, 1986; Benoufella-Kitous, 2005; Mohammedi-Boubekka, 2006; Belkahla et al., 2013; Benoufella-Kitous et al., 2014; Aroun, 2015; Labdaoui & Guenaoui, 2015; Lebbal & Laamari, 2016).

Citrus aphid species are widespread and four of them, *Aphis spiraecola* (Patch, 1914), *A. gossypii* (Glover, 1877), *Toxoptera aurantii* (Boyer de Fonscolombe, 1841) and *T. citricidus* (Kirkaldy, 1907), are especially abundant (Lapchin et al., 1994). Despite its presence in other Mediterranean countries, *T. citricida*, which is the vector the most implicated in the transmission of Tristeza disease (Lebdi Grissa, 2010), was not noted in the orchard of study. The complete elimination of Meyer lemon, the absence of the main vector *T. citricidus* and of natural transmission by other aphid species, have probably removed the risk of spreading the disease in Algeria (Larbi et al., 2009). Nevertheless, *A. spiraecola*, *A. gossypii*, *M. persicae*, and *T. aurantii* have some ability to transmit this virus (Bové, 1961; Ghosh et al., 2015). *A. gossypii* has been reported to cause major epidemics of CTV in the Mediterranean Basin (Yahiaoui, 2010)

**Table 1**: Number of individuals of each aphid species found on grapefruit in the examined orchard during 2012 and 2013

| Aphids / Years | 2012 | 2013 |
|---|---|---|
| *Aphis spiraecola* (Patch, 1914) | 388 | 1448 |
| *Aphis gossypii* (Glover, 1877) | 0 | 19 |
| *Toxoptera aurantii* (Boyer de Fonscolombe, 1841) | 3 | 0 |
| *Aphis nerii* (Boyer de Fonscolombe, 1841) | 0 | 5 |
| *Macrosiphum euphorbiae* (Thomas, 1878) | 0 | 1 |

The morphological characteristics of the identified aphids are described below

### 3.1 *A. spiraecola* (green citrus aphid or spiraea aphid)

This is a small yellow or greenish-yellow aphid with black siphunculi and cauda (Blackman & Eastop, 2007). Its body length ranges between 1.2 and 2.2 mm (Blackman & Eastop, 2006).

### 3.2 *A. gossypii* (cotton or melon aphid)

The coloration of adults, ranging in size from 0.8 to 1.5 mm, varies from light yellow or greenish to dark green. Their antenna are a little longer than half the (Célini, 2001). Cauda is lighter than siphunculi (Ilharco & Sousa-Silva, 2009).

### 3.3 *A. nerii* (oleander aphid)

Aptera are bright lemon yellow with dark antenna and legs, and black siphunculi and cauda (Blackman & Eastop, 2006). Antenna with terminal process more than three times length of base of VI (Stoetzel, 1994).

### 3.4 *T. aurantii* (black citrus aphid)

It is about 2.1 mm in length with striped legs (Fasulo & Halbert, 2015). Body of apterous form is dark-brown, while the apex of antennal segments III, IV and V, the apical half of base of VI and sometimes also the apex of terminal process are dark (Ilharco & Sousa-Silva, 2009).

### 3.5 *M. euphorbiae* (potato aphid)

It is a medium-sized to large, spindle shaped aphid, usually green but sometimes pink or magenta, the adult apterae often rather shiny in contrast to the immature stages, which have a light dusting of greyish-white wax (Blackman & Eastop, 2007). Siphunculi with a subapical zone of polygonal reticulation whereas the cauda is longer (Blackman & Eastop, 2000).

In our case, the most common species was *A. spiraecola* and with less degree *A. gossypii.* Tena & Garcia-Marí (2011) considered that these two species are the most harmful to citrus in the Mediterranean region. Its importance on citrus fruits has been mentioned, among others, in Algeria (Lebbal & Laamari, 2016), in Morocco (Elhaddad et al., 2016), in Syria (Abo Kaf, 2005) and in Turkey (Uygun & Satar, 2008). Whereas the weak infestation of *A. nerii, M. persicae* and *M. euphorbiae* on grapefruit in the studied orchard may be attributed to the competition from other species, particularly *A. spiraecola.*

On the other hand, ANOVA showed no significant difference of the infestation degree between months in 2012 (P = 0.156) and highly significant difference in 2013 (P = 0.000). The most important densities were recorded in spring and autumn (Figure 1), especially in April 2013 ($\bar{x}$ = 57.69 aphids / leaf), which coincide with the formation of new flushes and optimal temperatures.

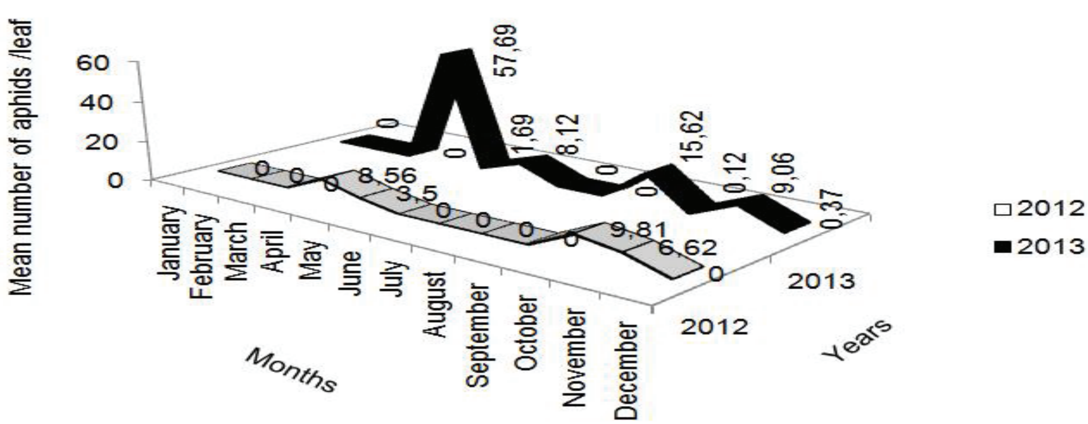



**Figure 1**: Mean number of aphids/leaf recorded on grapefruit in the examined orchard during 2012 and 2013

The correlation analysis for weather parameters and some citrus aphids indicated a significant negative correlation between minimum temperature and incidence of aphid species (Chavan & Singh, 2005). In addition, the presence and the abundance of citrus-dependent aphids depend on the population size of the different flushes (Saharaoui et al., 2015). Braham & Amor (2018) noticed a positive relationship between the number of new shoots per experimental tree and *A. spiraecola* infestation.

We observed an augmentation in the number of aphid species detected from two in 2012 to four species in 2013(Table 1). We also noticed big changes in the distribution of aphids between years (Figure 1). For example, aphids did not infest grapefruit trees in five months during 2012, and in eight months during 2013. It seems that changes in climatic parameters between years influenced the infestation level. In the present study, the minimum temperature ranged from 9.48 to 27.37 °C in 2012; and between 11.24 and 25.22 °C in 2013 according to climatic data provided by the meteorological station of Skikda (longitude 6° 54' E; latitude 36° 52' N; altitude 1.30 m). Aphids are particularly sensitive to temperature changes due to certain specific biological features of this group (Hullé et al., 2010). The effect of temperature on the biology of many aphid species has been demonstrated (De Reggi, 1972; Kaakeh & Dutcher, 1993; Wang & Tsai, 2000; Morgan et al., 2001; Brabec et al., 2014; Ranila et al., 2015). Dixon & Hopkins (2010) revealed that for each species, there is a temperature range where the aphid can grow and reproduce. For example, Komazaki (1982) found that the intrinsic rate of natural growth is highest at 22 ° C for *A. gossypii* and 27 ° C for *A. spiraecola*. In addition, the generation time of the latter species was 5.8 days at 25 ° C and 12.1 days at 15 ° C, on orange (Satar & Uygun, 2008).

## 4 CONCLUSION

The present study focused on grapefruit, which represents an underutilized fruit tree although its benefits, allows the obtaining of new data about the aphids attacking this citrus tree in Algeria.

Five aphid species were identified, of which four species are considered as vectors of CTV causing the quarantine disease Tristeza. Furthermore, population fluctuations of these insects were very variable. Consequently, regular surveys in orchards, especially in the spring and autumn, are necessary to execute control measures to limit the attacks by these pests.

## 5 REFERENCES


Abo Kaf, N. (2005). Quality and quantity diversity of aphids and its parasitoids on citrus in Coastal Regional of Syria. *Arab Journal of Plant Protection, 23*(2), 61-69.

Aroun, M. E. (1986). Les aphides et leurs ennemis naturels en verger d'agrumes de la Mitidja. *Annales de l'INA*, 59-66.

Aroun, M. E. (2015). *Le complexe aphides et ennemis naturels en milieux cultivé et forestiers en Algérie*. Doctoral dessertation, ENSA El Harrach.

Belkahla, H., Larbi, D., Bouafia, L., Moudoud, R., Guettouche, F. & Bouzidi, S. (2013). Serodetection of *Citrus Tristeza Closterovirus* (*CTV*) in Algeria. *American-Eurasian Journal of Sustainable Agriculture, 7*(1), 10-13.

Benoufella-Kitous, K. (2005). *Les pucerons des agrumes et leurs ennemis naturels à Oued Aïssi (Tizi-Ouzou)*. Magister dessertation, INA El Harrach.

Benoufella-Kitous, K., Doumandji, S. & Hance, T. (2014). Inventaire des aphides et de leurs ennemis naturels dans un verger d'agrumes. In: *10ème Conférence Internationale sur les Ravageurs en Agriculture*, 22 - 23 octobre 2014, Montpellier.

Biche, M. (2012). *Les principaux insectes ravageurs des agrumes en Algérie et leurs ennemis naturels*. Algérie, FAO.

Blackman, R. L. & Eastop, V. F. (2000). *Aphids on the world's crops: An identification and information guide*. United Kingdom, John Wiley and Sons.

Blackman, R. L. & Eastop, V. F. (2006). *Aphids on the world's herbaceous plants and shrubs*. United Kingdom, John Wiley & Sons.

Blackman, R. L. & Eastop, V. F. (2007). Taxonomic issues. In: H. F. van Emden & R. Harrington (Eds.), *Aphids as crop pests* (1–29), United Kingdom, CAB International.

Bové, J. M. (1961). Compte rendu du deuxième congrès international de virologie des citrus. *Fruits, 16*(4), 145-160.

Brabec, M., Honěk, A., Pekár, S. & Martinkova, Z. (2014). Population dynamics of aphids on cereals: Digging in the time-series data to reveal population regulation caused by temperature. *Plos One, 9*(9), 1-8. doi:10.1371/journal.pone.0106228

Braham, M., & Amor, N. (2018). Effect of pruning severity on the vegetative growth, density and population dynamics of the Spirea aphid, *Aphis spiraecola* in Citrus orchard. *Journal of Entomology and Zoology Studies, 6*(1), 311-319.

Célini, L. (2001). Le puceron du cotonnier *Aphis gossypii* (Glover) et son parasite *Aphelinus gossypii* Timberlake en République centrafricaine. *Insectes, 122*(3), 7-10.

Chavan, V. M., & Singh, S. J. (2005). Population dynamics and management of aphid vectors of citrus tristeza virus in Maharashtra. *Agricultural Science Digest*, *25*(2), 85-89.

Cœur D'acier, A., Hidalgo, N.P., & Petrović-Obradović, O. (2010). Aphids (Hemiptera, Aphididae). *BioRisk, 4*(1), 435-474. doi:10.3897/biorisk.4.57

Dartigues, D. (1991). Spatiotemporal distribution of aphids and influence of ants, on orange-trees in Kabylia. *Fruits, 46*(4), 461-469.

De Reggi, L. M. (1972). Développement larvaire du puceron *Myzus persicae* à une température anormalement élevée. *Journal of Insect Physiology, 18*(9), 1757-1761. doi:10.1016/0022-1910(72)90107-2

Dixon, A. F. G. & Hopkins, G. W. (2010). Temperature, seasonal development and distribution of insects with particular reference to aphids. In P. Kindlmann, A. F. G. Dixon & J. P. Michaud (Eds.), *Aphid biodiversity under environmental change: Patterns and processes* (pp. 129–147).Dordrecht, Springer. doi:10.1007/978-90-481-8601-3_8

Dixon, A.F.G., Kindlmann, P., Lepš, J., & Holman, J. (1987). Why there are so few species of aphids, especially in the tropics. *The American Naturalist, 129*, 580-592. doi:10.1086/284659

Elhaddad, A., ElAmrani, A., Fereres, A., & Moreno, A. (2016). Spatial and temporal spread of Citrus tristeza virus and its aphid vectors in the North western area of Morocco. *Insect science*, *23*(6), 903-912. doi:10.1111/1744-7917.12228

FAO (2017). Citrus fruit: *Fresh and processed statistical bulletin 2016.* Rome, FAO.

Fasulo T. R. & Halbert S. E. (2015). Aphid pests of Florida citrus, (University of Florida paper ENY 811). Retrieved from https://edis.ifas.ufl.edu/pdffiles/CH/CH05500.pdf

Ghosh, A., Das, A., Lepcha, R., Majumdar, K. & Baranwal, V. K. (2015). Identification and distribution of aphid vectors spreading *Citrus tristeza virus* in Darjeeling hills and Dooars of India. *Journal of Asia-Pacific Entomology*, *18*, 601-605. doi:10.1016/j.aspen.2015.07.001

Hales, D. F., Tomiuk, J., Wöhrmann, K., & Sunnucks, P. A. U. L. (1997). Evolutionary and genetic aspects of aphid biology: A review. *European Journal of Entomology*, *94*(1), 1-55.

Hanke, M.-V. & Flachowsky, H. (2010). Fruit crops. In F. Kempken & C. Jung (Eds.), *Genetic Modification of Plants* (pp. 307–348). Berlin, Springer. doi:10.1007/978-3-642-02391-0_17

Hullé, M., Cœur D'acier, A., Bankhead-Dronnet, S. & Harrington, R. (2010). Aphids in the face of global changes. *Comptes Rendus Biologies, 333*, 497-503. doi:10.1016/j.crvi.2010.03.005

Ilharco, F. A., & Sousa-Silva, C. R. (2009). *Toxoptera citricidus* (Kirkaldy, 1907)(Homoptera, Aphidoidea), the tropical citrus aphid in continental Portugal. *Options Méditerranéennes B65*, 53 - 58.

Kaakeh, W. & Dutcher, J. D. (1993). Survival of yellow pecan aphids and black pecan aphids (Homoptera: Aphididae) at different temperature regimes. *Environmental Entomology Journal, 22*(4), 810-817. doi:10.1093/ee/22.4.810

Kimball, D. A. (1999). *Citrus processing : A complete guide*. Maryland, Aspen Publishers. doi:10.1007/978-1-4615-4973-4

Komazaki, S. (1982). Effects of constant temperatures on population growth of three aphid species, *Toxoptera citricidus* (Kirdkaldy), *Aphis citricola* Van Der Goot and *Aphis gossypii* Glover (Homoptera: Aphididae) on *Citrus*. *Applied Entomology and Zoology, 17*(1), 75-81. doi:10.1303/aez.17.75

Labdaoui, Z. E. & Guenaoui, Y. (2015). The aphids infesting citrus orchards and their natural enemies in the Northwestern Algeria. In *Sixth International Scientific Agricultural Symposium" Agrosym2015", Jahorina, Bosnia and Herzegovina, October 15-18, 2015. Book of Proceedings* (pp. 787-792). University of East Sarajevo.

Lacirignola, C. & D'onghia, A. M. (2009). The Mediterranean citriculture: Productions and perspectives. *Options Méditerranéennes, B 65*, 13-17.

Lapchin, L., Guyot, H. & Brun, P. (1994). Spatial and temporal heterogeneity in population dynamics of citrus aphids at a regional scale. *Ecological Research, 9*, 57-66. doi:10.1007/BF02347242

Larbi, D., Ghezli, C. & Djelouah, K. (2009). Historical review of *Citrus tristeza virus* (CTV) in Algeria. *Options Méditerranéennes,B 65*, 107-110.

Lebbal, S. & Laamari, M. (2016). Population dynamics of aphids (Aphididae) on orange (Citrus sinensis 'Thomson Navel') and mandarin (Citrus reticulata 'Blanco'). *Acta agriculturaeSlovenica*, *107*(1), 137-145. doi:10.14720/aas.2016.107.1.14

Lebdi Grissa, K. (2010). *Etude de base sur les cultures d'agrumes et de tomates en Tunisie*. Regional integrated pest management program in the Near East. Rome, FAO.

Maserti, B.E., Del Carratore, R., Della Croce, C.M., Podda, A., Migheli, Q., Froelicher,Y., Luro,F., Morillon,R., Ollitrault,P., Talon, M. & Rossignol, M. (2011). Comparative analysis of proteome changes induced by the two spotted spider mite *Tetranychus urticae* and methyl jasmonate in citrus leaves. *Journal of Plant Physiology, 168*, 392-402. doi:10.1016/j.jplph.2010.07.026

Mohammedi-Boubekka, N. (2006). Biosystématique des Aphididae et leur place dans l'entomofaune de l'oranger dans la plaine de la Mitidja. Magister dessertation, INA El Harrach.

Morgan, D., Walters, K. F. A. & Aegerter, J. N. (2001). Effect of temperature and cultivar on pea aphid, *Acyrthosiphon pisum* (Hemiptera: Aphididae) life history. *Bulletin of Entomological Research, 91*(1), 47-52.

Mostefaoui, H., Mahmoude, A., AllalBenfekih, L., Petit, D. & Saladin, G. (2012). Fluctuations des abondances saisonnières des populations d'aphidiens du clémentinier en fonction de l'accumulation de leurs réserves énergétiques. In: *3ème Congrès de Zoologie et d'Ichtyologie*, 6 - 10 novembre 2012, Marrakech.

Ranila, A., Kanani, M. K., Bhut, J. B. & Borad, P. K. (2015). Population dynamics of *Aphis gossypii* Glover on coriander in relation to biotic and abiotic factors. *International Journal of Plant Protection, 8*(2), 372-374. doi:10.15740/HAS/IJPP/8.2/372-374

Rehman, S., Ahmad, J., Lanzoni, C., Rubies Autonell, C. & Ratti, C. (2016). The phytosanitary status of the National Collection of fruits and nuts of Afghanistan and the private Mother Stock Nurseries: A virus survey. *Advances in Horticultural Science, 30*(4), 239-248.

Saharaoui, L., Hemptienne, J. L. & Magro, A. (2015). Partage des ressources trophiques chez les coccinelles. *Bulletin de la Société Zoologique de France, 140*(1), 5-23.

Satar, S. & Uygun, N. (2008). Life cycle of *Aphis spiraecola* Patch (Homoptera: Aphididae) in East Mediterranean region of Turkey and its development on some important host plants. *IOBC/WPRS Bulletin, 38*, 216-224.

Skaria, M. (2004). People, arthropods, weather and citrus diseases. In S. A. M. H. Naqvi (Ed.), *Diseases of fruits and vegetables* (pp. 307–337). The Netherlands, Kluwer Academic Publishers. doi:10.1007/1-4020-2606-4_7

Stoetzel, M. B. (1994). Aphids (Homoptera: Aphididae) of potential importance on Citrus in the United States with illustrated keys to species. *Proceeding of the Entomology Society of Washington*, *96*, 74-90.

Uygun, N. & Satar, S. (2008). The current situation of citrus pests and their control methods in Turkey. *IOBC/WPRS Bulletin, 38*, 2-9.

Wang, J.-J. & Tsai, J. H. (2000). Effect of temperature on the biology of *Aphis spiraecola* (Homoptera: Aphididae). *Annals of Entomological Society of America, 93*(4), 874-883. doi:10.1603/0013-8746(2000)093[0874:EOTOTB]2.0.CO;2

Xiao, Y.-J., Hu, M. & Tomlinson, B. (2014). Effects of grapefruit juice on cortisol metabolism in healthy male Chinese subjects. *Food and Chemical Toxicology*, *74*, 85-90. doi:10.1016/j.fct.2014.09.012

Xu, W., Qu, W., Huang, K., Guo, F., Yang, J., Zhao, H. & Luo, Y. (2007). Antibacterial effect of Grapefruit Seed Extract on food-borne pathogens and its application in the preservation of minimally processed vegetables. *Postharvest Biology and Technology, 45*, 126-133. doi:10.1016/j.postharvbio.2006.11.019

Yahiaoui, D. (2010). *Assessment of genetic diversity of Mediterranean Citrus Tristeza Virus (CTV) isolates and genomic RNA variability associated to their vector transmission*. Doctoral dessertation, Università Degli Studi Di Catania.

Yin, X., Gyles, C. L. & Gong, J. (2012). Grapefruit juice and its constituents augment the effect of low pH on inhibition of survival and adherence to intestinal epithelial cells of *Salmonella enterica* serovar *Typhimurium* PT193. *International Journal of Food Microbiology, 158*, 232-238. doi:10.1016/j.ijfoodmicro.2012.07.022

Yoldaş, Z., Güncan, A. & Koçlut, T. (2011). Seasonal occurrence of aphids and their natural enemies in Satsuma mandarin orchards in Izmir, Turkey. *Türkiye Entomoloji Dergisi, 35*(1), 59-74.

doi:10.14720/aas.2018.111.3.07 Original research article / izvirni znanstveni članek

# Content of bioactive compounds and antioxidant activity in garlic (*Allium sativum* L.)

Marianna MICOVÁ[1*], Judita BYSTRICKÁ[2], Ján KOVAROVIČ[1], Luboš HARANGOZO[1], Adriana LIDIKOVÁ[3]

Received April 23, 2018; accepted November 30, 2018.
Delo je prispelo 23. aprila 2018, sprejeto 30. novembra 2018.

**ABSTRACT**

Garlic (*Allium sativum* L.) is highly regarded throughout the world for both its medicinal and culinary properties. With its bioactive compounds and sulphur containing compounds, high trace mineral content, garlic has shown anti-viral, anti-bacterial, anti-fungal and antioxidant abilities.

This work has focused on the measurement and comparison of the total polyphenols content, the total sulphur content and antioxidant activity of the studied varieties of garlic in the area of Nitra and Bardejov, Slovak Republic. The highest content of monitored indicators (TPC, TSC, AOA) was measured in the area of Nitra. TPC was determined using the spectrophotometric method of Folin-Ciocalteu agents. The total polyphenols content were determined in the range 621.13 to 763.28 mg $kg^{-1}$ in area of Nitra. The content of total sulphur compounds in the area of Nitra ranges from 0.562 to 0.800 % and in the second area ranges from 0.421 % to 0.658 %. Antioxidant activity was measured by the spectrophotometric method using a compound DPPH. The value of antioxidant activity ranged from 12.01 % to 20.22 % in both monitored locations. The content of TPC, TSC and AOA beside the variety may be affected by the localitylinked factors like climatic conditions and the agrochemical composition of the soil.

**Key words:** antioxidant activity; garlic; polyphenols; sulphur compounds

**IZVLEČEK**

**VSEBNOST BIOAKTIVNIH SNOVI IN ANTIOKSIDACIJSKA AKTIVNOST ČESNA (*Allium sativum* L.)**

Česen (*Allium sativum* L.) je zelo cenjen po celem svetu zaradi njegovih zdravilnih in kulinaričnih lastnosti. Na osnovi žveplo vsebujočih bioaktivnih snovi in elementov v sledeh ima protivirusne, protibakterijske, protiglivne in antioksidacijske lastnosti.

Raziskava se osredotoča na meritve im primerjave vsebnosti polifenolnih in žveplo vsebujočih snovi v preučevanih sortah česna na območju Nitre in Bardejova, Slovaška Republika. Največja vsebnost merjenih indikatorjev (TPC, TSC, AOA) je bila izmerjena na območju Nitre. TPC je bil določen s spektrofotometrično metodo z uporabo Folin-Ciocalteujevega reagenta. Celokupna vsebnost polifenolov, izmerjena na območju Nitre je bila v razponu od 621,13 do 763,28 mg $kg^{-1}$.Celokupna vsebnost žveplo vsebujočih snovi je bila na območju Nitre v razponu od 0,562 do 0,800 % in od 0,421 % do 0,658 % na drugem preučevanem območju. Antioksidacijska aktivnost je bila izmerjena spektrofotometrično z uporabo DPPH. Antioksidacijska aktivnost je bila v obeh preučevanih območjih v razponu od 12,01 % do 20,22 %. Na vsebnost TPC, TSC in AOA vplivajo poleg sorte z lokacijo povezani dejavniki kot so podnebne razmere, sestava tal in uporabljena agrotehnika.

**Ključne besede:** antioksidacijska aktivnost; česen; polifenoli; žveplove spojine

[1] Ing., Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Chemistry, Tr. A. Hlinku 2, 94976 Nitra, Slovak Republic; *Corresponding author: xlenkova@is.uniag.sk

[2] Assoc. Prof. Ing., PhD., Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Chemistry, Tr. A. Hlinku 2, 94976 Nitra, Slovak Republic

[3] Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Chemistry, Tr. A. Hlinku 2, 94976 Nitra, Slovak Republic

## 1 INTRODUCTION

Garlic (*Allium sativum* L.) is a plant of the genus Alliaceae and is one of the longest cultivated crop plants in the world. It is known that it was used for healing purposes in the Middle Ages. Garlic is an important raw material of plant origin with observed and proven positive effects on the human organism. Garlic has a versatile use as a spice and also uses its healing properties. It is believed to originate in Central Asia, where it originated from the wildly growing species *Allium longicuspis* Regel (Iciek et al., 2012). Garlic was extended to the West, South and East more than 6000 years ago. Garlic, as we know it today, has been used in Chinese medicine for 3000 years ago. Egyptians used garlic to increase immunity and protect against various diseases and to improve performance (Sultan et al., 2014). Louis Pasteur, the famous bacteriologist found that garlic juice kills bacteria propagated at a laboratory dish. From this time on, it has been shown many times that garlic destroys a very wide range of bacteria and fungi that adversely affect our health (Fulder, 2002). In the past, garlic was used as a medicine during various epidemics such as typhus, dysentery, cholera and influenza. At present, garlic is grown around the world. In our territory, it is used as an important preventive medicine, universal spice and food (Petrovská & Čekovská, 2010). Several studies have shown multifaceted health benefits of garlic, it is recommended as a dietary supplement worldwide. The evidence of health effects has increased its consumption especially in the culinary field (Suleria et al., 2015). Current knowledge indicates that nature around us is a great source of available health-promoting and chemopreventive agents. Chemoprotective substances are part of foods of plant origin where they occur as natural ingredients.

Garlic (*Allium sativum* L.) is a source of biologically active compounds such as phenolic acids, flavonoids, sulphur compounds, vitamins, minerals and others. Garlic is a good source of important vitamins. Although the amount of garlic consumed is not such as to cover the need for the body, sulphur compounds contained in garlic can enhance the effects of vitamins. Vitamin $B_1$ (thiamine) is easily absorbed in the intestines in combination with allicin. Garlic contains B vitamins ($B_1$, $B_3$, $B_5$ and $B_6$), vitamins C and E. The content of individual vitamins in garlic is different and depends on many factors - variety, storage, treatment and cultivation (Butt & Sultan, 2009).

Garlic belongs to the important and significant vegetables, characterized by significantly high content of polyphenolic compounds positively affecting human body. The majority of polyphenol compounds in garlic are phenolic acids and flavonoids (Piazzon et al., 2012; Obied, 2013). Polyphenols are heterogeneous oligo- or polymeric compounds combined with other compounds (Xiao et al., 2013). Polyphenols in plants fulfil important functions: they are carriers of flavours, fragrances and colorants, are building and structural components, and are defensive substances (from pests, various infections, pathogens and mechanical damage) (Knežević et al., 2012). Polyphenol compounds according to the chemical structure are divided into phenolic acids, flavonoids, lignans and stilbenes (Pereira et al., 2009). The total polyphenol content of the garlic can be influenced by the variety (Srivastava et al., 2013), as well as storage and technological processing of garlic (Süli et al., 2014). The physiological effect of polyphenols is wide ranging and shows antioxidant, anti-carcinogenic, anti-mutagenic, antibacterial, anti-parasitic, and antidiabetic activity (Obied, 2013).

For characteristic aroma and flavour of garlic are responsible sulphur compounds in garlic. These substances are physiologically active and used as antibiotics, particularly in the treatment of gastric cancer (Harris, 2004). The antibiotic value is influenced by the components: allicin, S-methylcysteine sulfoxide and S-propylcysteine sulfoxide. Allicin is the main biologically active ingredient of garlic (Lanzotti, 2006). Allicin is not found in garlic, it is produced by the enzyme allinase from alliin. After processing, such as cutting, crushing, chewing or dehydration, alliin is converted to allicin by allinase (Amagase et al., 2001; Bhuiyan et al., 2015). Sulphur compounds are very effective anticoagulant, prolong the clotting and promote blood circulation (Oberbeil & Lentzová, 2005). Sulphur compounds are characterized by their ability to support the immune system and also protect the cardiovascular system (Kačániová & Tančinová, 2012).

Garlic (*Allium sativum* L.) acts as a natural antioxidant and plays an important role in chemoprevention of many civilization diseases (Hamzah et al., 2013). Garlic is rich in antioxidant phytochemicals that prevent oxidative damage. In blood serum, bioactive compounds of garlic eliminate hydroxyl radicals and increase the activity of some enzymes with antioxidant effects. These include flavonoids and sulphur compounds soluble in water (S-allylcysteine and S-allylmercaptocysteine) and fat-soluble (allicin and its products) and selenium (Borek, 2001).

In the present work we evaluated the beneficial effects of the total polyphenols content, sulphur compounds and antioxidant activity in individual varieties (Mojmír, Lukan, Záhorský, Havran, Makoi) of garlic (*Allium sativum* L.) and we evaluated the impact of two sites

(Nitra, Bardejov) on the total content of polyphenols, the total content of sulphur compounds and antioxidant activity in selected garlic varieties.

## 2 MATERIALS AND METHODS

### 2.1 The local climate conditions

This study was performed in area of Nitra and Bardejov, Slovak Republic. Nitra is situated on the south-western Slovakia in the area of Podunajská lowland. Nitra belongs to warmer and dry areas with mild winters in Slovakia. The average annual rainfall is 550 – 600 mm and the average annual temperature is 9 - 10 ºC. Bardejov is located in the north-eastern Slovakia. Bardejov belongs to slightly warm and mountain-continental climate area. The average annual rainfall is 700 – 750 mm and the average annual temperature is 5 - 9 ºC. Nitra and Bardejov have very good natural and climatic conditions for crop growth, without any adverse effects.

### 2.2 Samples of plant material

The samples of plant material (5 variety of garlic – 'Mojmír', 'Záhorský', 'Lukan', 'Havran', 'Makoi') were collected in the phase of full ripeness from area of Nitra and Bardejov, Slovak republic. For analysis was used fresh material soil samples and plant. Samples were analysed by selected methodologies (determination of total polyphenols, total sulphur compounds and antioxidant activity). All samples of plant material were grown under the same conditions. The soil samples from the area, where was grown plant material, was analysed (Table 1 and Table 2). The analysis of soil samples was carried out four times in four sampling sites with pedological probe Geosampler Fisher. Only NPK fertilization (200 g $m^{-2}$) was used for the achievement of favourable soil macroelements content.

**Table 1:** Agrochemical characteristic of soil substrate in mg $kg^{-1}$, content of nutrients from locality Nitra and Bardejov

| Locality | K | Ca | Mg | P | $pH_{KCl}$ | Humus % | Cox % |
|---|---|---|---|---|---|---|---|
| Nitra | 399.2 ± 2.1 | 3861.4 ± 7.1 | 1312 ± 4 | 97.6 ± 1.6 | 6.91 ± 0.06 | 2.9 ± 0.1 | 1.68 ± 0.01 |
| Bardejov | 380.1 ± 2.7 | 2170.8 ± 2.2 | 259 ± 1.2 | 406.7 ± 1.7 | 5.51 ± 0.09 | 3.58 ± 0.03 | 6.17 ± 0.01 |

**Table 2:** Content of heavy metals (mg $kg^{-1}$) in soil substrate (extraction by aqua regia)

| Locality | Zn | Cu | Ni | Pb | Cd |
|---|---|---|---|---|---|
| Nitra | 55.2 ± 2.3 | 27.8 ± 1.4 | 42.6 ± 1.8 | 40.3 ± 1.6 | 4.2 ± 0.04 |
| Bardejov | 90.1 ± 2.9 | 33.5 ± 1.2 | 37.7 ± 1.6 | 19.2 ± 1.1 | 1.9 ± 0.1 |
| Limit[1] | 150 | 60 | 50 | 70 | 0.7 |

[1] Limit value for aqua regia – Law No. 220/2004

### 2.3 Sample preparation

Extract was prepared from the 25 g samples garlic, which were shaken (shaker GFL 3006, 125 rpm) in 50 ml of 80 % ethanol for sixteen hours. Samples were kept at laboratory room temperature in dark conditions until the analysis. Each determination was carried out in six replications.

### 2.4 Determination of total polyphenols

Total polyphenols content (TPC) was determined by the method according to Lachman et al. (2003). It is expressed as mg of gallic acid equivalent per kg of fresh matter. Total polyphenols content was determined using the Folin-Ciocalteu reagent. 2.5 ml of Folin-Ciocalteu reagent was added to 0.1 ml extract to volumetric flask. The content was mixed. After 3 minutes, 5 ml 20 % solution of sodium carbonate was added. Then the volume was adjusted to 50 ml with distilled water. After 2 hours, the samples were centrifuged (centrifuges UNIVERSAL 320, 15000 rpm) for 10 minutes. The absorbance was measured by use of spectrophotometer Shimadzu UV/VIS – 1240 at 765 nm. The concentration of polyphenols was calculated from a standard curve with known concentration of gallic acid.

### 2.5 Determination of total sulphur compounds

The determination of the total sulphur content is based on dry combustion in the presence of oxygen and allows for the quantitative conversion of sulphur to $SO_2$, the elimination of other combustion products including water and the separation of the generated gases. 50 mg of a lyophilized and homogenized sample is combusted in a tin crucible with a $V_2O_5$ catalyst in the elementar (Vario Macro Cube V 3.1.4, Elementar Analysensystem GmbH). After insertion of the crucible with the sample into the combustion tube, the oxygen stream produces a strong exothermic reaction, the temperature rises to 1250 ºC and the sample is combusted. Combustion products are conveyed along the combustion tube where the oxidation is complete. $SO_3$ is reduced to $SO_2$. The mixture of gases flows into the chromatographic column where the separation takes place. The gases are sent to the thermal conductivity detector where the electrical signals are processed by the software and provide the percentage of sulphur contained in the sample. Sulfanilamide is used as the calibration standard (Šapčanin et al., 2013).

### 2.6 Determination of antioxidant activity

Antioxidant activity (AOA) was measured according to Brand-Williams et al. (1995). The method is based on using DPPH˙ (2.2-diphenyl-1-picrylhydrazyl). DPPH˙ (3.9 ml) was pipetted into the cuvette and the absorbance was measured using the spectrophotometer Shimadzu UV/VIS – 1240 at 515.6 nm. The measured value corresponds to the initial concentration of DPPH˙ solution at the time $A_0$. Then 0.1 ml extract was added to start measuring dependence A = f (t). The content of cuvette was mixed and the absorbance was measured at 1, 5 and 10 minutes in the same way as DPPH˙ solution. The percentage of inhibition expresses how antioxidant compounds are able to remove DPPH˙ radical at the given period.

$$\text{Inhibition (\%)} = (A_0 - A_t / A_0) \times 100$$

### 2.7 Statistical analysis

Results were statistically evaluated by the Analysis of Variance (ANOVA – Multiple Range Tests, Method: 95.0 percent LSD) by using the statistical software STATGRAPHICS (Centurion XVI.I, USA).

## 3 RESULTS AND DISCUSSION

We analysed five varieties of garlic: Mojmír, Záhorský, Lukan, Havran and Makoi produced in two localities of Slovakia: Nitra and Bardejov. Each of the locations is characterized by a different annual average temperature, annual rainfall and agrochemical composition of the soil. The total content of polyphenols in the samples from the area Nitra ranged from 621.13 ± 4.45 to 763.28 ± 3.60 mg kg$^{-1}$ (Figure 1). In area Bardejov, the total polyphenols content in the studied samples ranged from 559.68 ± 2.26 to 682.94 ± 2.89 mg kg$^{-1}$ (Figure 1). Statistically the highest content of total polyphenols was measured in the variety of garlic Havran and the lowest content of total polyphenols was found in the variety Záhorský in both study areas. The higher content of total polyphenols in the variety Havran was found in the area of Nitra and was 1.12-times higher, compared with the locality Bardejov. In other studied varieties of garlic higher total polyphenols content was found in the area of Nitra. Chekki et al. (2014) indicated, that the total polyphenols content in garlic is in the wide range from 436 mg kg$^{-1}$ to 645 mg kg$^{-1}$, which good correlate with the results of this work. Some authors reported even a higher value of TPC in garlic: 812 mg kg$^{-1}$ (Charles, 2013), 1290 mg kg$^{-1}$ (Wangcharoen & Morasuk, 2009). In comparison with this study, lower content of TPC in garlic (493 mg kg$^{-1}$) was measured by Jastrzebski et al. (2007). The influence of the area on the content of total polyphenols was confirmed by the team of authors

Hamouz et al. (2010). The above mentioned authors determined the total content of polyphenols by the same spectrophotometric method using the Folin-Ciocalteu reagent.

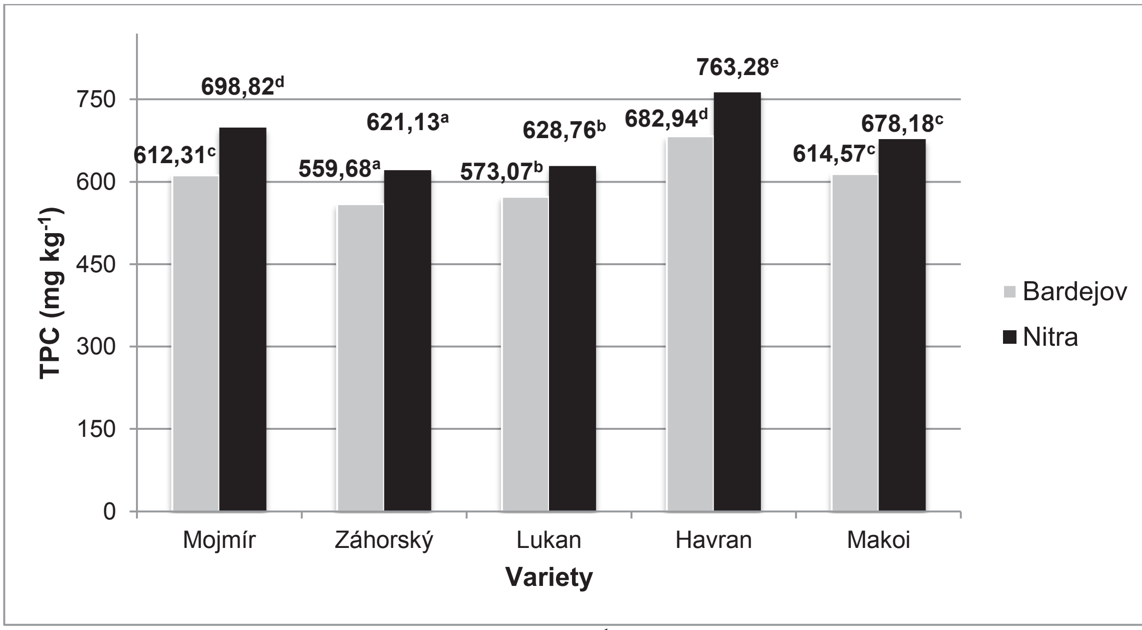



**Figure 1**: Average content of total polyphenols (TPC mg $kg^{-1}$) from area Nitra and Bardejov

* Multiple Range Tests, Method: 95.0 percent LSD, Different letters (a, b, c, d and e) between the factors show statistically significant differences ($p < 0.05$)

The values of antioxidant activity in the studied samples from the area Nitra are varied from 17.31 % to 20.22 % (Figure 2). The antioxidant activity of the samples from the area Bardejov ranged from 12.01 % to 13.61 % (Figure 2). Statistically the highest value of antioxidant activity was observed in the variety Havran and the lowest value of antioxidant activity was found in the variety Záhorský in both study areas. Higher value of antioxidant activity in the variety Havran was found in the area Nitra and was 1.13-times higher compared with the locality Bardejov. In other studied varieties of garlic was found higher value of antioxidant activity in the area Nitra. Statistically significant differences in the strength of antioxidant activity were also detected among all analysed varieties of garlic. Narendhirakannan & Rajeswari (2010) indicate that the antioxidant activity in garlic ranges from 12 % to 21 % and antioxidant activity of garlic determined in this study is within this range. According to Rai et al. (2015) the antioxidant activity in garlic is 21.5 %. Choi et al. (2014) measured lower values of antioxidant activity in garlic (7 %). The cited authors determined the antioxidant activity of the method using DPPH˙ and their results were comparably expressed in percentage as in our work.

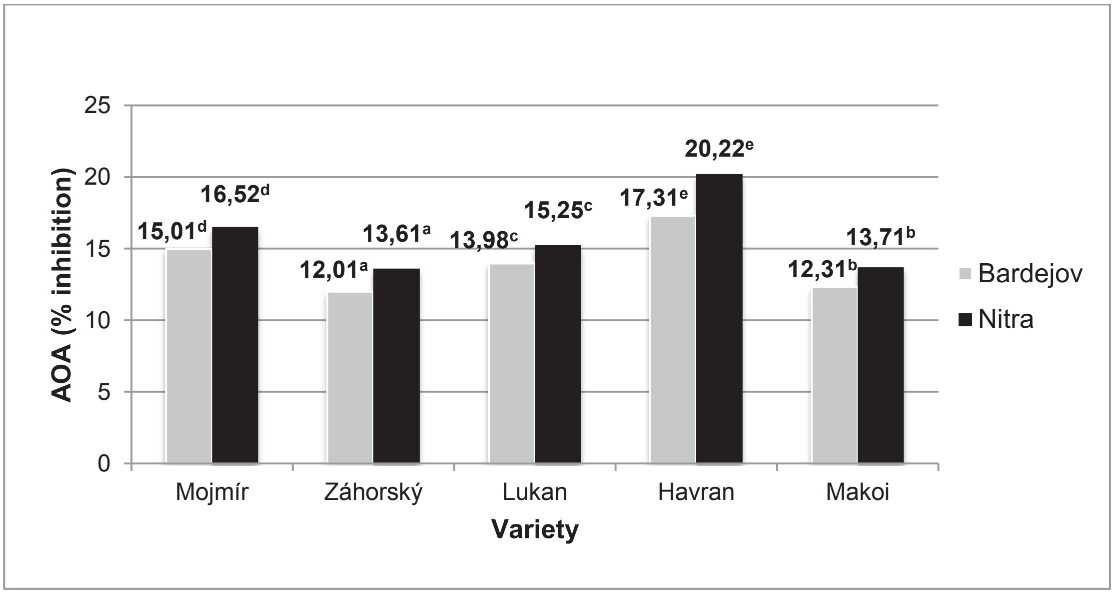



**Figure 2**: Average value of antioxidant activity (AOA %) from area Nitra and Bardejov

* Multiple Range Tests, Method: 95.0 percent LSD, Different letters (a, b, c, d and e) between the factors show statistically significant differences ($p < 0.05$)

The total sulphur content in the studied samples from area Nitra ranged from 0.562 ± 0.042 % to 0.800 ± 0.048 % (Figure 3). The total sulphur content in the studied samples from area Bardejov ranged from 0.421 ± 0.047 % to 0.658 ± 0.021 %. The highest content of total sulphur compounds of the analysed samples was measured in the variety Mojmír and the lowest total sulphur content was observed in the variety Záhorský in the both study areas. In other studied varieties of garlic was found higher total sulphur content in the area of Nitra, compared with the locality Bardejov. Benkeblia & Lanzotti (2007) recorded, that the total sulphur content in garlic is 0.56 %. Muradić et al. (2010) mentioned value 0.63 %, which good correlate with the results of this work. Mahmutović et al. (2014) indicate, that the content of total sulphur compounds in garlic is in the range of 0.63 % to 0.70 %. Munch (2013) report a lower total sulphur content in garlic (0.3 %).

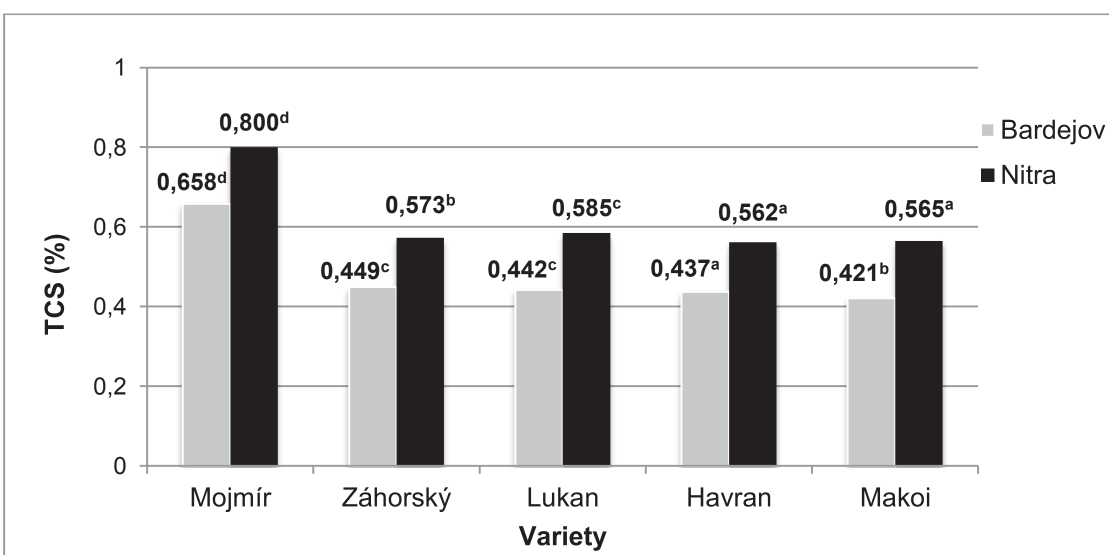



**Figure 3**: Average content of total sulphur compounds (TSC %) from area Nitra and Bardejov

* Multiple Range Tests, Method: 95.0 percent LSD, Different letters (a, b, c and d) between the factors show statistically significant differences ($p < 0.05$)

Khodadadi et al. (2015) in their experiment confirmed the site´s impact on the content of sulphur compounds and also on the total polyphenols content and the value of antioxidant activity. Chekki et al. (2016) reported that the content of bioactive compounds is affected by the climatic conditions of the area. Sárosi et al. (2011) reported that the content of bioactive constituents in plants is heavily influenced by climatic conditions. Changing climatic conditions leads to stress in the plant. The content of total polyphenols and antioxidant activity are affected by sunny and warmer weather. In their work, they determined a higher content of total polyphenols and a higher value of antioxidant activity in a site with higher temperature and direct sunlight. This is well in agreement with ours results. Viljevac Vuletić et al. (2017) have indicated that higher level of precipitation at the site has a positive impact on the content of bioactive substances in the plant, which we unfortunately did not confirm in this study. The higher content of total polyphenols, sulphur compounds and antioxidant activity was recorded in the locality with increasing average annual temperature in Nitra. This area is characterized by a higher number of sunny days with an average annual temperature of 9 to 10 °C and a lower annual rainfall (550-600 mm). Environmental influence on polyphenols synthesis is very important. Polyphenols, especially flavonoids are synthesized via the phenylpropanoid pathway with stimulation of light, which protects plants from harmful UV radiation acting as a protective filter. High temperatures during the season contributed to higher polyphenols content (Viljevac Vuletić et al., 2017).

In the locality of Nitra with a lower altitude (151 m a.s.l.) we found a higher content of bioactive compounds compared to the area of Bardejov with a higher altitude (323 m a.s.l.), which is in agreement with the results of Fertout-Mouri et al. (2014) and Ghasemi et al. (2011). Changes in the content of total polyphenols, sulphur compounds and antioxidant activity may be affected not only by climate conditions but as well as agrochemical composition of the soil and fertilization (Huchette et al., 2005). A higher content of total polyphenols, sulphur compounds and antioxidant activity was observed in the studied varieties of garlic in the area of Nitra, which may correlate with higher levels of potassium and magnesium in the soil. Hamouz et al. (2010) also pointed out that the content of total polyphenols is affected by the content of potassium and magnesium in the soil. Mudau et al. (2007) state that higher potassium content positively affects the content of total polyphenols, at higher doses of potassium were recorded higher values of total polyphenols. According to Ruan et al. (1999) the polyphenol content in studied spring and autumn tea was enhanced by potassium application, while magnesium apparently decreased the polyphenol content. Kraus et al. (2004) reported that the site conditions affect plant phenolic concentrations and changes in soil pH affect nutrient availability. According to Ali et al. (2012) cultivation factors such as soil type, com-posts, mulching and fertilisation influence the water and nutrient supply to the plant and affect the nutritional composition and antioxidant activity. In their study higher total polyphenol content was determined by high K treatment. Climatic factors (such as temperature, sunny days, rainfall) and environmental factors, such as soil type, nutrient level and application strategy, influence the nutrient supply to the plant and could thereby affect concentrations and composition of the bioactive compounds.

Relations among the total polyphenols content, total sulfur content and the antioxidant activity in studied varieties of garlic (Mojmír, Záhorský, Lukan, Havran and Makoi) were evaluated (Figure 4-23). The coefficient of correlation (r = 0.9315 - 0.9978) confirmed strong dependency between the content of polyphenols, total sulfur content and the antioxidant activity and the results are in good agreement with the findings of Mahmutović et al. (2014), who confirmed correlations between total polyphenols content, total sulfur content and antioxidant activity in garlic. Ramkissoon et al. (2012), Chekki et al. (2014) indicated correlations between total polyphenols content and antioxidant activity in garlic, onion and other vegetable..

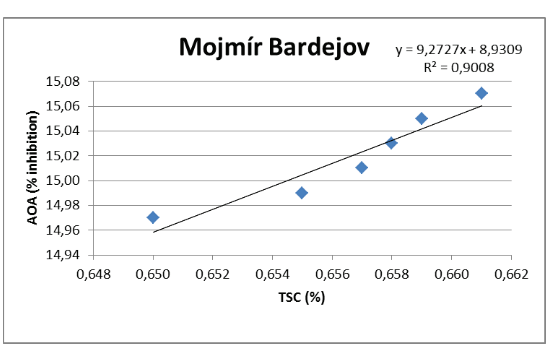



**Figure 4**: Relationship between TSC and AOA in 'Mojmír'

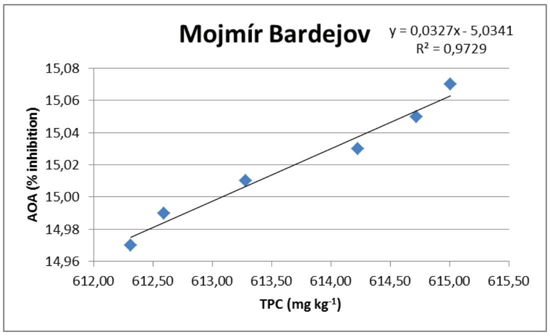



**Figure 5**: Relationship between TPC and AOA in 'Mojmír'

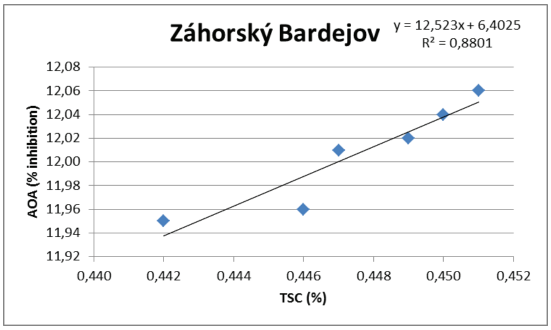



**Figure 6**: Relationship between TSC and AOA in 'Záhorský'

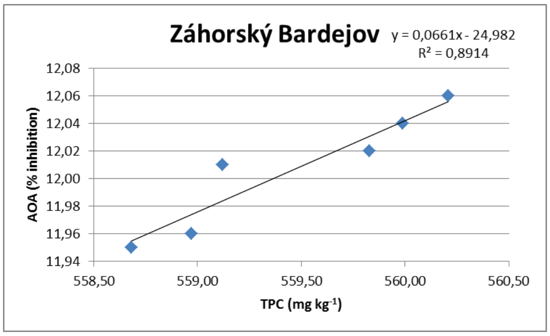



**Figure 7:** Relationship between TPC and AOA in 'Záhorský'

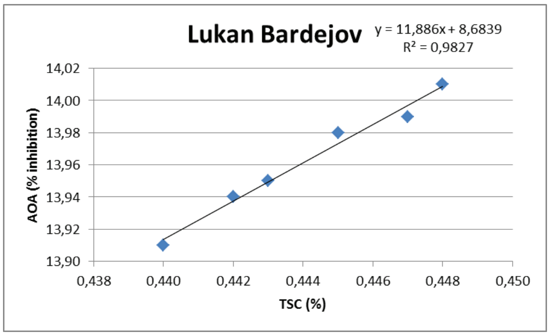



**Figure 8**: Relationship between TSC and AOA in 'Lukan'

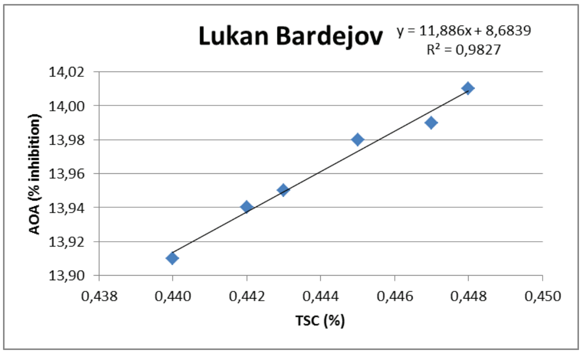



**Figure 9**: Relationship between TPC and AOA in 'Lukan'

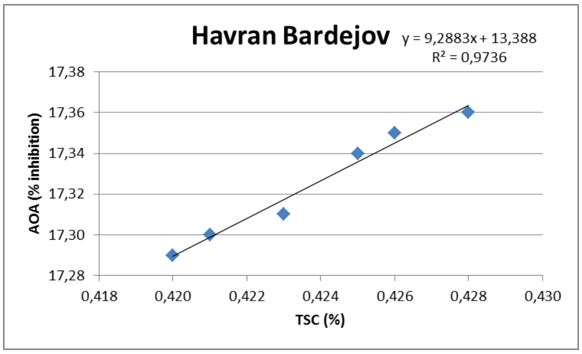



**Figure 10**: Relationship between TSC and AOA in 'Havran'

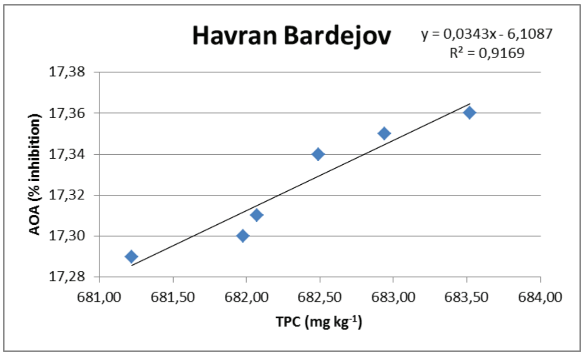



**Figure 11**: Relationship between TPC and AOA in 'Havran'

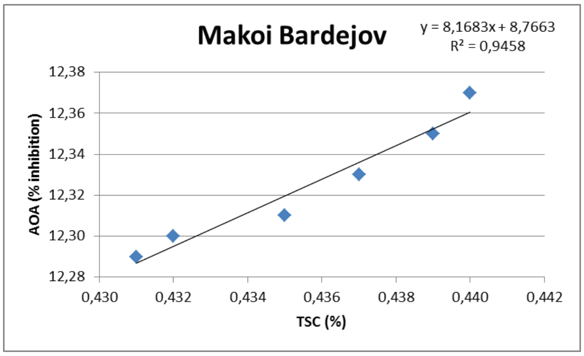



**Figure 12**: Relationship between TSC and AOA in 'Makoi'

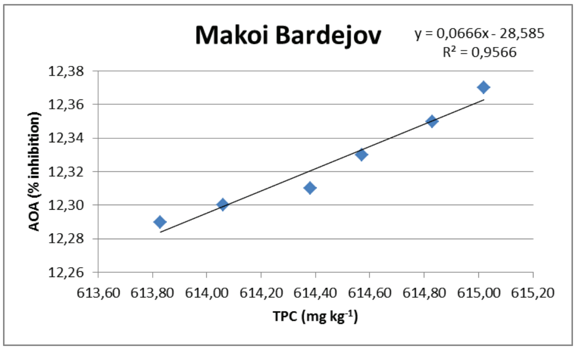



**Figure 13**: Relationship between TPC and AOA in 'Makoi'

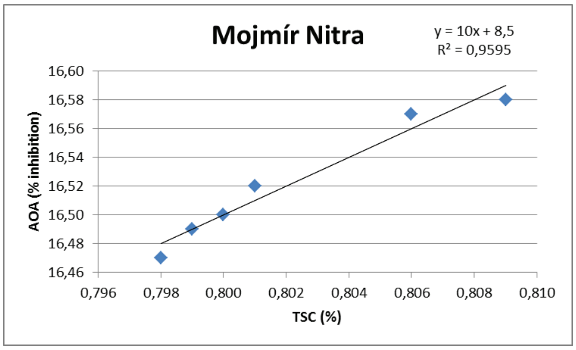



**Figure 14**: Relationship between TSC and AOA in 'Mojmír'

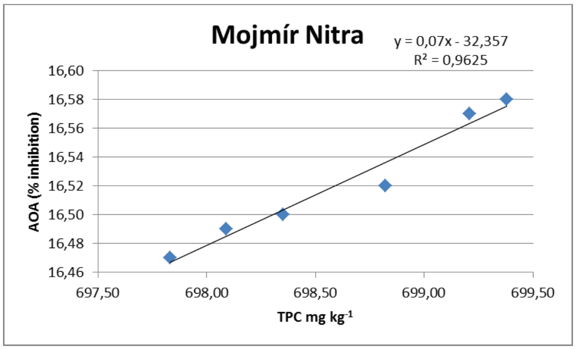



**Figure 15**: Relationship between TPC and AOA in 'Mojmír'

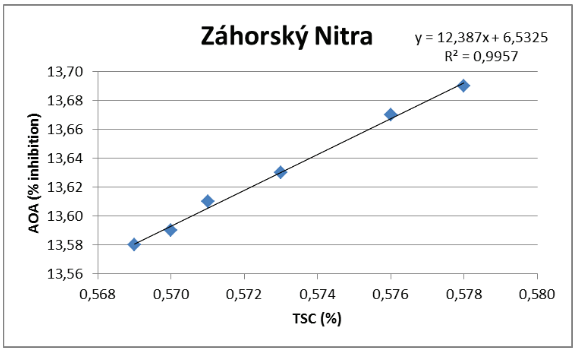



**Figure 16**: Relationship between TSC and AOA in 'Záhorský'

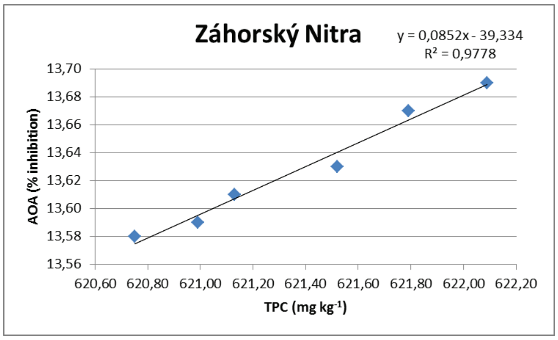



**Figure 17**: Relationship between TPC and AOA in 'Záhorský'

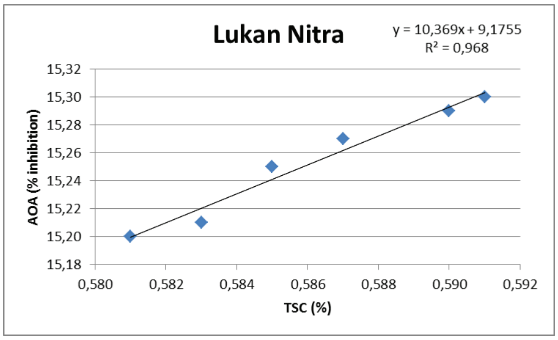



**Figure 18**: Relationship between TSC and AOA in 'Lukan'

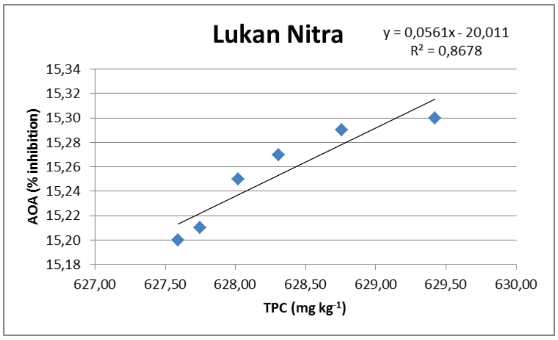



**Figure 19**: Relationship between TPC and AOA in 'Lukan'

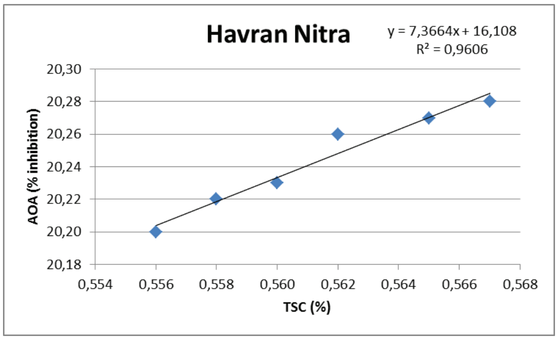



**Figure 20**: Relationship between TSC and AOA in 'Havran'

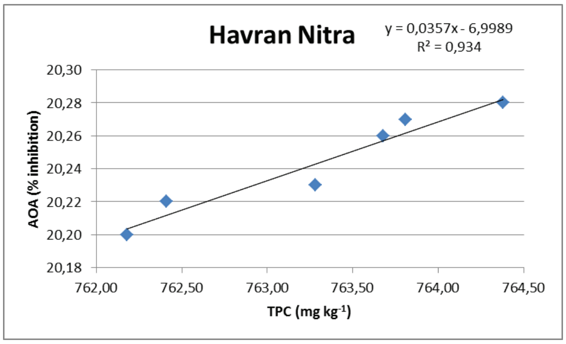



**Figure 21**: Relationship between TPC and AOA in 'Havran'

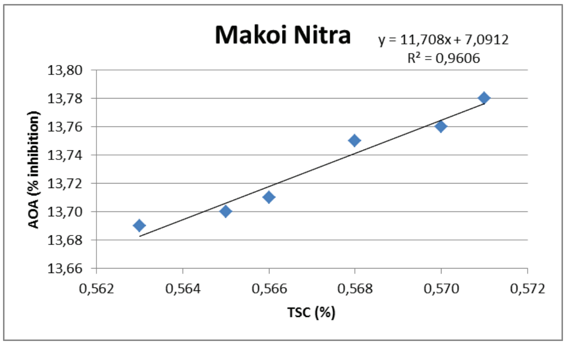



**Figure 22**: Relationship between TSC and AOA in 'Makoi'

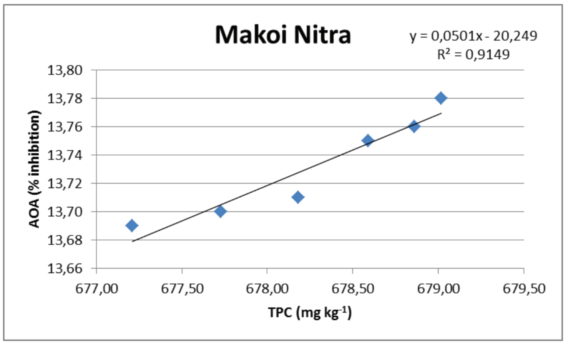



**Figure 23**: Relationship between TPC and AOA in 'Makoi'

## 4 CONCLUSIONS

The total polyphenols content, the total sulphur content and antioxidant activity in studed varieties of garlic in the both locality were comparable with the literature. We observed statistically significant differences between the analysed varieties of garlic and the monitored indicators. In this work was measured and evaluated the total polyphenols content, the total sulphur content and antioxidant activity of the studied varieties of garlic in the area of Nitra and Bardejov, Slovak Republic. Higher values of the monitored parameters (TPC, TSC, and AOA) were measured in the area of Nitra. This area is characterized by a higher number of sunny days with a higher average annual temperature, lower annual rainfall and lower altitude. The coefficient of correlation confirmed strong dependency between the total content of polyphenols, total sulphur content and the antioxidant activity. Climatic factors (such as temperature, sunny days, rainfall) and environmental factors, such as soil type, nutrient level and application strategy, influence the nutrient supply to the plant and could thereby affect concentrations and composition of the bioactive compounds in garlic.

## 5 ACKNOWLEDGEMENTS

This work was supported by scientific grant VEGA 1/0139/17, VEGA 1/0114/18, KEGA 011SPU-4/2017.

## 6 REFERENCES

Ali, L., Alsanius, B., Rosberg, A., Svensson, B., Nielsen, T., Olsson, M. E. (2012). Effects of nutrition strategy on the levels of nutrients and bioactive compounds in blackberries. *European Food Research and Technology, 234*(1), 33-44. doi:10.1007/s00217-011-1604-8

Amagase, H., Petesch, B. L., Matsuura, H., Kasuga, S., Itakura, Y. (2001). Intake of Garlic and Its Bioactive Components. *The Journal of Nutrition*, *131*, 955-962. doi:10.1093/jn/131.3.955S

Benkeblia, N., & Lanzotti, V. (2007). Allium Thiosulfinates: chemistry, biological properties and their potential utilization in food preservation. *Food, 1*(2), 193-201.

Bhuiyan, A., Papajani, V. T., Paci, M., Melino, S. (2015). Glutathione-Garlic Sulfur Conjugates: Slow Hydrogen Sulfide Releasing Agents for Therapeutic Applocations. *Molecules*, *20*, 1731-1750. doi:10.3390/molecules20011731

Borek, C. (2001). Antioxidant Health Effects of Aged Garlic extracts. *The Journal of Nutrition*, *131*(3), 1010-1015. doi:10.1093/jn/131.3.1010S

Brand-Williams, E., Cuverlier, M. E., Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. *Lebensmittel Wissenschaft und Techologie*, *11*, 925-929. doi:10.1016/S0023-6438(95)80008-5

Butt, S. M., & Sultan, T. M. (2009). Garlic: Nature´s Protection Against Physiological Threats. *Food Science and Nutrition*, *49*(10), 538-551. doi:10.1080/10408390802145344

Charles, D. J. (2013). *Antioxidant properties of spices, herbs and other sources*. New York: Springer science. doi:10.1007/978-1-4614-4310-0

Chekki, R. Z., Snoussi, A., Hamrouni, I., Bouzouita, N. (2014). Chemical composition, antibacterial and antioxidant activities of Tunisian garlic (Allium sativum) essential oil and ethanol extract. *Mediterranean Journal of Chemistry*, *3*(4), 947-956. doi:10.13171/mjc.3.4.2014.09.07.11

Chekki, R., Najjaa, H., Zouari, N., Máthé, Á., Bouzouita, N. (2016). Detection of organo-sulphur volatiles in Allium sativum by factorial design. *Natural Products Chemistry & Research*, *4*, 211. doi:10.4172/2329-6836.1000211

Choi, S., Cha, H. S., Lee, Y. S. (2014). Physicochemical and antioxidant properties of black garlic. *Molecules*, *19*, 16811-16823. doi:10.3390/molecules191016811

Fertout-Mouri, N., Latreche, A., Mehdadi, Z., Bénali, F. T., Bassou. (2014). The Effect of Altitude and Development stage on the Synthetic Activity of some Polyphenols in Teucrium polium L. in Tessala Mountains (Western Algeria). *Advances in Environmental Biology*, *8*, 193-201.

Fulder, S. (2002). *O česneku*. Praha: Pragma.

Ghasemi, K., Ghasemi, Y., Ehteshamia, A., Nabavi, S. M., Nabavi, S. F., Ebrahimzadeh, M. H., Pourmord, H. (2011). Influence of Environmental Factors on Antioxidant Activity, Phenol and Flavonoids Contents of walut (Juglans regia L.) green huskd. *Journal of Medicinal Plants Research*, *5*, 1128-1133.

Hamouz, K., Lachman, J., Hejtmánková, K., Pazderů, K., Čížek, M., Dvořák, P. (2010). Effect of natural and growing conditions on the content of phenolics in potatoes with different flesh color. *Plant, Soil and Environment*, *56*(8), 368-374. doi:10.17221/49/2010-PSE

Hamzah, R. U., Jigam, A. A., Makun, H. A., Egwin, E. C. (2013). Antioxidant properties of selected African vegetables, fruits and mushrooms: A Review. *Intech*, 203-250. doi:10.5772/52771

Harris, J. C. (2004). Antimicrobial properties of Allium sativum. *Revija Applied Microbiology and Biotechnology*, *57*(3), 282-286. doi:10.1007/s002530100722

Huchette, O., Kahane, R., Auger, J., Bellamy, C. (2005). Influence of environmental and genetic factors on the alliin content of garlic bulbs. *Acta Horticulturae*, *688*, 93-100. doi:10.17660/ActaHortic.2005.688.9

Iciek, M., Kwiecien, I., Chwatko, G., Jezewicz, M. S., Pachel, D. K., Rokita, H. (2012). The effect of garlic-derived sulphur compounds on cell proliferation, caspase 3 activity, thiol levels ad anaerobic sulphur metabolism in human hepatoblastoma. *Cell Biochemistry and Function*, *30*, 198-204. doi:10.1002/cbf.1835

Jastrzebski, Z., Leontowicz, M., Namiesnik, J., Zachwieja, Z., Barton, H., Pawelzik, E., Arancibia-Avila, P., Toledo, F., Gorinstein, S. (2007). The bioactivity of processed garlic (Allium sativum L.) as shown in vitro and in vivo studies on rats. *Food and Chemical Toxicology*, *45*(9), 1626-1633. doi:10.1016/j.fct.2007.02.028

Kačániová, M., & Tančinová, D. (2012). *Prediktívna mikrobiológia v potravinárstve*. Nitra: SPU.

Khodadadi, S., Nejadsattari, T., Naqinezhad, A., Ebrahimzadeh, M. A. (2015). Diversity in antioxidant properties and mineral contents of Allium paradoxum in the Hyrcanian forests, Northern Iran. *Biodiversitas*, *16*(2), 281-287. doi:10.13057/biodiv/d160224

Knežević, V. S., Blažeković, B., Bival, Š. B., Babac, M. (2012). Plant Polyphenols as Antioxidant Influencing the Human Health. *Phytochemicals as Nutraceuticals – Global Approaches to their Role in Nutrition and Health*, *9*, 156-180.

Kraus, T. E. C., Zasoski, R. J., Dahlgren, R. A. (2004). Fertility and pH effects on polyphenol and condensed tannin concentrations in foliage and roots. *Plant Soil*, *262*, 95–109. doi:10.1023/B:PLSO.0000037021.41066.79

Lachman, J., Hejtmánková, A., Dudjak, E. (2003). Content polyphenolic antioxidants and phenolcarboxylic acids in selected parts of yacon. *Vitamins 2003- Přírodní antioxidanty a volné radikály*. Pardubice.

Lanzotti, V. (2006). The analysis of onion and garlic. In *Journal of Chromatography A*, *1112*(1), 3-22. doi:10.1016/j.chroma.2005.12.016

Mahmutović, O., Tahirović, I., Copra, A., Memić, M., Ibragić, S., Karić, L. (2014). Correlation of Total Secondary Sulfur compounds, Total Phenols and Antioxidant capacity in the Ramsons and Garlic. *British Journal of Pharmaceutical Research*, *4*(23), 2662-2669. doi:10.9734/BJPR/2014/13977

Mudau, F. F., Saondy, P., Toit, E. S. (2007). Effects of nitrogen, phosphorus and potassium nutrition on total polyphenol content of Bush Tea (Athrixia phylicoides L.) Leaves in Shaded Nursery Environment. *Horticultural Science*, *42*, 334-338.

Munch, R. (2013). Deodorization of garlic breathe volatiles by food and food components. *Graduate Program in Food Science & Technology*, 66. doi:10.1111/1750-3841.12394

Muradić, S., Karačić, D., Mahmutović, O., Mutović, F., Sofić, E., Kroyer, G. (2010). Total sulphur and organosulphur compounds in garlic and ramsons plant organs at the end of vegetative period. *Planta Medica*, *76*, 292. doi:10.1055/s-0030-1264590

Narendhirakannan, R. T., Rajeswari, K. (2010). In vitro antioxidant properties of three varieties of Allium sativum L. extracts. *Journal of Chemistry*, *7*, 573-579. doi:10.1155/2010/283627

Oberbeil, K., & Lentzová, CH. (2005). *Ovocie a zelenina ako liek*. Bratislava: Fortuna.

Obied, H. K. (2013). Biography of biophenols: past, present and future. *Functional Foods In Health and Disease*, *3*(6), 230-241. doi:10.31989/ffhd.v3i6.51

Pereira, D., Valentao, P., Pereira, J. A., Andrade, P. B. (2009). Phenolics: From Chemistry to Biology. *Molecules*, *14*, 2202-2211. doi:10.3390/molecules14062202

Petrovská, B. B., & Čekovská, S. (2010). Extracts from the history and medical properties of garlic. *Pharmacognosy Review*, *4*(7), 106-110. doi:10.4103/0973-7847.65321

Piazzon, A., Vrhovšek, U., Masuero, D., Mattivi, F., Mandoj, F., Nardini, M. (2012). Antioxidant activity of Phenolic acids and their Metabolites: Synthesis and Antioxidant properties of the sulfate derivatives of ferulic and coffeic acids and of the acyl glucuronide of ferulic acids. *Journal of Pharmacy and Pharmacology*, *64*(8), 1119-1127. https://doi:10.1021/jf304076

Rai, CH., Bhattacharjee, S., Nandi, N., Bhattacharyya, S. (2015). Influence of blanching on antioxidant and antimicrobial activities of raw garlic (Allium sativum). *Indo American Journal of Pharmaceutical sciences*, *2*(6), 1071-1076.

Ramkissoon, H., Weiler, B., Smith, L. D. G. (2012). Place attachment and pro-environmental behaviour in national parks: the development of a conceptual framework. *Journal of Sustainable Tourism*, *20*(2), 257-276. doi:10.1080/09669582.2011.602194

Ruan, J. Y., Wu, X., Härdter, R. (1999). Effects of potassium and magnesium nutrition on quality components of different types of tea. *Journal of the Science of Food and Agriculture*, *79*(1), 47-52. doi:10.1002/(SICI)1097-0010(199901)79:1<47::AID-JSFA172>3.0.CO;2-A

Sárosi, S., Bernáth, J., Burchi, G., Antonetti, M. (2011). Effect of different plant origins and climatic conditions on the total phenolic content and total antioxidant capacity of self-heal (Prunella vulgaris L.). *Acta horticulturae*, *925*, 49-55. doi:10.17660/ActaHortic.2011.925.5

Srivastava, M. P., Tiwari, R., Sharma, N. (2013). Assessment of phenol and flavonoid content in the plant materials. *Journal on New Biological Reports*, *2*(2), 163-166.

Suleria, H. A. R., Butt, M. S., Khalid, N., Sultan, S., Raza, A., Aleem, M., Abbas, M. (2015). Garlic (Allium sativum): diet based therapy of 21st century. *Asian Pacific Journal of Tropical Disease*, *5*(4), 271-278. doi:10.1016/S2222-1808(14)60782-9

Sultan, M. T., Butt, M. S., Qayyum, M. M., Suleria, H. A. (2014). Imunnity: plants as effective mediators. *Critical Reviews in Food Science and Nutrition*, 54(10), 1298-1308. doi:10.1080/10408398.2011.633249

Süli, J., Homzová, K., Sobeková, A., Hrušková, T. (2014). Fyziologické účinky polyfenolov a ich metabolitov v strave. *Diabetologie Metabolismus Endokrinologie Vyživa*, *17*(3), 162-170.

Šapčanin, A., Jancan, G., Pazalja, M., Kresić, D., Pehlić, E., Uzunović, A. (2013). Determination of Total Sulphur Content in Biological Samples by Using High Performance Ion Chromatography and Elemental Analysis. *Bulletin of the Chemists and Technologists of Bosnia and Herzegovina*, *41*, 11-14.

Viljevac Vuletić, M., Dugalić, K., Mihaljević, I., Tomaš, V., Vuković, D., Zdunić, Z., Puškar, B., Jurković, Z. (2017) Season, location and cultivar influence on bioactive compounds of sour cherry fruits. *Plant, Soil Environment*, *63*, 389–395. doi:10.17221/472/2017-PSE

Wangcharoen, W., & Morasuk, W. (2009) Effect of heat treatment on the antioxidant capacity of garlic. Maejo *International Journal of Science and Technology*, *3*(01), 60-70. doi:10.2306/scienceasia1513-1874.2013.39.246

Xiao, J. B., Kai, G. Y., Yamamoto, K., Chen, X. Q. (2013). Advance in Dietary Polyphenols as α-glucosidases inhibitors: A Review on structure-activity relationship aspect. *Critical Reviews in Food Science and Nutrition*, *53*, 818-836. doi:10.1080/10408398.2011.561379

doi:10.14720/aas.2018.111.3.08 Original research article / izvirni znanstveni članek

# Salicylic acid and jasmonic acid alter physiological performance, assimilate mobilization and seed filling of soybean under salt stress

Kazem GHASSEMI-GOLEZANI[1*], Salar FARHANGI-ABRIZ[1], Ali BANDEHAGH[2]

Received April 30, 2018; accepted October 22, 2018.
Delo je prispelo 30. aprila 2018, sprejeto 22. oktobra 2018.

## ABSTRACT

This research was conducted to investigate the morpho-physiological effects of salicylic acid and jasmonic acid on soybean performance and productivity under salinity. Leaf chlorophyll content index, carotenoids and anthocyanins content, photosystem II efficiency, relative water content, leaf area, leaf mass, specific leaf area, water use efficiency, seed filling duration, assimilate mobilization efficiency and seed mass decreased, but leaf temperature, specific leaf mass and electrolytic leakage of leaves increased with enhancing salinity. Salicylic acid improved leaf chlorophyll content index, anthocyanins content, leaf area, specific leaf area, water use efficiency, seed filing duration, assimilate mobilization efficiency and seed mass under both saline and non-saline conditions. The superior effects of salicylic acid on some traits such as maximum quantum yield of PSII, relative water content and leaf electrolytic leakage only occurred under different salinity levels. Jasmonic acid improved leaf mass, specific leaf mass, carotenoids content, relative water content, seed filling rate and reduced chlorophyll content index, leaf temperature, leaf area, specific leaf area, seed filling duration, assimilates mobilization efficiency and relative electrolytic leakage of soybean, with no significant effects on photosystem II efficiency and seed mass. Application of salicylic acid was, therefore, the superior treatment for enhancing physiological performance and seed mass of soybean plants under different salinity levels.

**Key words:** chlorophyll content; jasmonic acid; salicylic acid; salinity; seed production; soybean

## IZVLEČEK

### SALICILNA IN JASMONSKA KISLINA SPREMINJATA FIZIOLOŠKE PROCESE, MOBILIZACIJO ASIMILATOV IN POLNJENJE SEMEN SOJE V RAZMERAH SOLNEGA STRESA

Raziskava je bila izvedena za preučitev morfo-fizioloških učinkov salicilne in jasmonske kisline na rast in produktivnost soje v razmerah slanosti. Parametri kot so indeks vsebnosti klorofila listov, vsebnost karotenoidov in antocianinov, učinkovitost fotosistema II, relativna vsebnost vode, listna površina, listna masa, specifična listna površina, učinkovitost izrabe vode, trajanje polnjenja semen, učinkovitost mobilizacije asimilatov in masa semen so se zmanjšali medtem, ko so se parametri kot so temperatura listov, specifična listna masa in puščanje elktrolitov povečali z naraščajočo slanostjo. Salicilna kislina je izboljšala indeks vsebnosti klorofila listov, povečala vsebnost antocianinov, listno površino, specifično listno površino, učinkovitost izrabe vode, trajanje polnjenja semen, učinkovitost mobilizacije asimilatov in maso semen v razmerah slanosti in brez nje. Večji učinki salicilne kisline na nekatere preučevane parametre kot so maksimalna učinkovitost PSII, relativna vsebnost vode in puščanje elektrolitov iz listov so se pojavili samo v nekaterih ravneh slanosti. Jasmonska kislina je povečala maso listov, specifično listno maso, vsebnost karotenoidov, relativno vsebnost vode, hitrost polnjenja semena in zmanjšala indeks vsebnosti klorofilov, temperaturo listov, listno površino, specifično listno površino, trajanje polnjenja semena, učinkovitost mobilizacije asimilatov in relativno puščanje elktrolitov brez značilnih učinkov na učinkovitost PSII in maso semen. Uporaba salicilne kisline je bilo primernejše obravnavanje za povečanje fiziološke aktivnosti in mase semen soje v različnih razmerah slanosti.

**Ključne besede:** vsebnost klorofila; jasmonska kislina; salicilna kislina; slanost; tvorba semena; soja

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

[2] Department of Plant Breading and Biotechnology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

# 1 INTRODUCTION

Soybean originates from China and is a major source of protein and oil for humans and as a high-quality animal feed. Soybean is a crop sensitive-to-moderately tolerant to salinity (Luo et al., 2005). Salinity enhances ion toxicity and reduces photosynthesis. The reduction in photosynthesis under salinity could be attributed to a decrease in chlorophyll and other photosynthetic pigments contents (Sali et al., 2015). Salinity can affect the chlorophyll content through inhibition of chlorophyll synthesis or an acceleration of its degradation (Reddy & Vora, 1986). The maximum quantum yield of PSII (Fv/Fm) could also be reduced by salt stress. Disturbances of photosynthesis at the molecular level are related to the restricted electron transport through PSII and/or with structural injuries to PSII (Ghassemi-Golezani & Lotfi, 2015). Under environmental stresses such as drought and salinity, plant leaves are dehydrated, and photosynthesis is decreased. The decrease in photosynthesis of dehydrated leaves usually results from a decrease in stomatal conductance and transpiration (Mirfattahi et al., 2017). As a consequence of the reduction in transpiration rate, leaf temperature increases (Mohammadian et al., 2015). Salinity can also reduce seed yield by decreasing physiological performance and accelerating seed filling period (Ghassemi-Golezani et al., 2010).

Growth inhibition, poor performance and decreasing seed yield of plants under salinity are attributed to osmotic stress imposed by salinity and to specific ion ($Na^+$) toxicity (Farhangi-Abriz & Ghassemi-Golezani, 2018). In soybean, the number of seeds, pods and yield are directly related to the number of flowers per nodes and node number can be reduced by environmental stresses (Jiang & Egli, 1993). Moreover, several reports have shown that salinity increases the reactive oxygen species (ROS) and decreases seed filling duration (Ghassemi-Golezani et al., 2010; Farhangi-Abriz & Ghassemi-Golezani, 2018). In soybean seeds, the dynamic seed 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 mass maturity. Mass maturity (end of seed filling phase) in soybean cultivars can be classified according to the duration of the life cycle, being early, semi-early, medium, late medium and late, according to Oya et al. (2004) and Ghassemi-Golezani et al. (2010). Ghassemi-Golezani et al. (2010) showed that salinity reduced seed filling period and yield in different cultivars of soybean.

Application of some growth regulators or plant hormones has been expanded to improve plant growth and development under stress and non-stress conditions. Salicylic acid (SA) is a plant phenolic compound and now considered as a hormone-like endogenous regulator. It plays diverse physiological roles in plants, which include plant growth, flower induction, nutrient uptake, stomatal movements, photosynthesis and enzyme activities (Hayat et al., 2007). Former reports show that SA plays important regulatory roles in plants against a wide range of environmental stresses (Choudhury & Panda, 2004; Gunes et al., 2007). Application of SA improves physiological performance of the crops (Shi et al., 2006; Farhangi-Abriz & Ghassemi-Golezani et al., 2018). Ghassemi-Golezani & Farhangi-Abriz (2018a) also reported that foliar application of SA stimulates the $H^+$-ATPase activity of tonoplast, nutrient uptake and salt tolerance of soybean.

Jasmonates are ubiquitously-occurring lipid-derived compounds with signal function in plant responses to abiotic and biotic stresses, as well as in plant growth and development (Wasternack, 2007). Foliar application of jasmonic acid (JA) modulates several physiological responses, improving resistance against abiotic stresses (Farhangi-Abriz & Ghassemi-Golezani, 2018). JA application to the stressed plants reduces the amount of lipid peroxidation and stimulates the synthesis of antioxidant enzymes, enhancing the physiological performance and seed yield of *Artemisia* (Aftab et al., 2011). Previous studies have helped to understand the effects of SA and JA on decreasing destructive effects of salt toxicity in plants (Farhangi-Abriz & Ghassemi-Golezani, 2018), however the possible effects of SA and JA on the productivity of soybean under salt stress were not documented so far. Thus, this research was conducted to investigate: 1) the effects of SA and JA on some morpho-physiological traits of soybean under salt toxicity and 2) the effects of these growth regulators on soybean grain filling and productivity under saline and non-saline conditions.

# 2 MATERIALS AND METHODS

## 2.1 Experimental conditions

This experiment was carried out in 2016 with a factorial arrangement on the basis of randomized complete block design with four replications at the greenhouse of the University of Tabriz, Iran. In each plastic pot, containing 1 kg perlite, seeds of soybean (M7 cultivar) were sown and then tap water (non-saline) and saline solutions (4, 7 and 10 dS $m^{-1}$) were added to achieve 100 % FC (Field capacity). All pots were placed in a glass greenhouse with a day-night mean temperatures of 28-26 ºC and 35-40 relative humidity, natural light intensify and photoperiod. During the growth period, the pots were weighed and the losses were made up with the Hoagland solution (electrical conductivity = 1.3 dS $m^{-1}$, pH 6.7–7.2). For avoiding extra electrical conductivity (EC), due to adding the Hoagland solution, perlites within the pots were washed every 30 days and non-saline and salinity treatments were reapplied. Salicylic acid (1 mM) and jasmonic acid (0.5 mM) were separately sprayed on plants at vegetative (V3) and flowering stages (R2).

## 2.2 Chlorophyll content index (CCI)

Leaf CCI was measured using a portable chlorophyll meter (CCM-200, Opti-Sciences, USA). Two plants were selected in each pot and the CCI of the upper, middle and lower leaves of each plant was measured at the beginning of pod development (R3). Subsequently, the mean CCI for each treatment at each replicate was determined.

## 2.3 Anthocyanins and carotenoids contents

About 0.2 g of leaf samples were homogenized in 4 ml acetone (80 %), and then centrifuged at 12,000 g for 20 min at 4 °C. The supernatant was collected and absorbance was recorded at 480 nm and 510 nm, using a spectrophotometer (Model Analytikjena Spekol 1500 Germany) (Maclachlan & Zalik, 1963). Another 0.2 g of leaf sample was homogenized in 3 ml extraction mixture (0.6 ml water, 2.37 ml methanol and 0.03 ml HCl) followed by centrifugation at 12,000 g for 20 min at 4 °C. The absorbance of supernatant was read at 530 nm and 657 nm (Mancinelli, 1984).

## 2.4 Maximum quantum yield of PSII (Fv/Fm)

At early pod filling stage, maximum quantum yield of PSII ($F_v/F_m$) was measured using a portable chlorophyll fluorometer (OS-30, Opti-Sciences, USA). Dark-adapted leaves (30 min) were initially exposed to the weak modulate measuring beam, followed by exposure to saturated white light to estimate the initial ($F_0$) and maximum ($F_m$) fluorescence values, respectively. Variable fluorescence ($F_v$) was calculated by subtracting $F_0$ from $F_m$.

## 2.5 Leaf temperature (LT)

Two plants were selected in each pot and LT (°C) of upper, middle and lower leaves were measured by an infrared thermometer (TES-1327) at beginning pod development stage. Then the mean temperature for each treatment was determined.

## 2.6 Leaf area (LA) and dry mass (LDM)

LA per plant was measured at the beginning pod formation stage using a leaf area meter (ADC-AM 300). Leaves per plant were dried in an oven at 80 °C for 48 h and then weighed. After determining leaf area and leaf mass, specific leaf area (SLA) and specific leaf mass (SLM) were calculated as:

SLA= LA ($mm^2$) / LDM (mg)
SLM= LDM (mg) / LA ($mm^2$)

## 2.7 Relative electrolytic leakage (REL)

About 1 g of leaf sample was washed with deionized water and then placed in tubes with 15 ml of deionized water and incubated for 2 hours at 25 °C. Subsequently, the electrical conductivity of the solution ($L_1$) was determined. Samples were then autoclaved at 100 °C for 30 minutes, and the conductivity ($L_2$) was recorded after cooling to 25°C. Then, the EL was calculated as (Lutts et al., 1996).

$$REL\ (\%) = \left(\frac{L1}{L2}\right) \times 100$$

## 2.8 Relative water content (RWC)

Relative water content of soybean leaves was determined according to Barrs and Weatherley. (1962) method. At the beginning of pod development, fresh mass ($F_M$) of the youngest fully expanded leaf was recorded. Turgid mass ($T_M$) was obtained after waterlogged the leaf for 24 h in distilled water. Finally, leaf dry mass ($D_M$) was determined after drying at the 80 °C for 24 h. The value of RWC was calculated using the following equation:

$$RWC = [(F_M - D_M) / (T_M - D_M)] \times 100$$

## 2.9 Water use efficiency (WUE)

Increasing seed mass per plant during seed development was estimated as the difference between the seed mass at the beginning and the end of the seed filling period. Crop water used up over these periods was estimated from the sum of the per diem water intake to the pots. WUE was determined as:

WUE (g $l^{-1}$) = (seed mass at final stage - seed mass at first stage)/total water used up

## 2.10 Rate and duration of seed filling

During seed filling from seed formation (R5) up to full maturity (R8), two plants were harvested from each pot in 10 days' intervals at five stages. Seeds of each sample were oven-dried at 80 ºC for 48 h and then seed dry mass was determined. Maximum seed mass and seed filling duration were estimated, using a two-piece regression model by SAS 9.1.3 software:

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

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

SFR = MSM/SFD

Where MSM is maximum seed mass and SFD is seed filling duration.

### 2.11 Assimilate mobilization efficiency

Assimilate mobilization efficiency in the seeds was calculated, using seed mass at the first and final stages of seed filling:

$AME = (SM_F - SM_P)/SW_F \times 100$

Where AME is the assimilate mobilization efficiency during seed filling, $SM_F$ is the amount of seeds mass at the final stage (mg) and $SM_P$ is the amount of assimilates at the primary stage (mg).

### 2.12 Yield components

At maturity (R8), two plants from each pot were harvested and pods and seeds per plant were determined.

### 2.13 Analysis of Variance

After testing the normality and homogeneity of variance by Shapiro–Wilk test, the data were analyzed and the means were compared at $p \leq 0.05$ by Duncan multiple range test, using MSTATC software. The figures were drawn by Excel software.

## 3 RESULTS

### 3.1 Chlorophyll content index (CCI)

Analysis of variance showed significant effects of salinity and hormonal application on soybean CCI. The CCI was decreased with increasing salinity. Plants with SA treatment showed the highest level of the CCI, but JA reduced the CCI (Table 1).

### 3.2 Carotenoids and anthocyanins

The carotenoids and anthocyanins in soybean leaves were significantly affected by salinity and hormonal treatments. Salinity reduced leaf carotenoids and anthocyanins, but treatment with JA increased these pigments. Treatment with SA only improved anthocyanins with no tangible effect on carotenoids (Table 1).

### 3.3 Leaf temperature

The effects of salinity and hormonal sprays on leaf temperature were significant. The leaf temperature increased as a consequence of enhancing salinity. Foliar application of SA and JA significantly reduced the leaf temperature (Table 1).

**Table 1**: **:** Means of morpho-physiological traits of soybean leaf under different levels of salinity and hormonal treatments

| Treatments | Chlorophyll content index | Carotenoid (mg $g^{-1}$ DM) | Anthocyanin (mg $g^{-1}$ DM) | Leaf temperature (°C) | Leaf area ($mm^2$) | Leaf dry mass (mg) | Specific leaf area ($mm^2$ $mg^{-1}$) | Specific leaf mass (mg $mm^{-2}$) |
|---|---|---|---|---|---|---|---|---|
| Salinity | | | | | | | | |
| 0 dS $m^{-1}$ | 20.63a | 0.71a | 0.28a | 20.26d | 26790a | 2110a | 12.68a | 0.078b |
| 4 dS $m^{-1}$ | 19.40b | 0.67b | 0.24b | 20.99c | 26860a | 2120a | 12.64a | 0.079b |
| 7 dS $m^{-1}$ | 18.24c | 0.55c | 0.20c | 23.15b | 21570b | 1910b | 11.27b | 0.090a |
| 10 dS $m^{-1}$ | 17.12d | 0.45d | 0.17d | 24.25a | 18610c | 1700c | 10.95c | 0.091a |
| Hormonal treatments | | | | | | | | |
| Con | 18.83b | 0.56b | 0.19c | 22.54a | 23430b | 1940b | 11.88b | 0.084b |
| SA | 20.06a | 0.58b | 0.22b | 21.85b | 24680a | 1980b | 12.45a | 0.081b |
| JA | 17.65c | 0.63a | 0.26a | 22.10b | 22260c | 2140a | 10.41c | 0.097a |

Con: Control, SA: Salicylic acid, JA: Jasmonic acid

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

### 3.4 Leaf area and mass

Leaf area, leaf dry mass, specific leaf area and specific leaf mass were significantly affected by salinity and hormonal treatments. Leaf area and dry mass were reduced with enhancing salinity but the difference between non-saline and low salinity conditions were not significant. Foliar application of SA improved the leaf area with no significant effect on leaf mass, but JA showed vice-versa effect, despite reducing leaf area, increased leaf dry mass. Specific leaf area was reduced, but specific leaf mass was elevated under moderate and severe salinity levels. Application of SA increased specific leaf area, but had no significant effect on specific leaf mass. Treatment of plants with JA reduced the specific leaf area and increased specific leaf mass in comparison with control plants (Table 1).

### 3.5 Maximum quantum yield of PSII (Fv/Fm)

Interaction of salinity × hormone for maximum quantum yield of PSII was significant. Fv/Fm was not changed significantly at 4 dS $m^{-1}$ salinity, but thereafter it was diminished with enhanced salinity. The Fv/Fm for JA and SA treated plants were statistically similar up to 7 dS $m^{-1}$, but SA treated plants in comparison with control and JA treatments showed the highest maximum quantum yield of PSII under severe salt stress (Fig. 1).

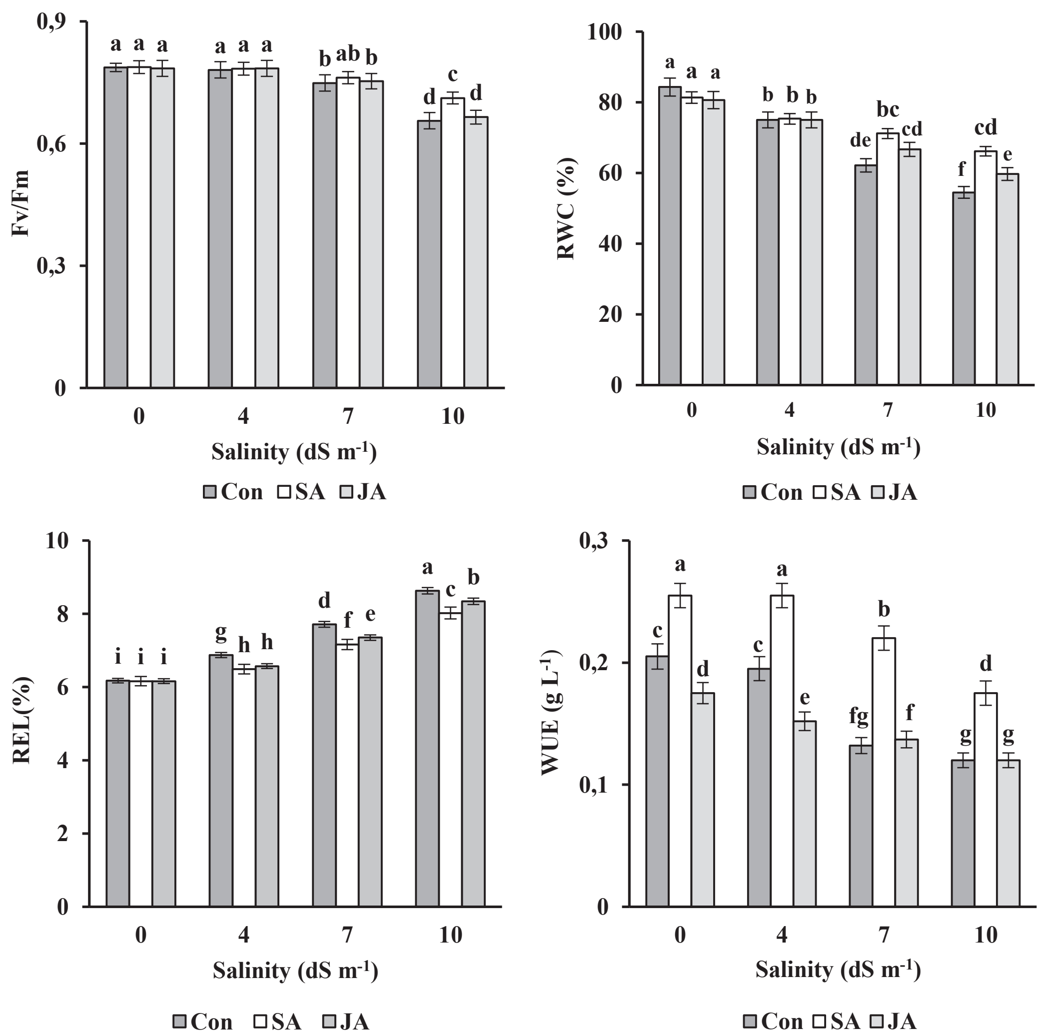



**Figure 1:** Changes in maximum quantum yield of PSII (Fv/Fm), relative electrolytic leakage (REL), water use efficiency (WUE) and relative water content (RWC) of soybean leaves under different levels of salinity and hormonal treatments. Different letters indicate significant difference at $p \leq 0.05$. Con: control, SA: salicylic acid and JA: Jasmonic acid

### 3.6 Leaf electrolytic leakage

Relative electrolytic leakage of soybean leaves was significantly enhanced by rising salt stress. Treatment of plants with SA and JA reduced the relative electrolytic leakage of soybean leaves under different levels of salt stress, but these hormonal treatments did not change this trait under non-saline condition (Fig. 1).

### 3.7 Relative water content (RWC)

The interaction of salinity × hormone for relative water content of soybean leaves was significant. Leaf relative water content was decreased as salt stress increased. Exogenous application of JA and SA under non-saline and low salinity conditions had no significant effect on RWC, but these treatments improved relative water content of soybean leaves under moderate and severe salinities (Fig. 1).

### 3.8 Rate and duration of seed filling

Seed mass was increased by increasing seed filling up to 39-56 days after flowering, depending on salinity level and hormonal treatments. Salinity reduced seed filling duration and increased seed filling rate. Maximum seed mass under salinity treatments was achieved about 6-12 days earlier than that under non-saline condition, and reduced with enhancing salinity levels. Treatment with SA under all salinity levels with rising seed filling duration increased maximum seed mass. Under non and low salinity levels, treatment with JA reduced seed filling duration, but in moderate and severe salinity levels, this reduction was not significant. JA increased seed filling rate of plants under all salinity levels, with no significant effect on control plants. Seeds from JA treated plants did not show significant difference with control plants in maximum seed mass (Fig. 2 and Tables 2 and 3).

**Table 2:** Means of soybean seed filling duration, rate and assimilate mobilization efficiency under different salinity and hormonal treatments

| Salinity (dS $m^{-1}$) | Hormonal treatments | Seed filling duration (day) | Seed filling rate (mg $day^{-1}$) | Assimilate mobilization efficiency (%) |
|---|---|---|---|---|
| 0 | Con | 44.75b | 2.28ef | 66.24d |
| | SA | 47.02a | 2.28ef | 68.78bc |
| | JA | 42.02d | 2.34e | 61.46f |
| 4 | Con | 42.91c | 2.46cd | 70.03b |
| | SA | 47.72a | 2.21f | 74.31a |
| | JA | 39.05f | 2.57ab | 64.08e |
| 7 | Con | 37.24g | 2.44cd | 63.77e |
| | SA | 42.02d | 2.38de | 65.70d |
| | JA | 36.86g | 2.61ab | 64.01e |
| 10 | Con | 34.35h | 2.51bc | 58.09g |
| | SA | 41.23e | 2.28ef | 68.07c |
| | JA | 33.61h | 2.63a | 58.65g |

Different letters in each column indicate significant difference at $p \leq 0.05$. Con: control, SA: salicylic acid and JA: Jasmonic acid

Salinity and hormonal treatments significantly affected assimilates mobilization efficiency to the seeds during the seed filling period. Assimilates mobilization efficiency to the seeds was increased by enhancing salinity up to 4 dS $m^{-1}$, but with further increase in salinity it was reduced. Under all levels of salinity and non-salinity, SA treatment significantly improved the mobilization efficiency; this advantageous effect was higher under severe salinity. The JA treatments under non and low salinity levels reduced the mobilization efficiency of assimilates to the seeds, however, under moderate and severe salinities, there was no tangible difference between the JA and control plants (Table 2).

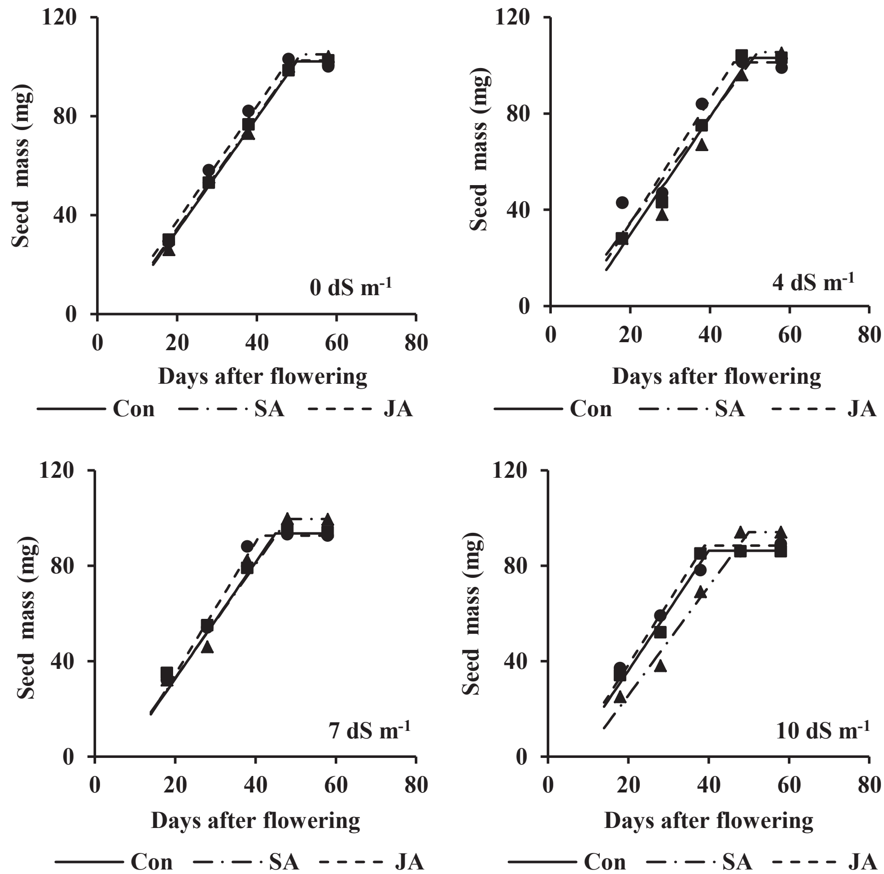



**Figure 2:** Changes in seed mass of soybean (M7 cultivar) in response to different salinity and hormonal treatments. Con: control, SA: salicylic acid and JA: Jasmonic acid

### 3.9 Yield components

The effects of salinity and hormonal treatments on pod and seed number per plant were significant. Pod and seed number per plant were decreased with rising salinity, however the difference in pod number between 0 dS $m^{-1}$ and 4 dS $m^{-1}$ was not significant. Treatment with SA increased pod and seed number per plant, but JA did not improve number of the pods per plant (Table 3).

**Table 3:** Means of soybean seed mass and yield components under different salinity and hormonal treatments

| Treatments | Pods per plant | Seeds per plant | Maximum seed mass (mg $seed^{-1}$) |
|---|---|---|---|
| Salinity | | | |
| 0 dS $m^{-1}$ | 10.16a | 33.62a | 103.52a |
| 4 dS $m^{-1}$ | 9.58a | 30.93b | 103.31ab |
| 7 dS $m^{-1}$ | 8.33b | 27.33c | 96.73b |
| 10 dS $m^{-1}$ | 7.33c | 23.60d | 89.32c |
| Hormonal treatment | | | |
| Con | 8.25b | 27.30b | 96.80b |
| SA | 9.81a | 32.53a | 104.37a |
| JA | 8.50b | 26.76b | 92.50b |

Different letters in each column indicate significant difference at $p \leq 0.05$. Con: control, SA: salicylic acid and JA: Jasmonic acid

## 4 DISCUSSION

The decrement in CCI under severe salinity could be attributed to a salt-induced weakening of protein-pigment-lipid complex and enhancing the activity of chlorophyllase. The decrease in chlorophyll content under salt stress is commonly reported phenomenon (Noreen et al., 2009). Increasing the CCI and anthocyanins content with SA treatment may be related to increasing nitrogen absorption with the enhancing nitrate reductase activity (Farhangi-Abriz & Ghassemi-Golezani, 2016) and increasing the chlorophyll stability index (Farhangi-Abriz & Ghassemi-Golezani, 2018).

The low Fv/Fm value under saline condition is the consequence of initial damage occurring in PSII, likely due to low water availability. This reduction in Fv/Fm under salt stress is dependent on damage to reaction centers and reducing electron transport capacity in PSII (Ghassemi-Golezani & Lotfi, 2015). Increasing PSII efficiency with SA treatment under severe salinity could be resulted from the effects of SA on decreasing the harmful effects of salt stress on plant performance (Farhangi-Abriz & Ghassemi-Golezani, 2018). Exogenous application of salicylic acid on mustard plants improved photosynthetic activities and growth through increasing ascorbate-glutathione metabolism and sulphur assimilation under salt stress (Nazar et al. 2015). Increasing Fv/Fm may be related to the effects of SA on inhibition of peroxidation of membrane lipid, decreasing electrolyte leakage and enhancement of electron transfer in membrane (Shi et al., 2006; Farhangi-Abriz & Ghassemi-Golezani, 2018).

Increasing leaf temperature under salinity is related to low relative water content. One of the early symptoms of salinity stress in plant tissue is the decrease of RWC. This reduction of RWC in stressed plants may be associated with a decrease in plant vigor and was observed in many plant species. The decrease in leaf water could be associated with ion toxicities, ion imbalance and osmotic stress (Farhangi-Abriz & Ghassemi-Golezani, 2018). Reduction in RWC leads to enhanced temperature via closing stomata and decreasing transpiration. Reducing the leaf temperature by SA application closely related with higher RWC of plants treated with this hormone. This may be resulted from the accumulation of so-called SA induced proteins that were found in all plant species and can have a helpful effect on the osmoregulation process in plants (Farhangi-Abriz & Ghassemi-Golezani, 2018).

Reduction of leaf area and leaf dry mass under salinity could be attributed to the nutritional imbalance due to an interference of salt ions, such as $Na^+$ and $Cl^-$ with $K^+$ involved in both uptake and translocation processes (Farhangi-Abriz & Ghassemi-Golezani, 2018). Potassium is a main plant macro-element that has some serious roles related to cell expansion and nutrient uptake. Restraining leaf expansion and reducing specific leaf area by salinity resulted in increasing specific leaf mass. Improving leaf area of soybean plants by SA application may be related to enhance essential nutrient uptake (Farhangi-Abriz & Ghassemi-Golezani, 2018) and inhibition of ethylene synthesis (Leslie & Romani, 1986). This leads to an increase in specific leaf area and a decrease in specific leaf mass. In contrast, JA application increases ethylene synthesis, which limits leaf area expansion, but increases leaf dry matter and specific leaf mass, probably with allocation of photo assimilate to produce secondary metabolites such as flavonoids and storing these metabolites in leaf cell vacuoles (Wasternack, 2007).

Increasing relative electrolytic leakage of leaves under salinity shows the extent of membrane damages due to salt toxicity. The lipid peroxidation and increasing relative electrolytic leakage under salinity are the serious adverse effects of the salinity on plant cells. Treatment with SA and JA decrease adverse effects of salinity by increasing anti-oxidants activity (Farhangi-Abriz & Ghassemi-Golezani, 2018), inhibiting ethylene synthesis (Leslie & Romani, 1986), increasing RWC, CCI and allocation of amino acids to producing non-structural protein in vegetative sinks (Farhangi-Abriz & Ghassemi-Golezani, 2016).

Decreasing WUE under salinity stress is largely related to limitation of crop growth. These reductions in growth of soybean under salinity might be caused by decreasing turgidity from high concentrations of $Na^+$ in the soil under salt stress. The negative effect of salinity on plants may provoke osmotic potential by salt in the culture medium, so root cells do not obtain required water from the soil. Consequently, the uptake of some mineral nutrients such as nitrogen dissolved in water is also restricted (Farhangi-Abriz & Ghassemi-Golezani, 2016). Increasing WUE by SA treatments could be the result of improving water uptake, translocation and increasing physiological performance and photosynthetic activities (Farhangi-Abriz & Ghassemi-Golezani, 2018). Treatment with JA in some plants, reduces water and nutrient uptake, imbalances nutrient content and WUE, and reduces plant growth and seed production (Creelman & Mullet, 1995; Ghassemi-Golezani & Farhangi-Abriz, 2018b).

Reduction in seed filling duration and increment in seed filling rate of plants with increasing salinity stress is the result of early plant senesces (Farhangi-Abriz & Torabian, 2017) and increasing ethylene synthesis (Cao et al., 2007). Yang et al. (2013) reported that a reformed hormonal balance in rice seeds during seed filling, especially a decrease in gibberellic acids and an increase in abscisic acid, enhances the remobilization of pre-stored assimilated to the seeds and accelerates the seed filling rate. The decreasing maximum seed mass of soybean seeds under salinity is related to limitation of water and nutrient uptake (Cao et al., 2007) and decreasing seed filling duration. Treatment with SA by preventing ethylene synthesis in plants and regulating hormonal balance (Leslie & Romani, 1986) increased seed filing duration and maximum mass of seeds. In contrast, JA treatment by enhancing ethylene synthesis (Creelman & Mullet, 1995) and seed filling rate, reduced seed filling duration (only on 0 and 4 dS $m^{-1}$). Consequently, the maximum mass of seeds did not significantly change by JA, compared with untreated plants.

Reduction of assimilates mobilization efficiency under moderate and high salinity levels could be attributed to decreasing water availability, seed filling duration and photosynthetic activities. Foliar spray of SA increased assimilates mobilization to the seeds by rising seed filling period and improving water use efficiency. Decreasing assimilates mobilization efficiency by JA under non and low salinity conditions most likely related to the reduction of the seed filing period and allocation of assimilates to the vegetative sinks (Farhangi-Abriz & Ghassemi-Golezani, 2016).

Pod number per plant is closely related with nod and flower number. Salinity could reduce nods and flowers per plant, leading to reduction in pods per plant. Treatment with SA increased number of pods and seeds per plant through improving flower-inducing factor (Hayat et al., 2007). Farhangi-Abriz and Ghassemi-Golezani (2018) reported that salinity can severely limit crop production, because high salinity reduces water potential and induces ionic stress. Large reductions in seed filling period, assimilate mobilization efficiency to the seeds, number of seeds and seed mass under saline conditions resulted in a considerable decrease in seed mass per plant. Exogenous application of SA improved seed mass via increasing morpho-physiological performance of plants, seed filling period, assimilate mobilization efficiency to the seeds, seed number per plant and seed mass. Foliar spray of SA decreases damages of salinity by increasing the activities of anti-oxidant enzymes, and also improving the general physiological performance of plants (Farhangi-Abriz & Ghassemi-Golezani, 2018), which result in higher seed mass.

## 5 CONCLUSIONS

Salt stress considerably reduced seed mass of soybean by decreasing morpho-physiological performance of plants and seed filling duration. Foliar application of SA improved seed production under different levels of salt stress. The advantages of SA treated plants in seed mass were directly related with improving physiological performance of soybean plants such as leaf growth, water use efficiency, seed filling duration and assimilate mobilization efficiency under non-saline and saline conditions.

## 6 REFERENCES

Aftab, T., Khan, M. M. A., Idrees, M., Naeem, M., Hashmi, N. (2011). Methyl jasmonate counteracts boron toxicity by preventing oxidative stress and regulating antioxidant enzyme activities and artemisinin biosynthesis in *Artemisia annua* L. *Protoplasma*, *248*, 601-612. doi:10.1007/s00709-010-0218-5

Barrs, H.D., Weatherley, P.E. (1962). A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Australian Journal of Biological Sciences*, *15*, 413-428. doi:10.1071/BI9620413

Cao, W.H., Liu, J., He, X.J., Mu, R.L., Zhou, H.L., Chen, S.Y., Zhang, J.S. (2007). Modulation of ethylene responses affects plant salt-stress responses. *Plant physiology*, *143*, 707-719. DOI: doi:10.1104/pp.106.094292

Choudhury, S., Panda, S.K. (2004). Role of salicylic acid in regulating cadmium induced oxidative stress in *Oryza sativa* L. roots. *Bulgarian Journal of Plant Physiology*, *30*, 95-110.

Creelman, R.A., Mullet, J.E. (1995). Jasmonic acid distribution and action in plants: regulation during development and response to biotic and abiotic stress. *Proceedings of the National Academy of Sciences*, *92*, 4114-4119. doi:10.1073/pnas.92.10.4114

Farhangi-Abriz, S., Ghassemi-Golezani, K. (2016). Improving amino acid composition of soybean under salt stress by salicylic acid and jasmonic acid. *Journal of Applied Botany and Food Quality*, *89*, 243-248.

Farhangi-Abriz, S., Ghassemi-Golezani, K. (2018). How can salicylic acid and jasmonic acid mitigate salt toxicity in soybean plants? *Ecotoxicology and Environmental Safety*, *147*, 1010-1016. doi:10.1016/j.ecoenv.2017.09.070

Farhangi-Abriz, S., Torabian, S. (2017). Antioxidant enzyme and osmotic adjustment changes in bean seedlings as affected by biochar under salt stress. *Ecotoxicology and environmental safety*, *137*, 64-70. doi:10.1016/j.ecoenv.2016.11.029

Ghassemi-Golezani, K., Farhangi-Abriz, S. (2018a). Foliar sprays of salicylic acid and jasmonic acid stimulate $H^+$-ATPase activity of tonoplast, nutrient uptake and salt tolerance of soybean. *Ecotoxicology and environmental safety*, *166*, 18-25. doi:10.1016/j.ecoenv.2018.09.059

Ghassemi-Golezani, K., Farhangi-Abriz, S. (2018b). Changes in Oil Accumulation and Fatty Acid Composition of Soybean Seeds under Salt Stress in Response to Salicylic Acid and Jasmonic Acid. *Russian Journal of Plant Physiology*, *65*, 229-236. doi:10.1134/S1021443718020115

Ghassemi-Golezani, K., Lotfi, R. (2015). The impact of salicylic acid and silicon on chlorophyll a fluorescence in mung bean under salt stress. *Russian journal of plant physiology, 62*, 611-616. doi:10.1134/S1021443715040081

Ghassemi-Golezani, K., Taifeh-Noori, M., Oustan, S., Moghaddam, M., Seyyed-Rahmani, S. (2010). Oil and protein accumulation in soybean grains under salinity stress. *Notulae Scientia Biologicae*, *2*, 64-69. doi:10.15835/nsb224590

Gunes, A., Inal, A., Alpaslan, M., Eraslan, F., Bagci, E. G., Cicek, N. (2007). Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity. *Journal of Plant Physiology, 164*, 728-736. doi:10.1016/j.jplph.2005.12.009

Hayat, S., Ali, B., Ahmad, A. (2007). Salicylic acid: biosynthesis, metabolism and physiological role in plants. In *Salicylic acid: A plant hormone*. Springer, Dordrecht. doi:10.1007/1-4020-5184-0

Jiang, H., Egli, D.B. (1993). Shade induced changes in flower and pod number and flower and fruit abscission in soybean. *Agronomy Journal, 85*, 221-225. doi:10.2134/agronj1993.00021962008500020011x

Leslie, C. A., Romani, R. J. (1986). Salicylic acid: a new inhibitor of ethylene biosynthesis. *Plant Cell Reports*, *5*, 144-146. doi:10.1007/BF00269255

Luo, Q., Yu, B., Liu, Y. (2005). Differential sensitivity to chloride and sodium ions in seedlings of *Glycine max* and *G. soja* under NaCl stress. *Journal of plant physiology*, *162*, 1003-1012. doi:10.1016/j.jplph.2004.11.008

Lutts, S., Kinet, J. M., Bouharmont, J. (1996). NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Annals of botany*, *78*, 389-398. doi:10.1006/anbo.1996.0134

Maclachlan, S., Zalik, S. (1963). Plastid structure, chlorophyll concentration, and free amino acid composition of a chlorophyll mutant of barley. *Canadian Journal of Botany*, *41*, 1053-1062. doi:10.1139/b63-088

Mancinelli, A.L. (1984). Photoregulation of anthocyanin synthesis: VIII. Effect of light pretreatments. *Plant Physiology*, *75*, 447-453. doi:10.1104/pp.75.2.447

Mirfattahi, Z., Karimi, S., Roozban, M.R. (2017). Salinity induced changes in water relations, oxidative damage and morpho-physiological adaptations of pistachio genotypes in soilless culture. *Acta agriculturae Slovenica*, *109*, 291-302. doi:10.14720/aas.2017.109.2.12

Mohammadian, R., Moghaddam, M., Rahimian, H. Sadeghian, S.Y. (2005). Effect of early season drought stress on growth characteristics of sugar beet genotypes. *Turkish journal of agriculture and forestry, 29,* 357-368.

Nazar, R., Umar, S., Khan, N.A. (2015). Exogenous salicylic acid improves photosynthesis and growth through increase in ascorbate-glutathione metabolism and S assimilation in mustard under salt stress. *Plant signaling & behavior*, 10. doi:10.1080/15592324.2014.1003751

Noreen, S., Ashraf, M., Hussain, M., Jamil, A. (2009). Exogenous application of salicylic acid enhances antioxidative capacity in salt stressed sunflower (*Helianthus annuus* L.) plants. *Pakistan Journal of Botany*, *41*, 473-479.

Oya, T., Nepomuceno, A. L., Neumaier, N., Farias, J. R. B., Tobita, S., Ito, O. (2004). Drought tolerance characteristics of Brazilian soybean cultivars. *Plant Production Science, 7*, 129-137. doi:10.1626/pps.7.129

Reddy, M.P., Vora, A.B. (1986). Salinity induced changes in pigment composition and chlorophyllase activity of wheat. *Indian Journal of plant physiology, 29*, 331-334.

Sali, A.L.I.U., Rusinovci, I., Fetahu, S., Gashi, B., Simeonovska, E., Rozman, L. (2015). The effect of salt stress on the germination of maize (*Zea mays* L.) seeds and photosynthetic pigments. *Acta agriculturae Slovenica*, *105*, 85-94. doi:10.14720/aas.2015.105.1.09

Shi, Q., Bao, Z., Zhu, Z., Ying, Q., Qian, Q. (2006). Effects of different treatments of salicylic acid on heat tolerance, chlorophyll fluorescence, and antioxidant enzyme activity in seedlings of *Cucumis sativa* L. *Plant growth regulation*, 48, 127-135. doi:10.1007/s10725-005-5482-6

Wasternack, C. (2007). Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. *Annals of botany*, *100*, 681-697. doi:10.1093/aob/mcm079

Yang, D.L., Yang, Y., He, Z. (2013). Roles of plant hormones and their interplay in rice immunity. *Molecular plant*, *6*, 675-685. doi:10.1093/mp/sst056

doi:10.14720/aas.2018.111.3.09 Original research article / izvirni znanstveni članek

# Impact of temperature stress on secondary metabolite profile and phytotoxicity of *Amaranthus cruentus* L. leaf extracts

Maria Elizabeth CAWOOD[1,*], Ingrid ALLEMANN[1], James ALLEMANN[2]

Received May 23, 2018; accepted November 07, 2018.
Delo je prispelo 23. maja 2018, sprejeto 07. novembra 2018.

**ABSTRACT**

In this study *Amaranthus cruentus* plants were grown under controlled optimal conditions (28/21 °C) for three months and then subjected to cold (14/7 °C) and hot (33/40 °C) temperatures. We investigated the influence of these temperature regimes on the metabolite profile of the leaves through analyses of data by TLC, HPLC and GC-MS spectrometry. The phytotoxic potential of a methanol-water (MW) and dichloromethane (DCM) extract from the aerial parts were examined through in vitro screening of germination and growth of lettuce and pepper. The optimal extracts displayed the highest diversity of secondary metabolites, and the highest total phenolics and flavonoids content. Through TLC and HPLC analysis the significantly lower phenolic content in the hot temperature treated samples was confirmed. A wide range of metabolites were detected in the DCM extracts through GC-MS analyses. The phytotoxicity of both the MW and DCM extracts were demonstrated, as germination and growth of pepper and lettuce were significantly inhibited, indicating the presence of more than one allelochemical compound which may affect the allelopathic activity of *A. cruentus* during changes in environmental temperatures.

**Key words:** *Amaranthus cruentus*; temperature; stress; phytotoxcitiy; metabolites; phenolic compounds

**IZVLEČEK**

**VPLIV TEMPERATURNEGA STRESA NA PROFIL IN FITOTOKSIČNOST SEKUNDARNIH METABOLITOV V LISTNEM IZVLEČKU ZRNATEGA ŠČIRA (*Amaranthus cruentus* L.).**

Rastline zrnatega ščira so bile za namene te raziskave gojene v nadzorovanih optimalnih temperaturnih razmerah tri mesece (28/21 °C) in nato izpostavljene hladu (14/7 °C) in vročini (33/40 °C). Preučevan je bil vpliv temperaturnih režimov na profil metabolitov v listih ščira z metodami kot so TLC, HPLC in GC-MS spektroskopija. Fitotoksični potencial metanolno-vodnih (MW) in diklormetanskih (DCM) izvlečkov nadzemnih delov ščira je bil analizirana preko *in vitro* analize kalitve vrtne solate in paprike. Optimalni izvlečki so imeli največjo raznolikost sekundarnih metabolitov in največjo vsebnost celokupnih fenolov in flavonoidov. S TLC in HPLC analizo je bila potrjena značilno manjša vsebnost fenolov v vročinsko obdelanih vzorcih. Z GC-MS analizo je bil ugotovljen širok nabor metabolitov v diklormetanskih izvlečkih (DCM). Fitotoksičnost MW in DCM izvlečkov se je izrazila v značilno zmanjšani kalitvi in rasti solate in paprike, kar kaže na prisotnost več kot ene alelokemične spojine. To lahko posledično vpliva na alelopatsko aktivnost zrnatega ščira med spremembami temperature v okolju.

**Ključne besede:** *Amaranthus cruentus*; temperatura; stres; fitotoksičnost; metaboliti; fenolne spojine

## 1 INTRODUCTION

Several studies documented on the increase of secondary compounds or changes in chemical profile within specimens of the same plant species growing under different or environmental stress conditions (Gobbo-Neto & Lopes, 2007; Ramakrishna & Ravishankar, 2011; Gouvea et al., 2012). The interaction between plants and their environment influence synthesis and accumulation of secondary

[1] Department of Plant Sciences, University of the Free State, Bloemfontein, South Africa *Corresponding author: cawoodme@ufs.ac.za

[2] Department of Soil, Crop and Climate Sciences, University of the Free State, Bloemfontein, South Africa

This article is part of a Master thesis entitled » Influence of abiotic stress on allelopathic properties of *Amaranthus cruentus* L. «, issued by Ingrid Allemann, Supervisor Dr Maria Elizabeth Cawood, Ph. D., Co-Supervisor Dr James Allemann, Ph.D.

metabolites and their roles as a response to the environment. (Rhoads et al., 2006). The exposure to various environmental stresses can strengthen the allelopathic potential of many plants (Einhellig, 1987, 1996; Gershenzon, 1984; Tang et al., 1995; Kobayashi, 2004) and can affect allelopathy in at least three ways: 1) the production of allelochemicals by the donor species, 2) their bioavailability and 3) modify the effect of an allelochemical on the target plant (Einhellig, 1996, Trezzi et al., 2016).

Amaranth is one of the few multi-purpose crops which can supply grain as well as tasty leafy vegetables of high nutritional quality (Mensah et al., 2008; Maiyo et al., 2010; Nana et al., 2012; Alemayehu et al., 2015). The chemical constituents and medicinal value of amaranth have been well described in the literature (Stintzing et al., 2004; Steffensen et al., 2011; Kraujalis et al., 2013). Most of the reported compounds include: carotenoids, steroids (Maiyo et al., 2010; Oboh et al., 2008; Bishop & Yokoto, 2001), terpenoids (Connick et al., 1989), ascorbic acid, betacyanins (Cai et al., 1998), α-spinasterol, spinoside, amaranthoside, amaracine (Shah, 2005), phenolic compounds (Kraujalis et al., 2013) and saponins (Vincken et al., 2007). Some of these compounds are considered to be allelochemicals which are able to affect surrounding plants once released into the environment (Rice, 1984; Waller, 1987). These phytotoxic compounds offer the opportunity to act as natural herbicides, since there is an increasing need for more cost-effective, safer, and more selective herbicides.

This study evaluated the influence of temperature on plant secondary metabolite production of *A. cruentus* L. and whether the chemical-mediated interaction is involved in *A. cruentus* allelopathy. Thus, phytotoxcitiy of *A. cruentus* was evaluated with extracts of the different temperature treatments.

## 2 MATERIALS AND METHODS

### 2.1 Plant material

*Amaranthus cruentus* 'Anna' seeds were planted in pots containing a soil-compost (80 : 22 v/v) mixture and grown at 28/21 °C; day/night temperatures in climate controlled chambers at The Department of Agriculture, University of the Free State as described by Allemann et al. (2017). Vegetable seeds used in this study were obtained from Starke Ayres: 'California Wonder' Sweet Pepper and 'Great Lakes' Lettuce.

### 2.2 Crude extracts

Methanol-water (70 : 30 v/v) and dichloromethane (DCM) were used as solvents. Ten grams of the powdered *A. cruentus* leaf material (oven dried at 40 °C) was extracted twice by shaking overnight in the different solvents (1 : 20 w/v). The pooled extracts were dried and kept at 4 °C until further analyses.

### 2.3 Allelopathy determination

A combination of the 'sandwich method' of Fujii et al. (2003) and Hill et al. (2007) was used to determine the *in vitro* phytotoxicity of the crude leaf extracts from the different temperature treatments of *A. cruentus* on the vegetable seeds. For this method 5 and 20 mg of each extract was dissolved in 1 ml of their own solvent, and 1 ml pipetted onto a filter paper. The filter papers were allowed to dry then placed on the bottom layer of agar resulting in 0.5 or 2 mg $ml^{-1}$ extract per well. Controls contain only the solvents on filter paper.

Lettuce (*Lactuca sativa* L.) and pepper (*Capsicum annuum* L.) seeds were surface sterilised as described by Allemann et al. (2017) and each of the experiments was done in triplicate and presented as the mean of the replicates.

### 2.4 Total phenolic and flavonoid content

Total phenolic content was evaluated in the methanolic extract, using the Folin-Ciocalteu method as reported by Singleton & Rossi (1965). The absorption was measured at 550 nm and the content in phenolics was expressed as mg galllic acid equivalents (GAE) of dry mass extract. Total flavonoid content was determined as reported by Zhishen et al. (1999). The absorption was measured at 510 nm and the content in flavonoids was expressed as mg quercetin equivalents (QE) of dry mass extract.

### 2.5 Thin Layer Chromatography

Thin layer chromatography (TLC) was carried out using silica gel 60 $F_{450}$–aluminium backed pre-coated plates. Extracts (50 mg $ml^{-1}$) were dissolved in their appropriate extraction solvents and 10 µl applied to the TLC. The mobile phase for development of the MW extracts was chloroform-methanol-water-acetic acid (65:35:5:1), while for the DCM extracts, plates were developed in toluene-ethyl acetate (93:7). Compounds resolved on the plate were visualized using ultraviolet light (UV) at 365 nm and 254 nm, ninhydrin (Pifrung, 2006), *p*-anisaldehyde-sulphuric/acetic acid, 5 % ferric chloride and dragendorf reagents, prepared according to the standard methods described by Wagner & Bladt (1996).

### 2.6 High Pressure Liquid Chromatography

The MW extracts (20 mg $ml^{-1}$) were separated and identified through high pressure liquid chromatography (HPLC) by comparing the retention times to standard phenolic compounds. Standards were prepared in methanol (3 mg $ml^{-1}$) and absorption measured between 200 and 400 nm. Ten micro litre of extracts and 2 µl of standards were injected while the flow rate was kept at 1 ml $min^{-1}$. A Shimadzu instrument with a Photo Diode Array Detector (PDA) and an elution procedure as described by Vidović et al. (2015) with a C18 column (Phenomenex C18, 250mm × 4.6mm, 5µm diameter), was used to achieve acceptable separation of all compounds. The mobile phase consisted of: A, acetonitrile and B, a mixture of acetic acid- acetonitrile-phosphoric acid-water (10:5:0.1:84:9, by vol.).

### 2.7 GC-MS analysis

The DCM extracts (10 mg) were dissolved in 1 ml hexane. Analyses was done through GC-MS using a Shimadzu GC-MS QP-2010 gas chromatography equipped with a DB-5 MS column (30 m length × 0.32 mm diameter × 0.25 µm film thickness) and injecting 1 µl of sample. The GC operating conditions were the following: 5 min at 60 °C, then gradually increased to 280 °C at a rate of 2 °C $min^{-1}$, and held for 10 min. Helium was used as the carrier gas (1.5 ml $min^{-1}$ flow rate). Spectra analysis was conducted using the library "National Institute of Standard and Technology (NIST) version 5.0.

### 2.8 Statistical analysis

The experiments were carried out adopting a completely randomized design with three replications. The results were expressed as means with least significant difference (LSD). Analysis of variance (ANOVA) was performed using SAS 9.3 (Institute Inc., Cary, NC, USA, 2008) statistical programme for data and Tukey-Kramer's LSD procedure for comparison of means. Significance of differences compared to the control groups was determined using the t-test (Steel & Torrie, 1980).

## 3 RESULTS AND DISCUSSION

### 3.1 Metabolites

Comparison of the compounds in the MW and DCM leaf extracts of the different temperature treatments of *A. cruentus* plants, are illustrated by TLC in Figure 1. It is clear that temperature played an obvious role in the production of secondary compounds, as clear differences in compounds between the treatments were visible in both the polar and non-polar extracts (Fig. 1A & B). Different compounds with varying $R_f$ values were visible when spraying the TLC's with *p*-anisaldehyde-sulphuric/acetic acid reagent. Colours of compounds range from green, yellow, pink, blue and purple with different $R_f$ values for the polar and non-polar extracts. The diverse coloured compounds with varying $R_f$ values visible in both polar and non-polar extracts on TLC (Fig. 1) may indicate many different compounds, including terpenes, saponins, sugars and flavonoids amongst others (Wagner & Bladt 1996).

In the optimal treatment of the MW extract, 11 compounds were noted, compared to 8 and 5 in the cold and hot treated samples respectively. Prominent spots, including a dark purple ($R_f$ = 0.055), a blue-purple ($R_f$ = 0.49) and a light blue spot ($R_f$ = 0.6) were only present in the optimal extract (Fig. 1A). From these results one can deduct that the stress temperatures, particularly the hot, inhibited the biosynthesis of some of the more polar compounds.

Differences were also visible in the non-polar samples (Fig. 2B), with a noticeable blue coloured compound visible at $R_f$ = 0.83, solely in the hot treatment DCM extract (Fig 1B). Less green pigment, probably chlorophyll was also observed in the hot treatment extract, indicating the effect the hot treatment had on photosynthesis.

Several studies were conducted on the impact of increased temperatures on secondary metabolite production of plants (Morrison & Lawlor, 1999). Phenolic compounds are important and common plant allelochemicals in the ecosystem and the main phenolic compounds are water soluble (Li et al., 2010). Kraujalis et al. (2013) reported on the antioxidant properties and phytochemical composition of amaranth extracts isolated by acetone and methanol-water from plant leaves, flowers, stems and seeds. They found that the methanol-water extract of the leaves possessed the highest antioxidant activities and various phenolic compounds and flavonoids e.g. rutin, nicotiflorin, isoquercitrin, 4-hydroxybenzoic and p-coumaric acids were identified as major constituents. In the review article by Mroczek (2015) it is reported that saponins were isolated from a diversity of Amaranthaceae genera and species.

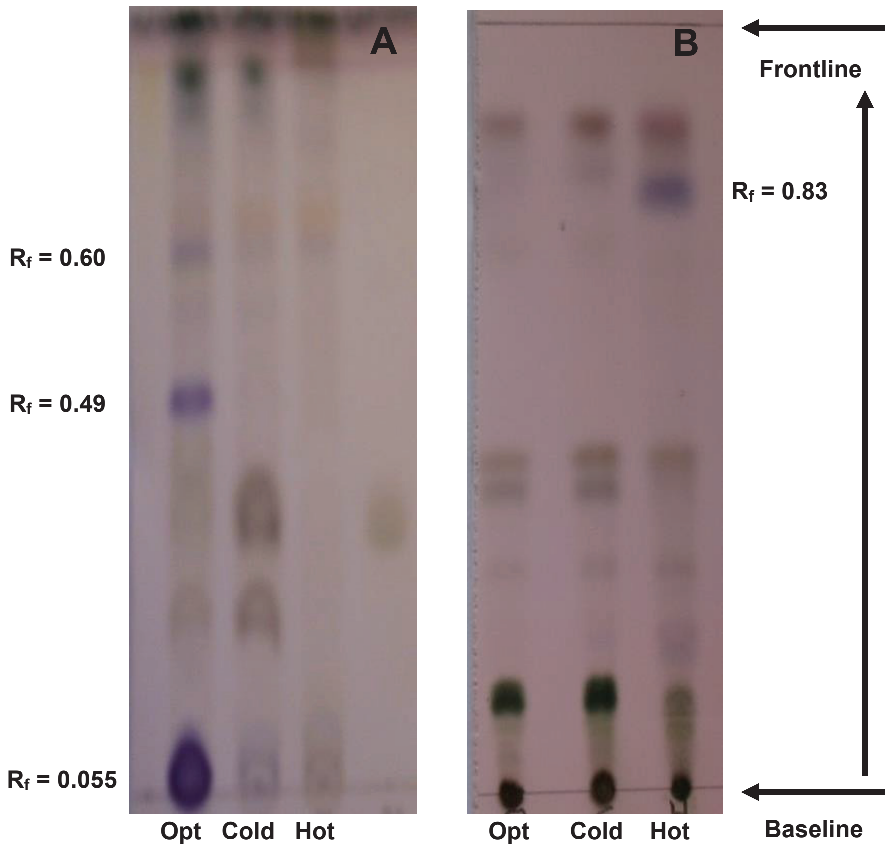



**Figure 1:** Qualitative TLC profiles of the optimal, cold and hot treated *A. cruentus* MW (A) and DCM (B) leaf extracts. Detection by *p*-anisaldehyde reagent

In this study, the total phenolic and flavonoid content significantly declined in *A. cruentus* plants exposed to hot temperatures compared to plants grown at the optimal temperature (Table 1). The decrease in total phenolic and flavonoid content are in contrast with findings of many authors who reported an increase in production of phytotoxic phenolic compounds in plant tissues exposed to high temperatures and solar radiation (Koeppe et al., 1969; Wender, 1970; Einhelig & Eckrich, 1984). Rudikovskaya et al. (2008), however reported that low growth temperature decreased the content of some phenolic compounds in pea seedling roots and according to Król et al. (2014), long-term drought stress caused a decrease in particular components of secondary metabolism in the leaves and roots of grapevine. It seems therefore that one cannot expect generalized patterns of phenolic compounds in stress situations.

**Table 1**: Total phenolic and flavonoid compounds in temperature stressed amaranth leaf material

| Treatment | Total phenolic content (mg GAE g $D.M^{-1}$.) | Total flavonoid content (mg QE g $D.M^{-1}$.) |
|---|---|---|
| Cold | 12.0 b | 5.4 a |
| Optimal | 18.8 a | 5.6 a |
| Hot | 10.1 b | 4.1 b |

Analyses by HPLC confirmed the decrease in phenolic compounds in the temperature stressed plants (Fig 2).

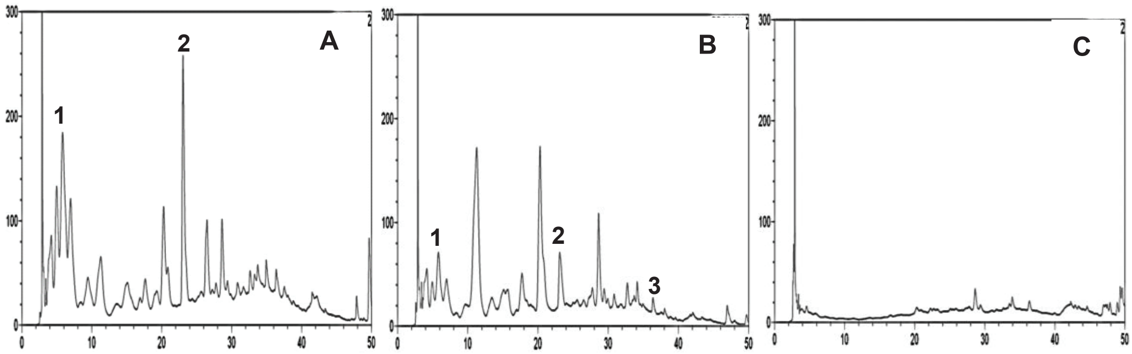



**Figure 2:** Comparison of HPLC-PDA chromatograms of optimal (A), cold (B) and hot (C) temperature treated *A. cruentus* methanol-water leaf extracts. 1 = Catechin; 2 = Rutin; 3 = Quercetin

Flavonoids are not usually seen as allelopathic compounds but they have other roles in plants such as attractants to pollinators, protection against ultraviolet light (Li et al., 1993) and as an anti-inflammatory, anti-allergic and anti-viral activities (Miller, 1996). Some flavonoids do however have allelopathic properties such as quercetin (Inderjit & Dakshini, 1995), catechin (Bais & Kaushik, 2010; Chobot et al., 2009) and rutin (Basile et al., 2000), which has been found in both *A. hybridus* and *A. cruentus*. From our HPLC results, catechin and rutin were identified in the optimal and cold treated amaranth MW leaf litter extracts (Fig 2A & B), while a small amount of quercetin was detected in only the cold treated sample (Fig 2B). The heat treated sample contained a reduced amount of unidentified compounds (Fig 2C), indicating the role temperature play on the biosynthesis of flavonoids and the possible consequence on allelopathy.

The influence of temperature on the expressed compounds in the different DCM extracts were clearly visible after analyses through gas chromatography and mass spectrometry (GC-MS). Major compounds made up a total composition of 75.69 % (9 compounds), 90.44 % (7 compounds) and 91.89 % (9 compounds), of the optimal, cold and heat treated samples respectively (Table 2). Neophytadiene and hexadecanoic acid were the only compounds present in all three extracts, although the concentrations of these compounds varied substantially between the treatments (Table 2). The highest concentration of neophytadiene (27.53 %) was found in the cold treated sample, while hexadecanoic acid (13.52 %) was maximum in the heat treatment extract. Squalene, trans-phytol and the phytosterol, stigmasta-7,22-dien-3-ol were present in only cold and heat treated samples.

Gamel et al. (2007) found high squalene concentrations in oil fractions of *A. caudatus* L. *and A. cruentus*, while Shah (2005) reported on the presence of stigmasta-7,22-dien-3-ol (α-spinasterol) in *A. spinosus* L.. According to Szakiel et al. (2010), lower soil temperatures triggered an increase in levels of steroidal furostanol and spirostanol saponins.

**Table 2:** GC-MS results of compounds present in optimal, cold and hot temperature treated DCM leaf extracts of *A. cruentus*

| Retention time (min) | Compound name* | Optimal area % | Cold area % | Hot area % |
|---|---|---|---|---|
| 63.315 | 16-Heptadecenal | 13.22 | - | - |
| 64.054 | Neophytadiene | 9.03 | 30.70 | 4.44 |
| 65.296 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | - | 5.19 | 3.72 |
| 66.193 | trans-Phytol | - | 10.04 | 6.01 |
| 70.621 | Hexadecanoic acid (Palmitic acid) | 11.01 | 3.29 | 13.52 |
| 77.243 | 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl | - | - | 7.82 |
| 78.458 | 9,12,15-Octadecatrienoic acid (α-Linolenic acid) | - | 27.40 | 29.68 |
| 79.695 | Octadecanoic acid (Stearic acid) | - | - | 2.30 |
| 81.156 | Tetrahydrofurano[6a,7a-b]-5-oxa-8-thiaphenanthrene | 9.86 | - | - |
| 97.733 | Hexahydrothunbergol | 7.07 | - | - |
| 102.751 | bis-Naphthylfuran | 5.47 | - | - |
| 103.841 | Methyl ester of decyclotrenudine | 5.02 | - | - |
| 103.992 | (-)-18-Noramborx | 4.84 | - | - |
| 104.067 | Benzyl methyl ether | 10.17 | - | - |
| 104.920 | Squalene | - | 3.73 | 6.22 |
| 121.002 | Stigmasta-7,22-dien-3-ol (α-Spinasterol) | - | 10.09 | 12.36 |
| Total | | 75.69 | 90.44 | 91.89 |

Area (%) of compound = height of peak x width of peak at ½height ≤ x Total area$^{-1}$
* Identification by Library: NIST 05. LIB

### 3.2 Phytotoxcitiy

Germination

Phytotoxic activity of *A. cruentus* extracts may be ascribed to a wide range of biologically active phytochemicals such as phenolic acids, flavonoids and fatty acids which are known for their phytotoxic and allelochemical activities. When these compounds are released into the soil by leaf litter decomposition there may be a change in both the physical and chemical properties and therefore affecting the organization and growth of plant communities. At different concentrations both the MW and DCM extracts of the different temperature treatments, significantly inhibited germination of both lettuce and pepper ($LSD_{(T \leq 0.05)}$ = 1.88) (Table 3). The polar MW extract was more effective in lowering germination percentages in both pepper and lettuce than the non-polar DCM extract (Table 3).

**Table 3:** Germination percentage of pepper and lettuce seeds exposed to increasing concentrations of MeOH-$H_2O$

| Extract [mg ml$^{-1}$] | Germination % of pepper temperature treatment | | | |
|---|---|---|---|---|
| MW | Optimal | Cold | Hot | Ave |
| 0 | 100 | 100 | 100 | 100 |
| 0.5 | 29 | 24 | 29 | 27 |
| 2 | 29 | 24 | 33 | 28 |
| Ave | 52 | 49 | 54 | |
| DCM | | | | |
| 0 | 100 | 100 | 100 | 100 |
| 0.5 | 49 | 29 | 56 | 44 |
| 2 | 40 | 22 | 47 | 36 |
| Ave | 63 | 50 | 68 | |

| Extract [mg ml$^{-1}$] | Germination % of lettuce temperature treatment | | | |
|---|---|---|---|---|
| MW | Optimal | Cold | Hot | Ave |
| 0 | 100 | 100 | 100 | 100 |
| 0.5 | 24 | 29 | 31 | 28 |
| 2 | 24 | 24 | 36 | 28 |
| Ave | 49 | 51 | 56 | |
| DCM | | | | |
| 0 | 100 | 100 | 100 | 100 |
| 0.5 | 69 | 67 | 42 | 59 |
| 2 | 42 | 24 | 36 | 34 |
| Ave | 70 | 64 | 59 | |

$LSD_{(T \leq 0.05)} = 1.88$. n = 96

Growth

Both the hypocotyl and seminal root were significantly inhibited when exposed to extracts of all the temperature treatments, however the cold stress treatment was the most detrimental (Table 4 & 5). The results also confirmed that root elongation was more sensitive to allelochemicals than stem (hypocotyl) elongation in both species. Furthermore, both polar and non-polar extracts, at 0.5 and 2 mg ml$^{-1}$, significantly reduced the growth of lettuce (Table 4) and pepper (Table 5). Allelopathy influences plant succession through root exudation, leaching and volatilization when the plant dies and starts to decompose (Rice 1984; Weston 2005; Minorsky 2002; Bertin et al. 2003). The most frequently reported morphological effects from allelochemicals on sensitive plants is the inhibition or retarded seed germination and retarded development of shoots and roots (Ghafarbi et al. 2012). It has been cited in literature that allelopathy was involved in many natural and manipulated ecosystems and that they play a role in the evolution of different plant communities (Ding et al., 2007). Abiotic stresses can lead to morphological, physiological, biochemical and molecular changes within the plants, and therefore has an impact on plant growth (Wang et al., 2003). Climate change and temperature play a role in the synthesis of allelochemicals, which can affect the growth processes of neighbouring plants (Li et al., 2010). Results by Amini (2009, 2012), proved that root exudates of *A. retroflexus* had inhibitory effects on shoot length of both crop (wheat) and vegetable (common bean) plants. Aqueous extracts from the leaves, roots and stems of *A. retroflexus* L. had inhibitory effects on the hypocotyl growth of maize (Konstantinović et al., 2014). Dhole et al. (2013) noticed that seed germination and seedling growth of maize were inhibited when using aqueous extracts from the root, stems and leaves of *A. tricolor* L..

Phenolic compounds take part in the regulation of seed germination and work together in regulating the growth of plants. The role of several phenolic compounds e.g. lignin, salicylic acid, flavonoids and phytoalexins play important roles in plant resistance, taking part in defence responses during biotic and abiotic stress (Kulbat, 2016). It is therefore possible that the reduction in phenolic compounds caused by temperature stress, could play a role in the germination and growth of lettuce and pepper seeds. Furthermore, it is clear from both literature and our results, that concentration plays a major role in the severity of allelopathic effects on different plants (Qasem, 1995; Obaid & Qasem, 2005).

**Table 4:** Hypocotyl and seminal root lengths of lettuce seeds exposed to increasing concentrations of MeOH-$H_2O$ and DCM leaf extracts of *A. cruentus* grown at optimal, cold and hot temperatures

| Extract [mg ml$^{-1}$] | Hypocotyl length of Lettuce (mm) | | | | Seminal root length of Lettuce (mm) | | | |
|---|---|---|---|---|---|---|---|---|
| MeOH-$H_2O$ | Temperature treatment | | | | Temperature treatment | | | |
| | Optimal | Cold | Hot | Ave | Optimal | Cold | Hot | Ave |
| 0 | 28.77±9.07 | 28.77±9.07 | 28.77±9.07 | 28.77 a | 26.31±11.2 | 26.31±11.2 | 26.31±11.2 | 26.31 a |
| 0.5 | 26.77±16.5 | 29.15±17.8 | 31.69±11.4 | 29.03 a | 17.54±12.5 | 13.23±7.25 | 15.08±6.45 | 15.28 b |
| 2 | 20.62±12.8 | 6.31±9.46 | 13.08±7.99 | 13.34 b | 8±7.43 | 3.15±3.31 | 3.85±2.44 | 5 c |
| Ave | 25.39 | 21.41 | 24.51 | | 17.28 | 14.23 | 15.08 | |
| LSD$_{(T \leq 0.05)}$ | T = ns | C = .23 | T×C = | | T = ns | C = .39 | T×C = ns | |
| DCM | | | | | | | | |
| 0 | 35.48±11.7 | 35.48±11.7 | 35.48±11.7 | 35.48 a | 27.63±12.4 | 27.63±12.1 | 27.62±11.8 | 27.63 a |
| 0.5 | 28.85±6.27 | 17.31±9.29 | 9.77±4.64 | 18.64 b | 19.62±5.04 | 15.77±9.49 | 8±4.16 | 14.46 b |
| 2 | 26.77±7.93 | 7.08±8.75 | 25.38±7.79 | 19.74 b | 14.08±5.99 | 9.08±8.84 | 16.69±6.71 | 13.28 b |
| Ave | 29.79 | 20.54 | 23.54 | | 19.49 | 17.82 | 17.44 | |
| LSD$_{(T \leq 0.05)}$ | T = 4.89 | C = 4.89 | T×C = 4.89 | | T = ns | C = .66 | T×C = 4.66 | |

Different letters along the column indicate significant differences at T ≤ 0.05 (Tukey test). Significant differences within each vegetable; n = 13.

**Table 5:** Hypocotyl and seminal root lengths of pepper seeds exposed to increasing concentrations of MeOH-$H_2O$ and DCM leaf extracts of *A. cruentus* grown at optimal, cold and hot temperatures

| Extract [mg ml$^{-1}$] | Hypocotyl length of pepper (mm) | | | | Seminal root length of pepper (mm) | | | |
|---|---|---|---|---|---|---|---|---|
| MeOH-$H_2O$ | Temperature treatment | | | | Temperature treatment | | | |
| | Optimal | Cold | Hot | Ave | Optimal | Cold | Hot | Ave |
| 0 | 20.23±15.3 | 20.23±15.3 | 20.23±15.3 | 20.23 a | 13.92±5.02 | 13.92±5.02 | 13.92±5.02 | 13.92 a |
| 0.5 | 2.69±4.55 | 5.62±3.91 | 11.31±7.78 | 6.54 b | 1.15±2.54 | 3.31±3.28 | 9.85±8.71 | 4.77 b |
| 2 | 1.08±1.80 | 3.46±2.26 | 5.15±1.99 | 3.23 b | 0.46±0.88 | 1.08±0.76 | 3.46±4.74 | 1.67 c |
| Ave | 8 | 9.77 | 12.23 | | 5.18 | 6.10 | 9.08 | |
| LSD$_{(T \leq 0.05)}$ | T=ns | C=4.35 | T×C=ns | | T=2.29 | C=2.29 | T×C=2.29 | |
| DCM | | | | | | | | |
| 0 | 16.08±6.21 | 16.08±6.21 | 16.08±6.21 | 16.08 a | 24.38±11.4 | 24.38±11.4 | 24.38±11.4 | 24.38 a |
| 0.5 | 6.31±2.56 | 4.31±5.75 | 2.46±1.61 | 4.36 b | 12.77±3.68 | 2.85±3.74 | 1.92±0.86 | 5.85 b |
| 2 | 3.69±2.66 | 4.31±5.02 | 8.77±7.33 | 5.59 b | 5±5.74 | 6.46±8.90 | 5.85±6.01 | 5.77 b |
| Ave | 8.69 | 8.23 | 9.10 | | 14.05 | 11.23 | 10.72 | |
| LSD$_{(T \leq 0.05)}$ | T = ns | C = 2.78 | T×C = 2.78 | | T = ns | C = 3.87 | T×C = 3.87 | |

Different letters along the column indicate significant differences at T ≤ 0.05 (Tukey test). Significant differences within each vegetable; n = 13.

Many studies have been done on allelopathy of the polar extracts of amaranth, but no information is available on the non-polar compounds. This is the first report on the *in vitro* phytotoxicity of a DCM extract of grain amaranth. The characteristics of allelochemicals are important and play a significant role in their fate in the environment. For example, the mobility of compounds within the soil are influenced by their water solubility; the vapour pressure can impact their volatilization and their chemical structure can affect their affinity with the soil surface (Souza Filho & Alves 2002). The outcome of all these complex interactions results in compounds with allelopathic properties which can be of potential agronomic use.

## 4 CONCLUSION

Temperature influenced the chemical composition of *A. cruentus* and *in vitro* bioassays proved the negative impact of the extracts on germination and growth of vegetables. This demonstrated that the environment for the cultivation of *A. cruentus* is important and that more than one compound were responsible for allelopathy, thus both polar and non-polar compounds were involved. Furthermore, with increased concentrations of extracts a decrease in germination and seedling development occurred. Consequently, if more plant residues are left behind in the soil, the growth of the next crop will often be affected with a subsequent decline in yield. It was also clear that vegetables displayed diversity in reaction towards the temperature treatments and type of extract. This information proves that a holistic understanding of the influence of abiotic environmental factors on the production of metabolites in various plant parts are of importance. The release of potentially phytotoxic compounds from *A. cruentus* leaf litter into soil deserves further investigation as well as the purification of extracts to determine unidentified natural compounds with herbicidal activity.

## 5 REFERENCES


Allemann, I., Cawood, M.E., Allemann, J. (2017). Influence of altered temperatures on allelopatic properties of Amaranthus cruentus L. *Acta agriculturae Slovenica, 109*(2), 465-471. doi:10.14720/aas.2017.109.2.29

Alemayehu, F.R., Bendevis, M.A., Jacobsen, S.E. (2015). The potential for utilizing the seed crop Amaranth (Amaranthus spp.) in East Africa as an alternative crop to support food security and climate change mitigation. Journal of Agronomy and Crop Science, 201, 321–329. doi:10.1111/jac.12108

Amini, R., An, M., Pratley, J., Azimi, S. (2009). Allelopathic assessment of annual ryegrass (Lolium rigidum): Bioassays. Allelopathy Journal, 24(1), 67-79.

Amini, R., Movahedpour, F., Ghassemi-Golezani, K., Mohammadi-Nasab, A.D., Zafarani-Moattar, P. (2012). Allelopathic assessment of common amaranth by ECAM. International Research Journal of Applied & Basic Sciences, 3(11), 2268-2272.

Bais, H.P., Kaushik, S. (2010). Catechin secretion & phytotoxicity. Communicative & integrative Biology, 3(5), 468-470. doi:10.4161/cib.3.5.12559

Basile, A., Sorbo, S., Giordano, S., Ricciardi, L., Ferrara, S., Montesano, D., Cobianchi, R.C., Vuotto, M.L., Ferrara, L. (2000). Antibacterial and allelopathic activity of extract from Castanea sativa leaves. Fitoterapia 7, Suppl., S110-S116. doi:10.1016/S0367-326X(00)00185-4

Bertin, C., Yang, X., Weston, L.A. (2003). The role of root exudates and allelochemicals in the rhizosphere. Plant and Soil, 256, 67-83. doi:10.1023/A:1026290508166

Bishop, G.J., Yokota, T. (2001). Plants steroid hormones, brassinosteroids: current highlights of molecular aspects on their synthesis/metabolism, transport, perception and response. Plant and Cell Physiology, 42(2), 114-120. doi:10.1093/pcp/pce018

Cai, Y., Sun, M., Corke, H. (1998). Colorant properties and stability of Amaranthus betacyanin pigments. Journal of Agricultural and Food Chemistry, 46(11), 4491-4495. doi:10.1021/jf980457g

Chobot, V., Huber, C., Trettenhahn, G., Hadacek, F. (2009). (±)-Catechin: Chemical weapon, antioxidant, or stress regulator? Journal of Chemical Ecology, 35(8), 980–996. doi:10.1007/s10886-009-9681-x

Connick, W.J., Bradow, J.M., Legendre, M.G. (1989). Identification and bioactivity of volatile allelochemicals from Amaranth residues. Journal of Agricultural and Food Chemistry, 37, 792-796. doi:10.1021/jf00087a049

Dhole, J.A., Lone, K.D., Dhole, G.A., Bodke, S.S. (2013). Allelopathic effect of aqueous and ethanolic extracts of some common weeds on seed health of Triticum aestivum L. (Wheat). International Journal of Current Microbiology and Applied Sciences, 2(6), 254-260.

Ding, J., Sun, Y., Xiao, C.L., Shi, K., Zhou, Y.H., Yu, J.Q. (2007). Physiological basis of different allelopathic reactions of cucumber and fig leaf gourd plants to cinnamic acid. Journal of

Experimental Botany, 58(13), 3765-3773. doi:10.1093/jxb/erm227

Einhellig, F.A., Eckrich, P.C. (1984). Interactions of temperature and ferulic acid stress on grain sorghum and soybeans. Journal of Chemical Ecology, 10, 161-170. doi:10.1007/BF00987653

Einhellig, F.A. (1987). Interactions among allelochemicals and other stress factors of the plant environment. ACS Symposium Series, 330, 343–357. doi:10.1021/bk-1987-0330.ch032

Einhellig, F.A. (1996). Interactions involving allelopathy in cropping system. Agronomy Journal, 88, 886–893. doi:10.2134/agronj1996.00021962003600060007x

Fujii, Y., Parvez, S.S., Parvez, M.M., Ohmae, Y., Iida, O. (2003). Screening of 239 medical plant species for allelopathic activity using the sandwich method. Weed Biology and Management, 3, 233-241. doi:10.1046/j.1444-6162.2003.00111.x

Gamel, T.H., Mesallam, A.S., Damir, A.A, Shekib, L.A, Linssen, J.P. (2007). Characterization of Amaranth seed oils. Journal of Food Lipids, 14, 323-334. doi:10.1111/j.1745-4522.2007.00089.x

Gershenzon, J. (1984). Changes in the levels of plant secondary metabolites under water and nutrient stress. Recent Advances in Phytochemistry, 18, 273-320. doi:10.1007/978-1-4684-1206-2_10

Ghafarbi, S.P., Hassannejad, S., Lotfi, R. (2012). Allelopathic effects of wheat seed extracts on seed and seedling growth of eight selected weed species. International Journal of Agricultural Research, 4(19), 1452-1457.

Gobbo-Neto, L., Lopes, N.P. (2007). Medicinal plants: factors of influence on the content of secondary metabolites. Quimica Nova, 30(2), 374-381. doi:10.1590/S0100-40422007000200026

Gouvea, D.R., Meloni, F., Ribeiro, A.B.B., Lopes, J.L.C., Lopes, N.P. (2012). A new HPLC-DAD-MS/MS method for the simultaneous determination of major compounds in the crude extract of Lychnophora salicifolia Mart. (Brazilian arnicão) leaves: Application of chemical variability evaluation. Analytica Chimica Acta, 748, 28-36. doi:10.1016/j.aca.2012.08.027

Hill, E.C., Ngouajio, M., Nair, M.G. (2007). Allelopathic potential of hairt vetch (Vicia villosa) and cowpea (Vigna unguiculata) methanol and ethyl acetate extracts on weeds and vegetables. Weed Technology, 21, 437-444. doi:10.1614/WT-06-167.1

Inderjit, A.U., Dakshini, K.M.M. (1995). On laboratory bioassays in allelopathy. The Botanical Review, 61, 28-44. doi:10.1007/BF02897150

Kobayashi, K. (2004). Factors affecting phytotoxic activity of allelochemicals in soil. Weed Biology and Management, 4, 1–7. doi:10.1111/j.1445-6664.2003.00112.x

Koeppe, P.E., Rohrbaugh, L.M., Wender, S.H. (1969). The effect of varying UV intensities on the concentration of scopolin and caffeoylquinic acids in tobacco and sunflower. Phytochemistry, 8, 889-896. doi:10.1016/S0031-9422(00)85879-3

Konstantinović, B., Blagojević, M., Konstantinivić, B., Samardžić, N. (2014). Allelopathic effect of weed species Amaranthus retroflexus L. on maize seed germination. Romanian Agricultural Research, 31, 315-321.

Kraujalis, P., Venskutonis, P.R., Kraujaliene, V., Pukalskas, A. (2013). Antioxidant properties and preliminary evaluation of phytochemical composition of different anatomical parts of Amaranth. Plant Foods for Human Nutrition, 68, 322–328. doi:10.1007/s11130-013-0375-8

Król, A., Amarowicz, R., Weidner, S. (2014). Changes in the composition of phenolic compounds and antioxidant properties of grapevine roots and leaves (Vitis vinifera L.) under continuous of long-term drought stress. Acta Physiologiae Plantarum, 36, 1491–1499. doi:10.1007/s11738-014-1526-8

Kulbat, K. (2016). The role of phenolic compounds in plant resistance. Biotechnology and Food Sciences, 80(2), 97-108.

Li, J., Ou-Lee, T.M., Raba, R., Amundsom, R.G., Last, R.L. (1993). Arabidopsis flavonoid mutants are hypersensitive to UV-B irradiation. The Plant Cell, 5(2), 171-179. doi:10.1105/tpc.5.2.171

Li, Z-H., Wang, O., Xiao, R., Cun, D.P., Jiang, D-A. (2010). Phenolics and plant allelopathy. Molecules, 15, 8933-8952. doi:10.3390/molecules15128933

Maiyo, Z.C., Ngure, R.M., Matasyoh, J.C., Chepkorir, R. (2010). Phytochemical constituents and antimicrobial activity of leaf extracts of three Amaranthus plant species. African Journal of. Biotechnology, 9(21), 3178-3182.

Mensah, J.K., Okoli, R.I., Ohaju-Obodo, J.O., Eifediyi, K. (2008). Phytochemical, nutritional and medical properties of some leafy vegetables consumed by Edo people of Nigeria. African Journal of. Biotechnology, 7(14), 2304-2309.

Miller, A.L. (1996). Antioxidant flavonoids: structure, function and clinical usage. Alternative Medicine Review, 1(2), 103-111.

Minorsky, V. (2002). Allelopathy and grain crop production. Plant Physiology, 130, 1745-1746. doi:10.1104/pp.900057

Morrison, J.I.L., Lawlor, D.W. (1999). Interactions between increasing $CO_2$ concentration and temperature on plant growth. Plant, Cell & Environment, 22, 659-82. doi:10.1046/j.1365-3040.1999.00443.x

Mroczek, A. (2015). Phytochemistry and bioactivity of triterpene saponins from Amaranthaceae family. Phytochemistry Reviews, 14, 577-605. doi:10.1007/s11101-015-9394-4

Nana, F.W., Hilou, A., Millogo, J.F., Nacoulma, O.G. (2012). Phytochemical composition, antioxidant and xanthine oxidase inhibitory activities of Amaranthus cruentus L. and Amaranthus hybridus L. extracts. Pharmaceuticals, 5(6), 613-628. doi:10.3390/ph5060613

Obaid, K.A., Qasem, J.R. (2005). Allelopathic activity of common weed species on vegetable crops grown in Jordan. Allelopathy Journal, 15(2), 221-236.

Oboh, G., Raddatz, H., Henle, T. (2008). Antioxidant properties of polar and non-polar extracts of some tropical green leafy vegetables. Journal of the Science of Food and Agriculture, 88, 2486–2492. doi:10.1002/jsfa.3367

Qasem, J.R. (1995). The allelopathic effect of three Amaranthus spp. (pigweeds) on wheat (Triticum durum). Weed Research, 35(1), 41-49. doi:10.1111/j.1365-3180.1995.tb02015.x

Pifrung, M.C. (2006). The Synthetic Chemists Companion. Wiley. Online Library. http//www.onlinelibrary.wiley.com/book/10.1002/9780470141045 (15/09/2013).

Ramakrishna, A., Ravishankar, G.A. (2011). Influence of abiotic stress signals on secondary metabolites in plants. Plant Signaling & Behavior, 6(11), 1720-1731. doi:10.4161/psb.6.11.17613

Rhoads, D.M., Umbach, A.L., Subbaiah, C.C., Siedow, J.N. (2006). Mitochondrial reactive oxygen species. Contribution to oxidative stress and interorganellar signaling. Plant Physiology, 141, 357–366. doi:10.1104/pp.106.079129

Rice, E.L. (1984). Allelopathy. 2nd ed. Orlando, Florida, USA: Academic Press; p. 67-68.

Rudikovskaya, E.G., Fedorova, G.A., Dudareva, L.V., Makarova, L.E., Rudikovskii, A.V. (2008). Effect of growth temperature on the composition of phenols in pea roots. Russian Journal of Plant Physiology, 55(5), 712-715. doi:10.1134/S1021443708050178

Shah, A.H.A. (2005). Spinoside, new coumaroyl flavone glycoside from Amaranthus spinosus. Archives of Pharmacal Research, 27(12), 1216-1219.

Singleton, V.L., Rossi, Jr J.A. (1965). Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16, 144-158.

Souza Filho, A.P.S., Alves, S.M. (2002). Allelopathy: Basic principles and general aspects. EMBRAPA Amazônia Oriental: Belém, Brazil, p. 206.

Steel, R.G.D., Torrie, J.H. (1980). Principles and procedures of statistics, 2nd ed. New York: McGraw-Hill; p. 633.

Steffensen, S.K., Rinnan, Å., Mortensen, A.G., Laursen, B., de Troiani, R.M., Noellemeyer, E.J., Janovska, D., Dusek, K., Délano-Frier, J., Taberner, A., et al. (2011). Variations in the polyphenol content of seeds of field grown Amaranthus genotypes. Food Chemistry, 129, 131–138. doi:10.1016/j.foodchem.2011.04.044

Stintzing, F.C., Carle, R. (2004). Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. Trends in Food Science & Technology, 15, 19-38. doi:10.1016/j.tifs.2003.07.004

Szakiel, A., Paczkowski, C., Henry, M. (2010). Influence of environmental abiotic factors on the content of saponins in plants. Phytochemistry Reviews, 10, 471-491. doi:10.1007/s11101-010-9177-x

Tang, C-H., Cai, W-F., Kohl, K., Nishimote, R.K. (1995). Plant stress and allelopathy. In: Inderjit AU, Dakshini KMM, Einhellig FA, editors. Allelopathy, Organisms, Processes and Applications. American Chemical Society: Washington, DC; p. 142–157.

Trezzi, M.M., Vidal, R.A., Balbinot, Junior A.A., Bittencourt, H.H., da Silva Souza Filho, A.P. (2016). Allelopathy: driving mechanisms governing its activity in agriculture. Journal of Plant Interactions, 11(1), 53-60. doi:10.1080/17429145.2016.1159342

Vidović, M., Morina, F., Milić, S., Jovanović, S.V. (2015). An improved HPLC-DAD method for simultaneously measuring phenolics in the leaves of Tilia platyphyllos and Ailanthus altissima. Botanica Serbica, 39(2), 177-186.

Vincken, J.P., Heng, L., De Groot, A., Gruppen, H. (2007). Saponins, classification and occurrence in the plant kingdom. Phytochemistry, 68, 275–97. doi:10.1016/j.phytochem.2006.10.008

Wagner, H., Bladt, S. (1996). Plant Drug Analysis: A thin layer chromatography atlas. 2nd ed. Springer-Verlag Berlin Heidelberg, Germany; p. 384. doi:10.1007/978-3-642-00574-9

Waller, G.R. (1987). Allelochemicals: role in agriculture and forestry. ACS Symposium Series, 330, American Chemical Society, Washington, D. C. doi:10.1021/bk-1987-0330

Wang, W., Vinocur, B., Altman, A. (2003). Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta, 218, 1-14. doi:10.1007/s00425-003-1105-5

Wender, S.H. (1970). Effects of some environmental stress factors on certain phenolic compounds in tobacco. Recent Advances in Phytochemistry, 3, 1-29.

Weston, L.A. (2005). History and current trends in the use of allelopathy for weed management. Hort Technology, 15(3), 529-534.

Zhishen, J., Mengcheng, T., Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry, 64, 555-559. doi:10.1016/S0308-8146(98)00102-2

doi:10.14720/aas.2018.111.3.10 Original research article / izvirni znanstveni članek

# Waterlogging effects on some antioxidant enzymes activities and yield of three wheat promising lines

Fereshteh ALIZADEH-VASKASI[1], Hemmatollah PIRDASHTI[2*], Ali CHERATI ARAEI[3], Sara SAADATMAND[1]

Received May 31, 2018; accepted October 25, 2018.
Delo je prispelo 31. maja 2018, sprejeto 25. oktobra 2018.

**ABSTRACT**

Waterlogging is one of the most important environmental stresses that have negative effects on wheat growth and yield. The purpose of this study was to investigate the effect of waterlogging (0, 7, 14 and 21 d) at tillering (ZG21) and stem elongation (ZG31) stages on the content of photosynthetic pigments, proline, malondialdehyde (MDA), antioxidant enzymes, grain yield and yield components of three wheat promising lines (N-93-19, N-93-9 and N-92-9). Increasing waterlogging stress reduce the photosynthetic pigments contents and the activity of catalase enzyme while increase the proline content, MDA, superoxide dismutase and peroxidase enzymes in three wheat genotypes in both tillering and stem elongation stages. Waterlogging also reduced yield and yield components in three wheat genotypes. The results showed that N-92-9 genotype had better response than other two genotypes in all studied traits under waterlogging conditions.

**Key words:** waterlogging stress; antioxidant enzymes; wheat; proline; yield; yield components

**IZVLEČEK**

**UČINKI ZASTAJANJA VODE V TLEH NA AKTIVNOST NEKATERIH ANTIOKSIDACIJSKIH ENCIMOV IN PRIDELEK TREH OBETAJOČIH LINIJ PŠENICE**

Zastajanje vode v tleh je eden od najpomembnejših okoljskih stresov, ki ima negativni učinek na rast in pridelek pšenice. Namen raziskave je bil preučiti učinke zastajanja vode v tleh (0, 7, 14 in 21 dni) v fazah razraščanja (ZG21) in bilčenja (ZG31), prolina, malondialdehida (MDA), antioksidacijskih encimov, pridelek zrnja in komponenete pridelka treh obetajočih linij pšenice (N-93-19, N-93-9 in N-92-9). Povečanje stresa zaradi zastoja vode v tleh zmanjša vsebnost fotosinteznih pigmentov in aktivnost katalaze in poveča vsebnost prolina in MDA, poveča aktivnost superoksid dismutaze in peroksidaze pri vseh treh genotipih pšenice v vseh preučevanih fazah razvoja. Zastajanje vode v tleh je tudi zmanjšalo pridelek in njegove komponente pri vseh treh genotipih pšenice. Rezultati so pokazali, da se je genotip N-92-9 bolje odzval na razmere zastajanja vode v tleh v vseh preučevanih znakih kot ostala dva analizirana genotipa.

**Ključne besede:** stres zaradi zastajanja vode v tleh; antioksidacijski encimi; pšenica; prolin; pridelek: komponenete pridelka

## 1 INTRODUCTION

Waterlogging, which is due to poor drainage of the soil under inappropriate irrigation practices or heavy rainfall, is one of the abiotic stresses and considered as one of the main limitation factors for the growth and yield of crops. Therefore, excess water around the plant's root causes a decrease in oxygen concentration and the plant encounters oxygen deficiency, which is known as hypoxia (Dennis et al., 2000). Plants need to exchange atmospheric gases to maintain their natural growth and photosynthesis and respiration processes (Suzuki and Mittler, 2006). Therefore, under flooding conditions, excess water around the root reduces gas

[1] Department of Biology, Basic Science Campus, Science and Research Branch, Islamic Azad University, Tehran, Iran

[2] Department of Agronomy, Genetics and Agricultural Biotechnology Institute of Tabarestan, Sari agricultural science and natural resources university, Sari, Iran; *corresponding author: Pirdasht@yahoo.com

[3] Agricultural and Natural Resources Research Center of Mazandaran, Iran

diffusion, which directly affects the absorption of nutrients, plant growth and yield (Fukao and Bailey-Serres, 2004).

The tolerance to flooding conditions varies in crops that depend on biochemical and anatomical adaptations (Liu et al., 2005). The closure of the stomata to prevent water loss along with the adjustment of photosynthetic apparatus is one of the main responses of the plant under waterlogging stress (Arbona et al., 2008). In addition, under environmental stresses, antioxidant defense systems play an important role in plant tolerance to stress. Reactive oxygen species (ROS) damage the living tissues through the oxidation of biological macromolecules such as lipids, proteins, and nucleic acid (Mittler et al., 2004). By converting ROS to harmless compounds by enzymatic and non-enzymatic antioxidants, the toxic effect of ROS is reduced. The first cellular defense line is superoxide dismutase (SOD) followed by catalase (CAT) and peroxidase (POD) (Edreva, 2005). In various studies, increased activity of antioxidant enzymes has been reported under various environmental stresses (Kumutha et al., 2009; Amador et al., 2012; Gerami et al., 2018). Van Toai and Bolles (1991) showed that high SOD activity with superoxide detoxification could help tolerate the plant under flood stress.

Wheat (*Triticum aestivum* L.) is one of the most important crops that is widely cultivated under different climatic conditions. Wheat is grown in areas with different moisture conditions where rainfall ranges from 250 to 1800 millimeters. However, the most areas of wheat cultivation receive an average annual rainfall of 380 and 880 millimeters (Herzog et al., 2016). Wheat cultivation requires adequate moisture during the growing season; however, excessive irrigation or precipitation causes waterlogging. According to estimates, more than 10 to 15 million ha of wheat fields are under waterlogging threat (Sayre et al., 1994). Olgun et al. (2008) reported that wheat yield reduced under waterlogging stress. In other study, Collaku and Harrison (2002) examined the effect of different levels of waterlogging (0, 10, 20 and 30 d) on nine wheat cultivars, indicating that waterlogging stress reduced the yield of nine wheat cultivars from 35 to 60 %.

The purpose of this study was to evaluate the effects of waterlogging on biochemical traits such as content of chlorophyll a and b, proline and antioxidant enzymes activity of three wheat-promising lines in two tillering and stem elongation stages. In addition, at physiological maturity, grain yield, number of seeds per spike, grain mass per spike and number of spikes per square meter were investigated.

## 2 MATERIALS AND METHODS

Seeds (20 seeds per pot) of three wheat-promising lines (N-93-19, N-93-9 and N-92-9) were sown in plastic pots (19 cm depth, 23 cm diameter). The pots were filled with 4 kg of sterilized field soil amended with 2.4 g $K_2O$, 3.2 g $P_2O_5$ and 4 g $(NH_4)_2SO_4$ per pot (240 pots). After germination, plants were thinned to 10 plants per pot. Waterlogging treatments included 0, 7, 14 and 21 days in tillering (ZG21) and stem elongation (ZG31) stages. In the stem elongation stage, plants of all three genotypes after 21 days of waterlogging were completely died, which were not sampled. Waterlogging stress was applied by placing the pots in larger pots (30 cm depth, 30 cm diameter) filled with water to 2 cm above the surface of the soil. The control plants were watered as much as needed to avoid waterlogging stress and drought stress. At the end of the waterlogging treatments, half of the pots (5 pots) were harvested to measure the biochemical traits, while the remaining pots were grown until physiological maturity. Then seeds/spike, seeds mass/spike, spikes/$m^2$ and grain yield were measured according to Ceylan (1994).

To measure chlorophyll (a and b) and carotenoids, extracts of fresh leaves with 80 % acetone according to method of Lichtenthaler and Wellburm (1983) were used. For proline measurement, fresh leaves were extracted with methanol (40 %). Then 1 ml of methanolic extract was mixed with 25 ml of ninhydrin and 1 ml of the mixture of orthophosphoric acid (6M) and glacial acetic acid (2.3 v/v). The tubes were incubated at 100 °C and after cooling, toluene (5 ml) was added. By reading the absorbance of supernatant at 528 nm using spectrophotometer (Varian, Carry 300, California, USA), free proline content was calculated according to Bates et al. (1973) method. In order to measure lipid peroxidation, malondialdehyde (MDA) content was determined using thiobarbituric acid method according to Heath and Packer (1968) and an extinction coefficient of 155 $mM^{-1}$ $cm^{-1}$.

Fresh leaf samples (10 g) were homogenized in 100 mM Tris–HCl (pH 7.5) comprising 5mM dithiothreitol (DTT), 10 mM magnesium chloride ($MgCl_2$), 1mM ethylenediaminetetraacetic acid (EDTA), 5mM $Mg(CH_3COO)_2$ (magnesium acetate), 1.5 % polyvinylpyrrolidone (PVP), 1 mM phenylmethanesulfonyl fluoride (PMSF), and 1 µg $ml^{-1}$ aproptinin. As the enzyme source, the supernatant separated after centrifugation of homogenate at 15000 g for 20 minutes has been used.

The superoxide dismutase (SOD) activity was measured using photo-reduction of NBT (nitrobluetetrazolium). The amount of protein used to inhibit 50 % of the NBT photo-reduction at 560 nm was considered as one unit of activity of SOD enzyme. The protocol of Luck (1971) has been applied for determination of catalase (CAT) activity by using the rate of decrease in absorbance at 240 nm for two minutes. Meahly and Chance (1954) method was used to measure the peroxidase (POD) activity based on the oxidation of guaiacol in the presence of hydrogen peroxide ($H_2O_2$). All spectrophotometric experiments were performed using a Varian, Carry 300, California, USA spectrophotometer.

Two-way ANOVA has been used for the analysis of the results by with SAS 9.1.3 software and means were compared with the LSD test ($P < 0.05$).

## 3 RESULTS

### 3.1 Photosynthetic pigments

In tillering and stem elongation stages, waterlogging treatments reduced the content of chlorophyll a and b in all three wheat genotypes. The highest reduction of chlorophylls was observed during 14 and 21 d waterlogging in tillering and stem elongation stages, respectively. In all treatments, N-93-19 and N-92-9 genotypes had the highest and lowest reductions, respectively, as compared to the corresponding control (Fig 1 and 2). In the tillering stage, carotenoids contents decreased in N-93-19 genotype by 15.45, 37.55 and 67.38 %, in N-93-9 genotype by 14.88, 26.48 and 53.61 %, in N-92-9 genotypes by 11.03, 15.86 and 28.96 % under treatment of 7, 14 and 21 d waterlogging, respectively as compared to the corresponding controls (Fig 3 A). 7 d waterlogging in stem elongation stage reduced carotenoids in all three wheat genotypes, but there was no significant difference between the three wheat genotypes. 14 d waterlogging treatment reduced carotenoids contents in N-93-19, N-93-9 and N-92-9 by 49.14, 35.5 and 32.15 %, respectively, as compared to the corresponding controls (Fig 3 B).

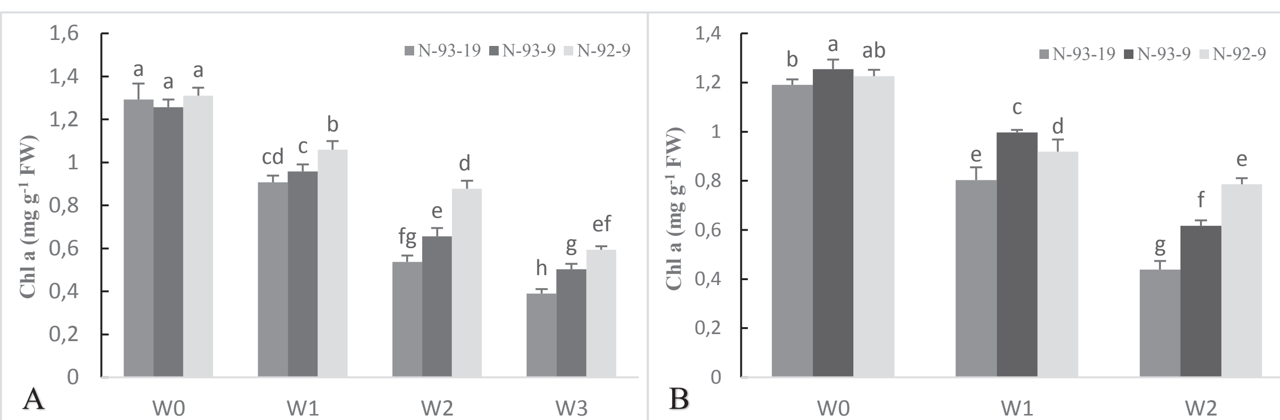



**Figure 1:** Effect of waterlogging (W0: 0, W1: 7, W3: 14 and W4: 21 d) on contents of chlorophyll a in wheat genotypes in tillering (A) and stem elongation (B) stages. Means followed by the same letter are not significantly different ($P < 0.05$) according to LSD test ($n = 5$)

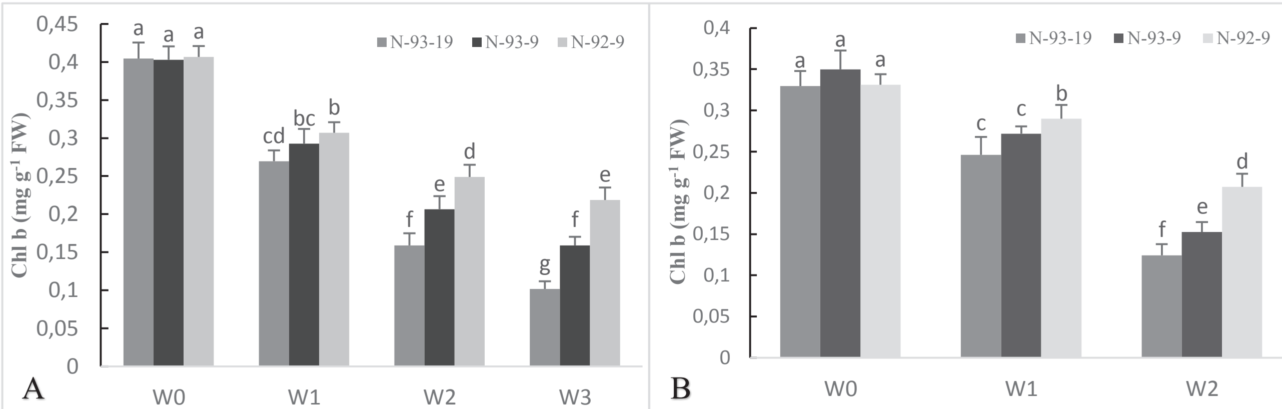



**Figure 2:** Effect of waterlogging (W0: 0, W1: 7, W3: 14 and W4: 21 d) on contents of chlorophyll b in wheat genotypes in tillering (A) and stem elongation (B) stages. Means followed by the same letter are not significantly different (P < 0.05) according to LSD test (n = 5)

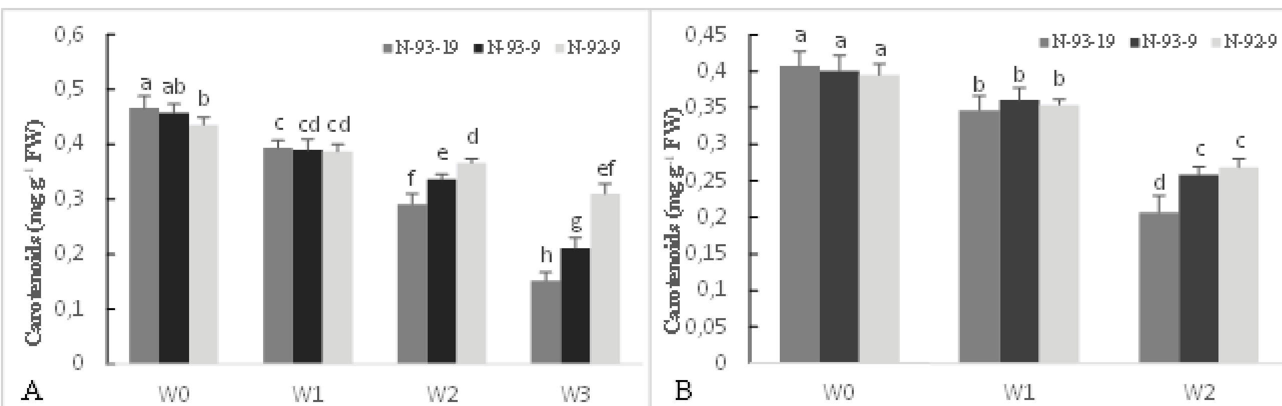



**Figure 3:** Effect of waterlogging (W0: 0, W1: 7, W3: 14 and W4: 21 d) on contents of carotenoids in wheat genotypes in tillering (A) and stem elongation (B) stages. Means followed by the same letter are not significantly different (P < 0.05) according to LSD test (n = 5)

### 3.2 Proline and MDA contents

Waterlogging treatments in tillering stage (4, 7 and 21 d) and stem elongation stage (7 and 14 d) resulted in significant increase of proline contents in all three wheat genotypes. The highest increase was observed in N-92-9 genotype (Fig 4). The waterlogging-induced oxidative stress results in lipid peroxidation and damage to the membrane, evaluated as MDA, representing possible damage. MDA contents in both growth stages under waterlogging treatment showed a significant increase in all three wheat genotypes as compared to corresponding controls and N-93-19 genotype showed the highest increase compared to other wheat genotypes (Fig 5). Proline and MDA contents also increased with increasing waterlogging duration, so that the highest proline and MDA contents were observed in the tillering stage under 21 d waterlogging and in stem elongation stage under 14 d waterlogging (Fig 4 and 5).

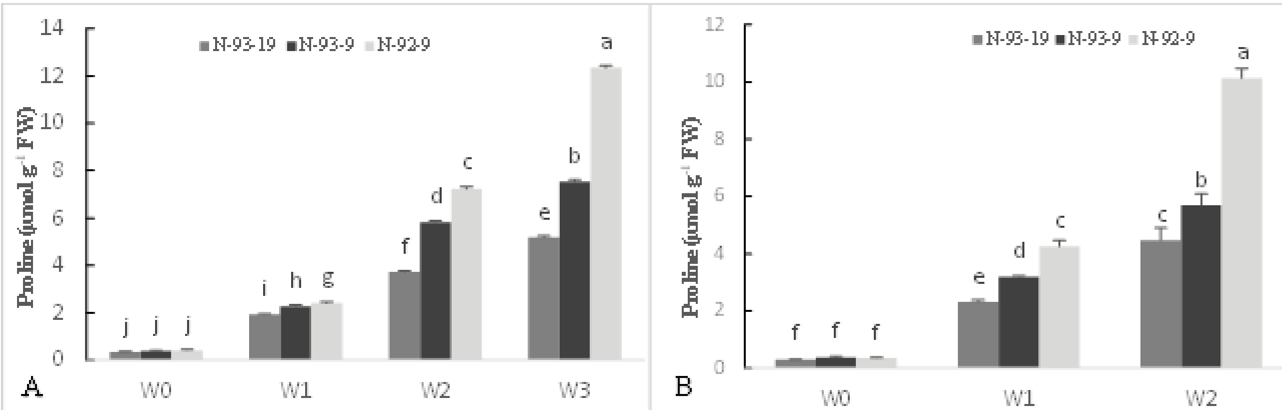



**Figure 4:** Effect of waterlogging (W0: 0, W1: 7, W3: 14 and W4: 21 d) on contents of proline in wheat genotypes in tillering (A) and stem elongation (B) stages. Means followed by the same letter are not significantly different (P < 0.05) according to LSD test (n = 5)

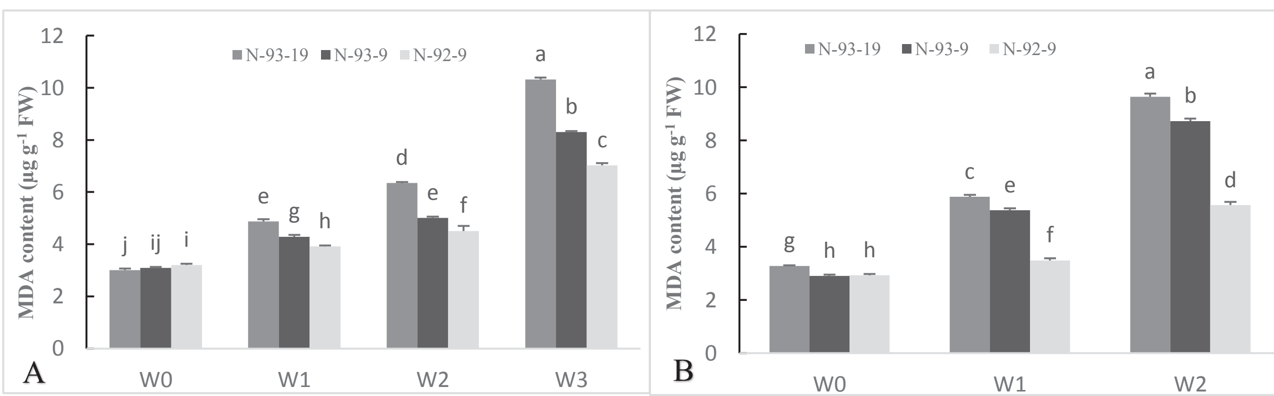



**Figure 5:** Effect of waterlogging (W0: 0, W1: 7, W3: 14 and W4: 21 d) on contents of MDA in wheat genotypes in tillering (A) and stem elongation (B) stages. Means followed by the same letter are not significantly different (P < 0.05) according to LSD test (n = 5)

### 3.3 Antioxidant activity

Waterlogging treatment in the tillering stage significantly increased the activity of SOD enzyme in all three wheat genotypes. At tillering stage, the highest increase in N-93-9, N-92-9 and N-93-19 genotypes observed under 21 d waterlogging by 73.67, 79.55 and 66.99 %, respectively as compared to the corresponding control (Fig 6 A). In stem elongation stage, the waterlogging stress increased the activity of the SOD and the highest activity of SOD observed in N-92-9 genotype under 14 d waterlogging (Fig 6 B). In all three wheat genotypes, waterlogging treatment significantly reduced the activity of POD enzyme in stem elongation and tillering stages. There was no significant difference between wheat genotypes in the tillering stage, however, in the stem elongation stage, the highest decrease of POD activity observed in N-93-19 genotype under 14 d waterlogging (Fig 7). The results of CAT activity in the tillering stage indicated that the waterlogging treatment reduced the activity of CAT in N-93-19 and N-93-9 genotypes, while the activity of this enzyme under the waterlogging conditions in N-92-9 genotype had a significant increase as compared to the control (Fig 8 A). The results of CAT enzyme activity in stem elongation stage indicated that wheat genotypes responded differently to waterlogging stress. In N-93-19 genotype, waterlogging treatment reduced the activity of CAT enzyme and the highest reduction observed for 14 d waterlogging. CAT enzyme activity in N-93-9 genotype significantly increased under 7 d waterlogging than control, but decreased under 14 days of waterlogging. There was no significant difference in CAT activity in N-92-9 genotype under different waterlogging durations (Fig 8 B).

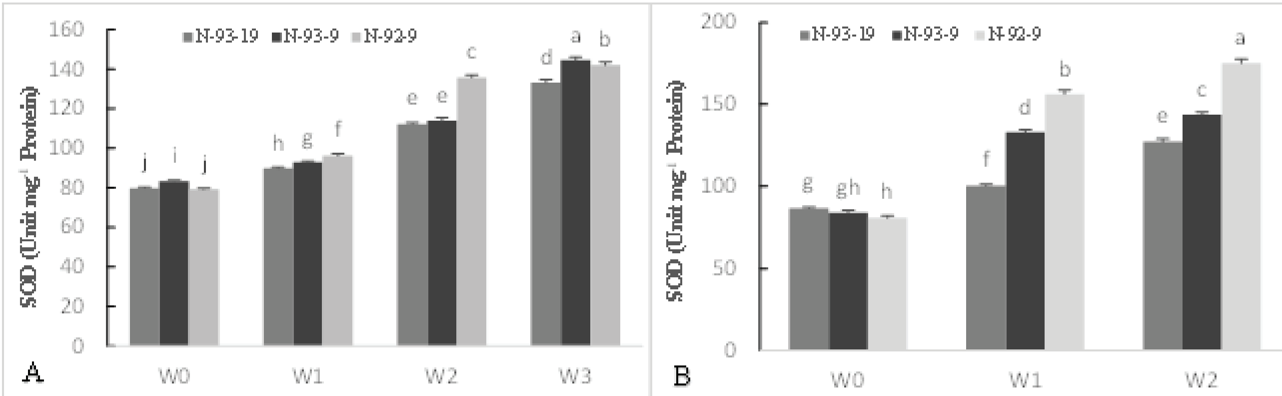



**Figure 6:** Effect of waterlogging (W0: 0, W1: 7, W3: 14 and W4: 21 d) on SOD activity in wheat genotypes in tillering (A) and stem elongation (B) stages. Means followed by the same letter are not significantly different ($P < 0.05$) according to LSD test ($n = 5$).

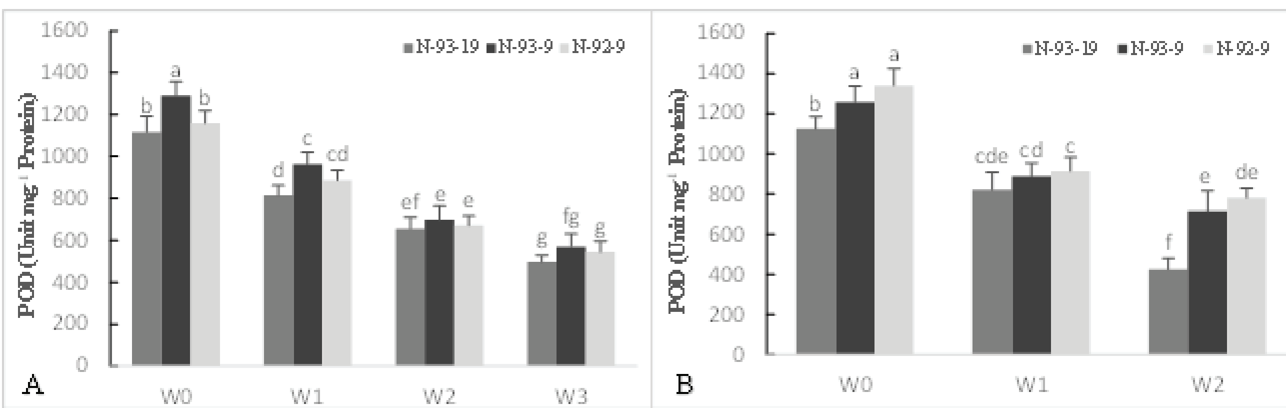



**Figure 7:** Effect of waterlogging (W0: 0, W1: 7, W3: 14 and W4: 21 d) on POD activity in wheat genotypes in tillering (A) and stem elongation (B) stages. Means followed by the same letter are not significantly different ($P < 0.05$) according to LSD test ($n = 5$).

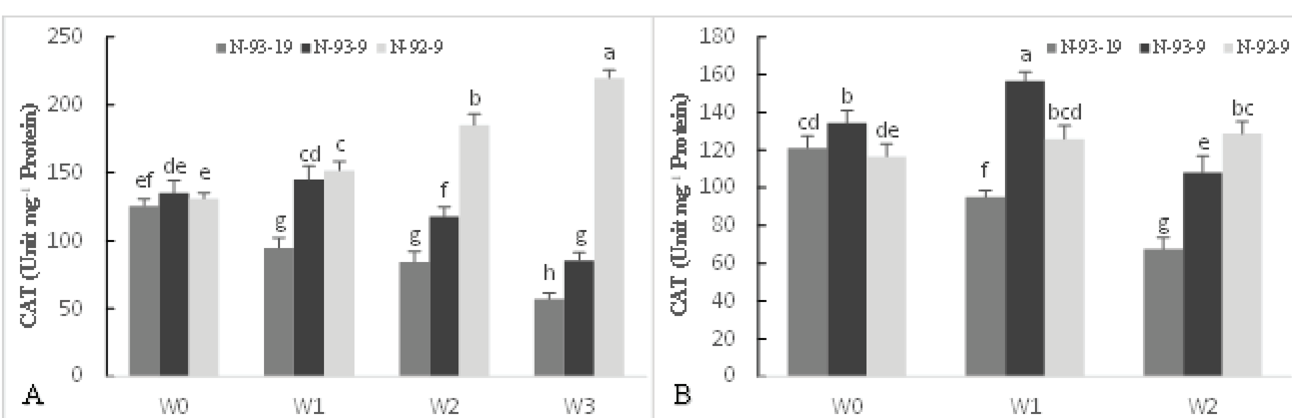



**Figure 8:** Effect of waterlogging (W0: 0, W1: 7, W3: 14 and W4: 21 d) on CAT activity in wheat genotypes in tillering (A) and stem elongation (B) stages. Means followed by the same letter are not significantly different ($P < 0.05$) according to LSD test ($n = 5$)

### 3.4 Yields and yield components

In tillering and stem elongation stages, waterlogging treatments decreased kernels/spike and kernels mass/spike in all three wheat genotypes. The highest decline of kernels/spike and kernels mass/spike were observed during 14 and 21 d waterlogging in tillering and stem elongation stages, respectively. In all treatments, N-93-19 and N-92-9 genotypes had the highest and lowest reductions, respectively as compared to the corresponding control (Fig 9 and 10). Waterlogging treatment in the tillering stage significantly reduced grain yield in all three wheat genotypes. At tillering stage, the highest decline in N-93-9, N-92-9 and N-93-19 genotypes observed under 21 d waterlogging by 60.15, 56.01 and 37.08 %, respectively as compared to the corresponding control (Fig 11 A). In stem elongation stage, the waterlogging stress decreased the grain yield and the highest reduction observed in N-93-19 genotype under 14 d waterlogging (Fig 11 B). The spikes/m$^2$ is an important criterion for assessing the effect of stress on wheat yield. In tillering and stem elongation stages, waterlogging treatments reduced the spikes/m$^2$ in all three wheat genotypes. The highest reduction in spike/m$^2$ were observed during 14 and 21 d waterlogging in tillering and stem elongation stages, respectively. In all treatments, N-92-9 and N-93-19 genotypes had the lowest and highest reductions, respectively as compared to the corresponding control (Fig 12).

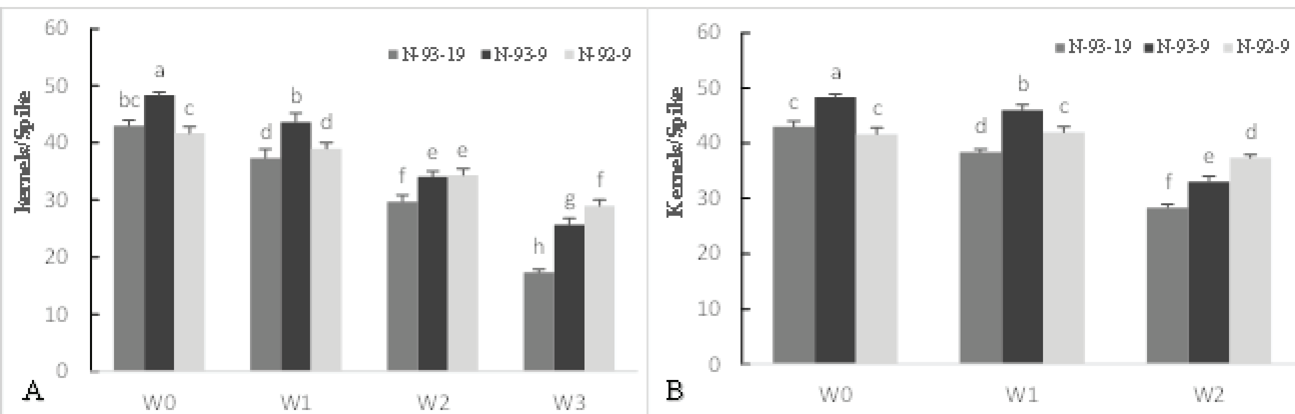



**Figure 9:** Effect of waterlogging (W0: 0, W1: 7, W3: 14 and W4: 21 d) on kernels/spike in wheat genotypes in tillering (A) and stem elongation (B) stages. Means followed by the same letter are not significantly different (P < 0.05) according to LSD test (n = 5)

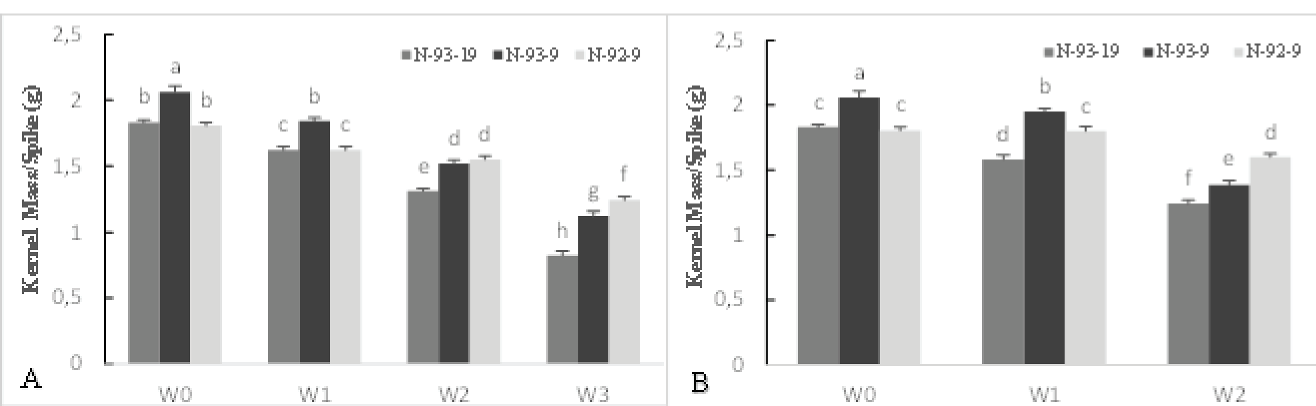



**Figure 10:** Effect of waterlogging (W0: 0, W1: 7, W3: 14 and W4: 21 d) on kernels mass/spike in wheat genotypes in tillering (A) and stem elongation (B) stages. Means followed by the same letter are not significantly different (P < 0.05) according to LSD test (n = 5)

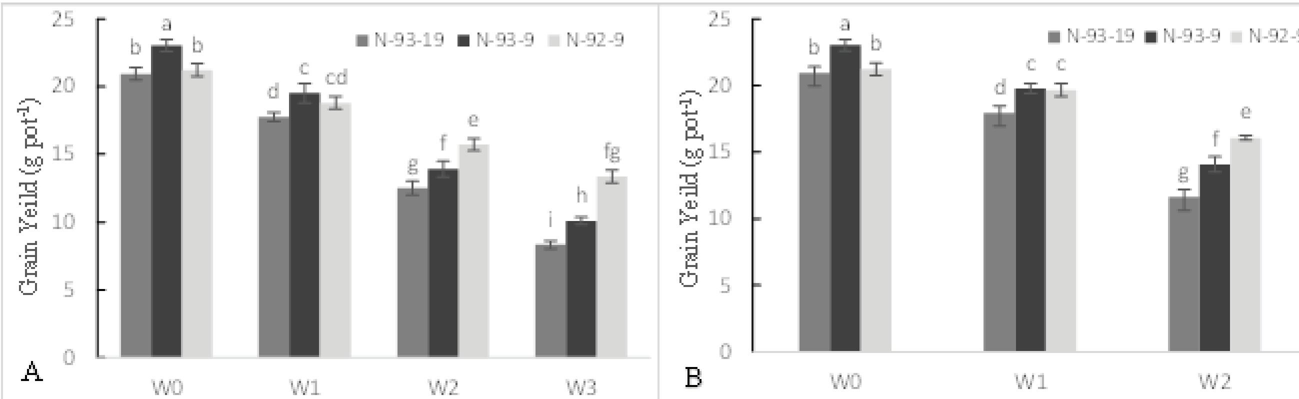



**Figure 11:** Effect of waterlogging (W0: 0, W1: 7, W3: 14 and W4: 21 d) on grain yield in wheat genotypes in tillering (A) and stem elongation (B) stages. Means followed by the same letter are not significantly different (P < 0.05) according to LSD test (n = 5)

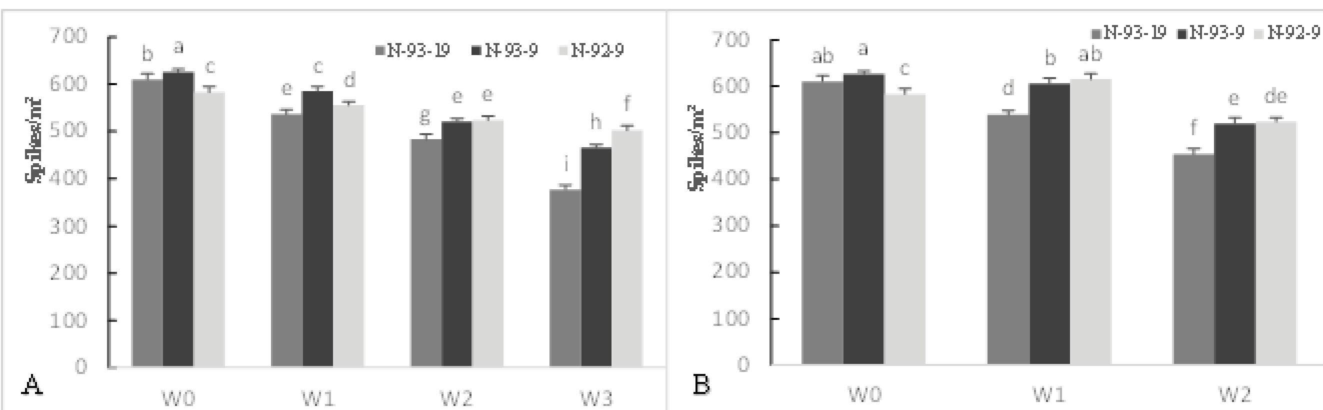



**Figure 12:** Effect of waterlogging (W0: 0, W1: 7, W3: 14 and W4: 21 d) on spikes/m$^2$ in wheat genotypes in tillering (A) and stem elongation (B) stages. Means followed by the same letter are not significantly different (P < 0.05) according to LSD test (n = 5)

## 4 DISCUSSION

High chlorophyll content under waterlogging conditions can be the most appropriate way for achieving high yield under waterlogging conditions (Gardner et al., 1993). Our results indicated that waterlogging treatments in tillering and stem elongation stages significantly reduced photosynthetic pigments (chl a, b and carotenoids) in all three wheat genotypes, however, N-92-9 genotype had higher photosynthetic pigments than other genotypes under all waterlogging duration. Pang et al. (2004) stated that waterlogging stress reduced the content of chlorophyll and consequently reduced the rate of $CO_2$ assimilation. In another report, Smethurst and Shabala, (2003) indicated that the content of chlorophyll a and b reduced in genotypes under waterlogging treatments. Screening of the effect of waterlogging duration on chlorophyll and carotenoids content presented that by increasing the duration of waterlogging, the content of chlorophyll and carotenoids more decreased, which is according to the results of Olgun et al. (2008).

The maintaining of water balance of the plant under environmental stresses is very important for plant tolerance, which proline is one of the most compatible solutes (Claussen, 2005; Ghorbani et al., 2018). Our results showed that proline contents under waterlogging stress increased in tillering and stem elongation stages. The increase of proline contents in N-92-9 genotype was significantly higher than the other two varieties under same waterlogging duration. Increasing of waterlogging duration significantly increased the contents of proline, which is in accordance to results of Olgun et al. (2008). Increasing proline contents in plants under waterlogging treatments can be due to oxidation inhibition, loss of inhibition of feedback and protein synthesis impairment. Videmšek et al. (2006) reported that wheat genotypes have different ability to synthesize and accumulate proline, which affects their tolerance to waterlogging.

The stomatal closure and the reduction of $CO_2$ availability resulting from waterlogging conditions increase the production of reactive oxygen species (ROS) and thus induce oxidative stress (Gossett et al., 1994). Our results showed that waterlogging stress increased MDA production, which indicating the degree of damage to membrane lipids. However, there was a significant difference between wheat genotypes, which indicates their different defensive capacity against waterlogging-induced ROS. Increasing the activity of antioxidant enzymes such as SOD, CAT and POD can play an important role in plant tolerance to waterlogging stress. The results of this study showed a continuous increase in the activity of SOD and a decrease in the activity of POD in all three wheat genotypes under waterlogging stress. However, CAT activity under waterlogging treatments in N-92-9 genotype showed a continuous increase while there was decline in CAT activity in two genotypes of N-93-19 and N-93-9. Different researchers have suggested that the activity of antioxidant enzymes under waterlogging conditions increases to take care of hypoxia-induced oxidative stress (Arbona et al., 2008; Kumutha et al., 2009). Our results are in accordance with the results of Blokhina et al. (2001) who found more oxidative stress under waterlogging and increased antioxidant enzymes could reduce ROS production. It is clear from the results that wheat genotypes suffered from ROS under waterlogging stress that intensified with increasing the waterlogging duration. Therefore, the rapid increase in the activity of antioxidant enzymes in plants under waterlogging stress can protect the plant against the oxidative stress caused by waterlogging. Many researchers have been previously reported increased activity of CAT (Ushimaru et al., 1997), POD (Meloni et al., 2003) and SOD (VanToai and Bolles, 1991, Biemelt et al., 2000) enzymes under waterlogging conditions.

Kernels mass/spike is one of the most important components of wheat yield. Our results showed that waterlogging treatments reduced the kernels/spike and kernels mass/spike traits of all three studied wheat genotypes in both tillering and stem elongation stages, however, the lowest decrease was observed in N-92-9 genotype. These findings are consistent with the results of Collaku and Harrison (2002) who stated that increasing the duration of waterlogging reduce the kernels/spike. They also showed that tolerant genotypes had higher kernels/spike compared to other genotypes. In another report, Saqib et al. (2004) showed that the kernels mass/spike in wheat genotypes under waterlogging stress was reduced by 50-80 % as compared to control treatments. The results related to yield and spikes/$m^2$ showed that these traits significantly decreased with increasing waterlogging duration in all three genotypes and the highest decrease was observed in N-93-19 genotype. Reducing the yield of wheat genotypes under waterlogging according to Barret-Lennard (2003) and Setter et al. (2001) who showed that increasing waterlogging duration reduced wheat yield from 10 to 80 %. Collaku and Harrison (2002) also reported that waterlogging stress reduced the spikes/$m^2$ in winter wheat.

## 5 CONCLUSION

In general, the results showed that waterlogging stress caused oxidative stress in wheat genotypes that enhanced with duration of exposure. N-92-9 genotype with higher activity of antioxidant enzymes (CAT, SOD and POD) which resulted in higher grain yield and yield components under waterlogging conditions was more tolerant to waterlogging stress.

## 6 REFERENCES

Amador, M.L., Sancho, S., Bielsa, B., Gomez-Aparisi, J., Rubio-Cabetas, M.J. (2012). Physiological and biochemical parameters controlling waterlogging stress tolerance in Prunus before and after drainage. *Physiologia Plantarum, 144*, 357–368. doi:10.1111/j.1399-3054.2012.01568.x

Arbona, V., Hossain, Z., Lo´pez-Climent, M.F., Pe´rez-Clemente, R.M., Go´mez-Cadenas, A. (2008). Antioxidant enzymatic activity is linked to waterlogging stress tolerance in citrus. *Physiologia Plantarum, 132*, 452–466. doi:10.1111/j.1399-3054.2007.01029.x

Barret-Lennard, E.G. (2003). The interaction between waterlogging and salinity in higher plants: causes, consequences and implications. *Plant and Soil, 253*, 35-54. doi:10.1023/A:1024574622669

Bates, L., Waldren, R.P., Teatre, J.D. (1973). Rapid determination of free proline for water stress studies. *Plant and Soil, 39*, 205-207. doi:10.1007/BF00018060

Biemelt, S., Keetman, U., Mock, H.P., Grimm, B. (2000). Expression and activity of isoenzymes of superoxide dismutase in wheat roots in response to hypoxia and anoxia. *Plant, Cell & Environment, 23*, 135-144. doi:10.1046/j.1365-3040.2000.00542.x

Blokhina, O.B., Fagerstedt, K.V., Chirkova, T.V. (1999). Relationships between lipid peroxidation and anoxia tolerance in a range of species during post-anoxic reaeration. *Physiologia Plantarum, 105*, 625-632. doi:10.1034/j.1399-3054.1999.105405.x

Ceylan, A. (Ed) (1994). *Field Crop Production*. Aegean University Press, Izmir, pp. 520.

Claussen, W. (2005). Proline as a measure of stress in tomato plants. *Plant Science, 168*, 241–248. doi:10.1016/j.plantsci.2004.07.039

Collaku, A., Harrison, S.A. (2002). Losses in wheat due to waterlogging. *Crop Science, 42*, 444-450. doi:10.2135/cropsci2002.4440

Dennis, E.S., Dolferus, R., Ellis, M., Rahman, M., Wu, Y., Hoeren, F.U., Grover, A., Ismond, K.P., Good, A.G., Peacock, W.J. (2000). Molecular strategies for improving waterlogging tolerance in plants. *Journal of Experimental Botany, 342*, 89–97. doi:10.1093/jexbot/51.342.89

Edreva, A. (2005). Generation and scavenging of reactive oxygen species in chloroplasts: a submolecular approach. *Agriculture, Ecosystems & Environment, 106*, 119–133. doi:10.1016/j.agee.2004.10.022

Fukao, T., Bailey-Serres, J. (2004). Plant responses to hypoxia – is survival a balancing act? *Trends in Plant Science, 9*, 449–456. doi:10.1016/j.tplants.2004.07.005

Gardner, W.K., Flood, R.G. (1993). Less waterlogging damage with long season wheats. *Cereal Research Communications, 21*, 337-343.

Gerami, M., Ghorbani, A., Karimi, S. (2018). Role of salicylic acid pretreatment in alleviating cadmium-induced toxicity in *Salvia officinalis* L. *Iranian Journal of Plant Biology*, *10*(1), 81-95.

Ghorbani, A., Razavi, S.M., Ghasemi Omran, V.O., Pirdashti, H. (2018). *Piriformospora indica* inoculation alleviates the adverse effect of NaCl stress on growth, gas exchange and chlorophyll fluorescence in tomato (*Solanum lycopersicum* L.). *Plant Biology, 20*(4), 729-736. doi:10.1111/plb.12717

Gossett, D.R., Millhollon, E.P., Lucas, M.C. (1994). Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. *Crop Science, 34*, 706–714. doi:10.2135/cropsci1994.0011183X003400030020x

Herzog, M., Striker, G.G., Colmer, T.D., Pedersen, O. (2016). Mechanisms of waterlogging tolerance in wheat--a review of root and shoot physiology. *Plant, Cell & Environment, 39*(5), 1068-86. doi:10.1111/pce.12676

Kumutha, D., Ezhilmathi, K., Sairam, R.K., Srivastava, G.C., Deshmukh, P.S., Meena, R.C. (2009). Waterlogging induced oxidative stress and antioxidant activity in pigeonpea genotypes. *Physiologia Plantarum, 53*(1), 75-84. doi:10.1007/s10535-009-0011-5

Lichtenthaler, H., Wellburm, A.R. (1983). Determination of total carotenoids and chlorophyll a and b of leaf extracts in different solvents. *Biochemical Society Transactions, 603*, 591–593. doi:10.1042/bst0110591

Liu, F., Van Toai, T., Moy, L.P., Bock, G., Linford, L.D., Quackenbush, J. (2005). Global transcription profiling reveals comprehensive insights into hypoxic response in Arabidopsis. *Plant Physiology, 137*, 1115–1129. doi:10.1104/pp.104.055475

Heath, R.L., Packer, L. (1968). Photoperoxidation in isolated chloroplasts. *Archives of Biochemistry and Biophysics, 125*(1), 189-198. doi:10.1016/0003-9861(68)90654-1

Luck, H. 1971. *Catalase*. In: Bergmeyer HU, editor. Methods of Enzymatic Analysis. New York: Academic Press. pp. 885–894.

Maehly, A.C., Chance, B. (1954). The assay of catalases and peroxidases. *Methods of Biochemical Analysis, 1*, 357–424. doi:10.1002/9780470110171.ch14

Meloni, D.A., Oliva, M.O., Martinez, C.A., Cambraia, J. (2003). Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environmental and Experimental Botany, 49*, 69–76. doi:10.1016/S0098-8472(02)00058-8

Mittler, R., Vanderauwera, S., Gollery, M., Van Breusegem, F. (2004). Reactive oxygen gene network of plants. *Trends in Plant Science, 9*, 490–498. doi:10.1016/j.tplants.2004.08.009

Olgun, M., Kumlay, A.M., Adiguzel, M.C., Caglar, A. (2008). The effect of waterlogging in wheat (*T.aestivum* L.). *Acta Agriculturae Scandinavica Section B- Soil and Plant Science,* 58(3), 193-198.

Pang, J., Zhou, M., Mendham, N., Shabala, S. (2004). Growth and physiological responses of six barley genotypes to waterlogging and subsequent recovery. *Australian Journal of Agricultural Research, 55*, 895-906. doi:10.1071/AR03097

Saqib, M., Akhtar, J., Qureshi, R.H. (2004). Pot study on wheat growth in saline and waterlogged compacted soil. *Soil & Tillage Research, 77*, 169-177. doi:10.1016/j.still.2003.12.004

Sayre, K.D., Van Ginkel, M., Rajaram, S., Ortiz-Monasterio, I. (1994). Tolerance to waterlogging losses in spring bread wheat: effect of time of onset on expression. *Annual Wheat Newsletter, 40*, 165–171.

Setter, T.L., Burgess, P., Waters, I., Kuo, J. (2001). Genetic diversity of barley and wheat for waterlogging tolerance in Western Australia. In: *Proceedings of the 10th Australian Barley Technical Symposium*, Canberra, 16-20 September 2001, ACT, Australia.

Smethurst, C.F., Shabala, S. (2003). Screening methods for waterlogging tolerance in lucerne: comparative analysis of waterlogging effects on chlorophyll fluorescence, photosynthesis, biomass and chlorophyll content. *Functional Plant Biology, 30*, 335-343. doi:10.1071/FP02192

Suzuki, N., Mittler, R. (2006). Reactive oxygen species and temperature stresses: a delicate balance between signaling and destruction. *Physiologia Plantarum, 126*, 45–51. doi:10.1111/j.0031-9317.2005.00582.x

Ushimaru, T., Maki, Y., Sano, S., Koshiba, K., Asada, K., Tsuji, H. (1997). Induction of enzymes involved in the ascorbate-dependent antioxidative system, namely ascorbate peroxidase, mono dehydroascorbate reductase and dehydroascorbate reductase, after exposure to air of rice (*Oryza sativa*) seedlings germinated under water. *Plant and Cell Physiology, 38*, 541-549. doi:10.1093/oxfordjournals.pcp.a029203

Van Toai, T.T., Bolles, C.S. (1991). Postanoxic injury in soybean (*Glycine max*) seedlings. *Plant Physiology,* 97, 588-592. doi:10.1104/pp.97.2.588

Videmšek, U., Turk, B., Vodnik, D. (2006). Root aerenchyma–formation and function. *Acta Agriculturae Slovenica, 87*, 445-453.

doi:10.14720/aas.2018.111.3.11 Original research article / izvirni znanstveni članek

# The quality of sour cherry fruits *(Prunus cerasus* L.), treated with chitosan solution before storage

Olena VASYLYSHYNA[1]

Received June 08, 2018; accepted November 14, 2018.
Delo je prispelo 08. junija 2018, sprejeto 14. novembra 2018.

**ABSTRACT**

The article shows the results of the research into marketability evaluation of sour cherry *(Prunus cerasus* L.) fruits, treated with chitosan solution. To achieve this goal, fruits were treated with 0.5 % or 1 % chitosan solution, and stored at 5 °C for 21 days. To assess the shelf life of fruit, physical and chemical parameters were determined: mass loss, reduction of sugar, titrated acidity and ascorbic acid content. Treatment with chitosan solution significantly reduced the mass loss, content of sugar, acids, ascorbic acid and respiration rate. The treatment with chitosan solution extended the shelf life and improved the quality of sour cherry fruit. After 21-day storage of sour cherries, mass loss was 4.6 % with the product output accounting for 85.5 %. The obtained results are approved by physiological and chemical changes in the fruits during storage: respiration rate of fruits decreases, loss of sugar does not exceed 6.7 %, acids – 33 % and ascorbic acid – 18 %. It has been found that post-harvest treatment with 1 % chitosan solution has a positive influence on commercial quality of sour cherry fruits.

**Key words:** sour cherry fruit; storage; mass loss; commercial quality; chitosan

**IZVLEČEK**

**KAKOVOST PLODOV VIŠNJE *(Prunus cerasus* L.), TRETIRANIH Z RAZTOPINO HITOZANA PRED SHRANJEVANJEM**

Prispevek obravnava tržno ovrednotenje plodov višnje *(Prunus cerasus* L.) tretiranih z raztopino hitozana. Plodovi so bili tretirani z 0,5 % in 1 % raztopino hitozana in shranjeni pri 5 °C za 21 dni. Za ovrednotenje poličnega trajanja plodov so bili izmerjeni naslednji fizikalni in kemični parametri: izguba mase, zmanjšanje vsebnosti sladkorjev in askorbinske kisline ter zmanjšanje titracijske kislosti. Obdelava z raztopino hitozana je značilno zmanjšala izgubo mase, vsebnost sladkorjev, celokupnih kislin, askorbinske kisline in velikost dihanja. Obdelava s hitozanom je podaljšala polično trajanje višenj in izboljšala njihovo kakovost. Po 21-dneh shrambe se je masa višenj zmanjšala za 4,6 %, celoten pridelek pa na 85,5 %. To so potrdile naslednje fiziološke in kemične spremembe v plodovih med shranjevanjem: upad dihanja plodov, izguba sladkorjev, ki ni presegla 6,7 %, vsebnost kislin se je zmanjšala za 33 % in askorbinske kisline za 18 %. Ugotovljeno je bilo, da je imela obdelava plodov višenj z 1 % raztopino hitozana po obiranju pozitivni vpliv na njihovo tržno kakovost.

**Ključne besede:** plodovi višenj; shranjevanje; izguba mase; tržna kakovost; hitozan

## 1 INTRODUCTION

Sour cherry (*Prunus cerasus* L.) is one of the most appreciated fruit by consumers due to its excellent quality. Sour cherry fruits are the source of acids, vitamins and anthocyanins (Chernozubenko, 1993). However, they have a limited shelf life, which is only 15 days. To prolong the shelf life, pre- and postharvest treatment with substances of antimicrobial action, *Aloe vera* (L.) Burm.f. cover, or 1-methylcyclopropene (1-MCP) are used (Paronian et al., 2003; Yen et al., 2008; Ahmed et al., 2009). However, the use of these substances is not fully studied and 1-MCP is expensive to use. Therefore, chitosan is more widely applied for post-harvest treatment of fruit before storage, including sweet cherry, strawberries, grapes (Romanazzi, 2010).

Chitosan as polysaccharide is a biopolymer and is used in agriculture, biotechnology, food industry, due to its biocompatibility and bioactivity (Wu et al., 2005). In 2001 chitosan was included in the list of secure compounds and foodstuffs in the USA. This natural polymer is derived from chitin by deacetylation and can form a film on treated surfaces, slowing down respiration rate, reducing mass loss, improving quality and prolonging shelf life (Romanazzi et al., 2003;

[1] Uman National University of Horticulture, Institutska 1, Uman city, 20305, Ukraine; e-mail: elenamila@i.ua

Romanazzi, 2010; Romanazzi et al., 2013; Romanazzi et al., 2015). Moreover, chitosan films have selective penetrability to carbon dioxide and oxygen, antibacterial and fungitoxic properties.

Besides other, acting as protective barriers, these films can be used as carriers of bioactive compounds with antioxidant or antibacterial properties (Genskowsky et al., 2015). Therefore, it is used for pre-harvest treatment of grapes, strawberries, cherries and others (El Ghaouth et al., 1991; Romanazzi et al., 2006; Romanazzi et al., 2007; Hernandez-Munoz, 2008; Romanazzi, 2010). According to the literature (El Ghaouth et al., 1991), 1 % and 1.5 % chitosan coatings are used for treatment of strawberries, while storing them at a temperature 4 °C. After 29 days its commercial quality is reduced to 19−22 % compared to 33 % in untreated fruits. In addition, respiration rate of treated fruits is also lower.

Chitosan combined with ethanol or warm water or calcium is applied asa postharvest treatment and helps to reduce diseases of grapes and sweet cherries (Romanazzi et al., 2007; Hernandez-Munoz, 2008; Dang et al., 2010; Chailoo et al., 2011). Research of Romanazzi et al. (2002) has shown that chitosan can replace fungicides to combat postharvest damage of grapes.

Taking into account a widespread use of chitosan for storing fruits, we set the goal to study its influence on storage duration and quality of sour cherries fruits.

## 2 MATERIALS AND METHODS

### 2.1 Preharvest and postharvest treatments

Sour cherry variety *Shpanka*, collected in 2016 at the stage of eating ripeness, was used for storage. Four trees were selected randomly according to the following variants: control (without treatment), treatment with chitosan solution in concentrations 0.5 % and 1.0 %. On the day before harvesting, the fruits were sprayed with 0.5 % or 1 % chitosan solution with a mechanical mist sprayer (7.5 l $tree^{-1}$) and dried naturally. Fruits were loaded in box №5 with a capacity of 5 kg and kept at a the temperature of 5 °C ± 0.5 and relative humidity not lower than 95 ± 1 %.

One week before harvesting 100 fruits from each tree (or variants) were selected for further analytical determinations: mass loss, sugar content decrease reduction, total acidity, ascorbic acid and respiration rate. Analytical determinations were made in recently-harvested fruits (day 0) and in fruits that were stored for 7, 15, and 21 days at 5 °C + 1 day at 20 °C by using one lot for each variant and treatment selected randomly from the storage room. Experiment was repeated three times and then the mean values of all results were calculated.

### 2.2 Analytical methods

After storing the commercial quality of products was determined. To determine commercial quality, the sour cherry fruits of the first commercial quality were selected after storage. By their appearance they were typical in form and color for the pomological variety. Fruits were homogeneous in degree of maturity, not overripe and no smaller than 16 mm in size. The number of fruits without a peduncle and with healed damages was not higher than 4 %. The fruits were of the same size, color and shape without damages (average diameter is about 15.51 ± 0.05 mm).

### 2.3 Fruit mass loss

At the end of storage, natural mass loss was measured by weighting. Mass loss was calculated as the difference of two weightings before and after storage expressed as a percentage. The criteria for the end of storage was the mass loss no more than 6 %.

### 2.4 Determining respiration rate

Sour cherry samples weighing 200 g were put into exicator for 2 hours, meanwhile generated $CO_2$ reacted with 20 ml 0.4 M NaOH. The respiration rate was determined by titration with 0.2 M oxalic acid and expressed as milliliter $CO_2$ $kg^{-1}$ (fresh mass) per hour. Three repetitions were carried out for each sample.

The ferricyanide method was used for sugar content analysis (Naichenko, 2001). The content of titrated acids was determined by titration with a solution of 0.1 M NaOH. Ascorbic acid content was determined using the modified method of Tillmans. The solution was titrated with 2.6-dichloroindophenol under acid conditions (Naichenko, 2001).

### 2.5 Statistical analysis

The data were statistically processed using a single and two factors analysis of variance (ANOVA) at significance level $P < 0.05$ on the PC program Statistica (Mamchich, 2006). Variances were the time of storage and type of treatment.

## 3 RESULTS AND DISCUSSION

Results (Table 1) have shown that storage duration of sour cherries in the control variant was 15 days, in the experimental variants was 21 days, which is 1.4 times longer.

**Table 1:** The output of standard products from sour cherry fruits

| Variant | Shelf life (days) | Mass loss (%) | The standard number of products (%) |
|---|---|---|---|
| Control | 15 | 5.7 ± 1.6 | 79.6 ± 2.3 |
| 0.5 % chitosan | 21 | 5.2 ± 1.5 | 81.6 ± 3.1 |
| 1 % chitosan | 21 | 4.6 ± 1.3 | 85.5 ± 2.2 |
| $LSD_{05}$ | | 0.8 | 0.6 |

An important indicator of preservation is natural mass loss. The experimental results show that for sour cherries in the control variant, the average mass loss was 5.7 %. The mass loss of treated fruits were 5.2 % and 4.6 % (0.5 % and 1 % solution of chitosan), which is 8.8 % and 19 % less than control.

An important indicator of fruit quality after storage is marketability evaluation, which determines the competitiveness of products in the market. According to the research results, output of marketable products after 15 days of storage in the control was 79.6 %. Whereas for fruits treated with chitosan solution, storage duration was longer by 6 days and made up 21 days. The output of standard products in the fruits treated with 0.5 % chitosan solution was higher by 2 % and treated with 1 % solution of chitosan − by 5.9 % and accounted for 85.5 %. Results are consistent with the results of El Ghaouth (1991) and Romanazzi et al. (2003).

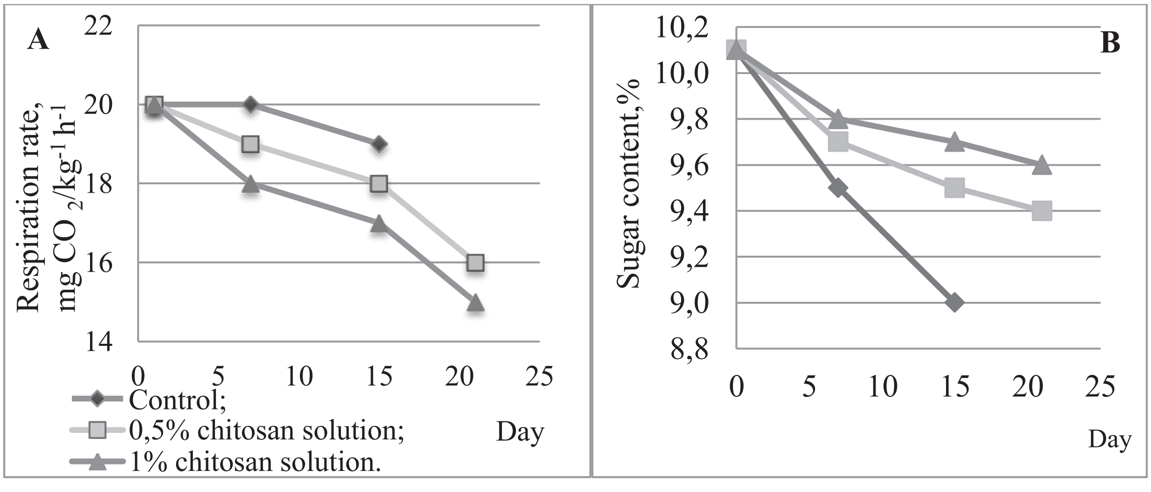



**Figure 1:** Changes in respiration rate (A) ($LSD_{05}$ = 1.2) and total sugar content (B) ($LSD_{05}$ = 0.2) in sour cherries treated with chitosan solution during storage

Chitosan treatment changed the respiration rate of sour cherry (Figure 1). In particular, from the 1st to the 7th day of storage, it remained similar in all variants. On the 7th, 15th and 21st day of storage the respiration rate of chitosan treated fruitwas by 1−3 mg $CO_2$ / $kg^{-1}h^{-1}$ lower. The obtained results are consistent with the data of Petriccione et al. (2015) and Romanazzi et al. (2013). It could be explained by the fact that the chitosan coating forms a semipermeable film on the surface of fruits and vegetables, slowing respiration rate, reducing weight loss, improving quality and prolonging storage.

One of the main substrates of respiration of fruits are sugars and acids. Figure 1B shows the change in the total content of sugars during the storage. The content of total sugar in sour cherry fruit treated with 1 or 0.5 % chitosan solutions decreased throughout the storage period by 5 %. Content of sugar depended on treatment. It remained the highest in fruit treated with 1 % chitosan solution and made up 9.6 %, which is 6.7 % higher than control.

Figure 2A shows the change in the acid content of the fruits during storage. After 15 days of storage there was a threefold decrease in the content of titrated acids in the fruits of the control variant. Acid loss in sour cherry fruit treated with 0.5 % solution of chitosan is lower by 44 %, and lower by 33 % in fruits treated with 1 % solution of chitosan.

During storage, the content of ascorbic acid also significantly decreased (Figure 2B). The content of ascorbic acid decreased throughout the storage period by 1.7 times. In addition, the content of ascorbic acid in fruits, treated with 0.5 % and 1 % chitosan solution decreased by 27 % and 18 %. Our results are in agreement with previous studies (Dang et al., 2010; Kerch, et al., 2011 and Petriccione et al., 2015).

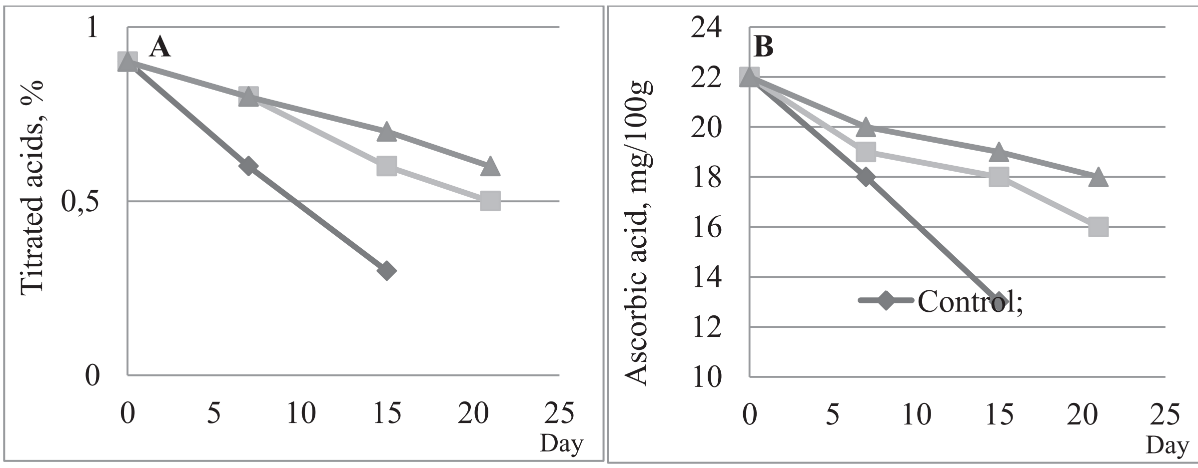



**Figure 2:** : Influence of chitosan solution on the content of titrated acids (A) (LSD $_{05}$ = 0.2) and ascorbic acid (B) (LSD $_{05}$ = 1.5) in sour cherries during storage

## 4 CONCLUSIONS

It has been established that postharvest treatment of sour cherries with 1 % solution of chitosan has a positive influence and improves the commercial quality of fruits. After 21 days of storage of sour cherry fruits the mass loss made up 4.6 % with 85.5 % output of marketable products. The obtained results are consistent with physiological and chemical changes in the fruits during storage. The respiration rate of the fruits decreased, the loss of sugars does not exceed 6.7 %, acids – 33 %, and ascorbic acid – 18 %.

## 5 REFERENCES

Ahmed, M.J., Singh, Z., Khan, A.S. (2009). Postharvest *Aloe vera* gel-coating modulates fruit ripening and quality of 'Arctic Snow' nectarine kept in ambient and cold storage. *International Journal of Food Science & Technology, 44*, 1024−1033. doi:10.1111/j.1365-2621.2008.01873.x

Chailoo, M.J., Asghari, M.R. (2011). Hot water and chitosan treatment for the control of postharvest decay in sweet cherry (*Prunus avium* L.) cv. *Napoleon* (Napolyon). *Journal of Stored Products and Postharvest Research*, *2*(7), 135−138.

Chernozubenko, N.K. (1993). *Determination of suitability of new varieties of black currants and cherries for storage and processing*. Thesis. Kyiv.

Dang, Q.F., Yan, J.Q., Li, Y., Cheng, X.J., Liu, C.S., Chen, X.G. (2010). Chitosan acetate as an active coating material and its effects on the storing of *Prunus avium* L. *Journal of Food Science, 75*(2), 125−131. doi:10.1111/j.1750-3841.2009.01483.x

El Ghaouth, A., Arul, J., Wilson, C., Benhamouc, N. (1997). Biochemical and cytochemical aspects of the interactions of chitosan and *Botrytis cinerea* in bell pepper fruit. *Postharvest Biology and Technology, 12*(2), 183−194. doi:10.1016/S0925-5214(97)00056-2

El Ghaouth, A., Arul, J., Ponnamapalam, R., Boulet, M. (1991). Chitosan coating effect on storability and quality of fresh strawberries. *Journal of food science*, *56*(6), 1618−1620. doi:10.1111/j.1365-2621.1991.tb08655.x

Genskowsky, E., Puente, L.A., Perez-Alvarez, J.A., Fernandez-Lopez, J., Munoz, L.A., Viuda-Martos, M. (2015). Assessment of antibacterial and antioxidant properties of chitosan edible films incorporated with maqui berry (*Aristotelia chilensis*). *Food Science and Technology, 64,* 1057–1062.

Hernandez-Munoz, P., Almenar, E., Del Valle, V., Velez, D., Gavara, R. (2008). Effect of chitosan coating combined with postharvest calcium treatment on strawberry (*Fragaria ananassa*) quality during refrigerated storage. *Food Chemistry*, *110*, 428–435. doi:10.1016/j.foodchem.2008.02.020

Kerch, G., Sabovics, M., Kruma, Z., Kampuse, S., Straumite, E. (2011). Effect of chitosan and chitooligosaccharide on vitamin C and polyphenols contents in cherries and strawberries during refrigerated storage. *European Food Research and Technology*, *233*(2), 351–358. doi:10.1007/s00217-011-1525-6

Mamchich, T.I., Olenko, A.Ya., Osipchuk, M.M., Shportyuk, V.G. (2006). *Statistical analysis of data with the STATISTICA package*. Drohobych.

Naichenko, V.M. (2001). *Practicum on technology of storage and processing of fruits and vegetables*. Kyiv.

Paronian, V.H., Kureghian, T.P., Komarov, N.V. (2003). Progressive ways of processing fruits and vegetables before storing. *Storage and processing of agricultural raw materials, 7*, 23–24.

Petriccione, M., De Sanctis, F., Pasquariello, M.S., Mastrobuoni, F., Rega, P., Scortichini, M.,Mencarelli, F. (2015). The effect of chitosan coating on the quality and nutraceutical traits of sweet cherry during postharvest life. *Food and Bioprocess Technology*, *8*(2), 394–408. doi:10.1007/s11947-014-1411-x

Romanazzi, G. (2010). Chitosan treatment for the control of postharvest decay of table grapes, strawberries and sweet cherries. *Fresh Produce, 4,* 111−115.

Romanazzi, G., Feliziani, E., Santini, M., Landi, L. (2013). Effectiveness of postharvest treatment with chitosan and other resistance inducers in the control of storage decay of strawberry. *Postharvest Biology and Technology*, *75*, 24−27. doi:10.1016/j.postharvbio.2012.07.007

Romanazzi, G., Feliziani, E., Baños, S.B., Sivakumar, D. (2017). Shelf life extension of fresh fruit and vegetables by chitosan treatment. *Critical Reviews in Food Science and Nutrition*, *57*, 579–601. doi:10.1080/10408398.2014.900474

Romanazzi, G., Karabulut, O.A., Smilanick, J.L. (2007). Combination of chitosan and ethanol to control postharvest gray mold of table grapes. *Postharvest Biology and Technology*, *45*(1), 134−140. doi:10.1016/j.postharvbio.2007.01.004

Romanazzi, G., Nigro, F., Ippolito, A., Venere, D. DI, Salerno, M. (2002). Effects of pre- and postharvest chitosan treatments to control storage grey mold of table grapes. *Journal of Food Science*, *67*(5), 1862–1867. doi:10.1111/j.1365-2621.2002.tb08737.x

Romanazzi, G., Gabler, F.M., Smilanick, J.L. (2006). Preharvest chitosan and postharvest UV irradiation treatments suppress gray mold of table grapes. *The American Phytopathological Society*, *90*(4), 445–450. doi:10.1094/PD-90-0445

Romanazzi, G., Nigro, F., Ippolito, A. (2003). Short hypobaric treatments potentiate the effect of chitosan in reducing storage decay of sweet cherries. *Postharvest Biology and Technology*, *29*, 73−80. doi:10.1016/S0925-5214(02)00239-9

Romanazzi, G., Feliziani, E., Bautista Banos, S., Sivakumar, D. (2015). Shelf life extension of fresh fruit and vegetables by chitosan treatment. *Critical Reviews in Food Science and Nutrition*, 57, 579–601. doi:10.1080/10408398.2014.900474

Wu, B.H., Quilot, B., Genard, M., Karvella, J., Li, S.H. (2005). Changes in sugar and organic acid concentrations during fruit maturation in peaches *P. davidiana* and hybrids as analyzed by principal component analysis. *Scientia Horticulturae, 103*, 429−439. doi:10.1016/j.scienta.2004.08.003

Yen, M.T., Yang, J.H., Mau, J.L. (2008). Antioxidant properties of chitosan from crab shells. *Carbohydrate Polymers*, *74*, 840−844. doi:10.1016/j.carbpol.2008.05.003

doi:10.14720/aas.2018.111.3.12 Original research article / izvirni znanstveni članek

# The effect of sublethal concentrations of malathion on some biological parameters of the ectoparasitoid wasp, *Habrobracon hebetor* (Say, 1836)

Amin ASADI[1], Jaber KARIMI[2*], Habib ABBASIPOUR[3]

Received June 11, 2018; accepted October 29, 2018.
Delo je prispelo 11. junija 2018, sprejeto 29. oktobra 2018.

### ABSTRACT

The ectoparasitoid wasp, *Habrobracon hebetor* (Say, 1836) (Hym.: Braconidae) is one of the most important natural enemies of many pests in Iran. In this study, the effects of sublethal concentrations of malathion ($LC_{10}$ and $LC_{25}$) on biological parameters of the adult wasp including parasitism percentage, male production and longevity of the parasitoid in different growth stages (larvae, pupae and adult) were investigated. The experiments were conducted at 26 ± 1 °C, 60 ± 5 % RH, and 16L:8D h photoperiods. The effect of sublethal concentrations of malathion on adults was determined by exposure of wasp to insecticide, and in the case of larvae and pupae, immersion method was used. Based on the results, with the increase of toxic dose, the percentage of parasitism in adult decreased from 95.6 5% to 87.78 %, compared to the control treatment. Male production increased from 22.73 % in control to 56.38 % at $LC_{25}$ concentration. Also, the percentage of parasitism of the malathion-impregnated wasp larvae decreased from 87.78 to 22.92%, compared to the control treatment. Male production from 30.94 % in the control increased to 39.63 % at the $LC_{25}$ concentration. In addition, the percentage of parasitism in the wasp pupae impregnated with malathion was reduced from 93.33 to 88.89 %, respectively. Male production increased from 22.98 in the control to 37.23 % in the $LC_{25}$ concentration. The data showed that pupae were more susceptible to sublethal concentrations than the larvae.

**Key words:** malathion; sublethal concentration; *Habrobracon hebetor*; biology; biological parameters

### IZVLEČEK

**UČINEK SUBLETALNIH KONCENTRACIJ MALATIONA NA NEKATERE BIOLOŠKE PARAMETRE EKTOPARAZITSKE OSICE *Habrobracon hebetor* (Say, 1836)**

Ektoparazitska osica *Habrobracon hebetor* (Say, 1836), (Hymenoptera: Braconidae) je eden izmed najpomembnejših naravnih sovražnikov mnogih škodljivih žuželk v Iranu. V raziskavi so bili preučevani učinki subletalnih koncentracij malationa ($LC_{10}$ in $LC_{25}$) na biološke parametre odraslih osic vključno z odstotkom parazitiranja, razvojem samcev in življenjsko dobo parazitoida v različnih razvojnih štadijih (ličinke, bube in imagi). Poskus je bil izveden pri 26 ± 1 °C, 60 ± 5 % RH in osvetlitvi 6 : 8 dan/noč. Učinek subletalnih koncentracij malationa na image je bil določen z izpostavitvijo osic insekticidu, v primeru ličink in bub je bila uporabljena metoda potapljanja. Rezultati so pokazali, da je z naraščanjem koncentracije insekticida odstotek parazitiranja upadel iz 95,65 % na 87,78 % v primerjavi s kontrolo. Razvoj samcev se je iz 22,73 % v kontroli povečal na 56,38 % pri koncentraciji $LC_{25}$. Tudi odstotek parazitiranja z malationom impregniranih ličink osic se je zmanjšal iz 87,78 % na 22,92 % v primerjavi s kontrolo. Razvoj samcev se je iz 30,94 % v kontroli povečal na 39,63 % pri koncentraciji $LC_{25}$ . Dodatno se je odstotek parazitiranja bub impregniranih z malationom zmanjšal iz 93,33 na 88,89 %. Razvoj samcev se je povečal iz 22.98 v kontroli na 37,23 % pri koncentraciji $LC_{25}$. Podatki so pokazali, da so bile bube bolj občutljive na subletalne koncentracije kot ličinke.

**Ključne besede:** malation; subletalna koncentracija; *Habrobracon hebetor*; biologija; biološki parametri

[1] Postgraduate MSc. Student, Department of Plant Protection, Faculty of Agricultural Sciences, Shahed University, Tehran, Iran

[2] Assistant Professor, Department of Plant Protection, Faculty of Agricultural Sciences, Shahed University, Tehran, Iran; *Correspondent author: karimi_jaber@yahoo.com

[3] Professor, Department of Plant Protection, Faculty of Agricultural Sciences, Shahed University, Tehran, Iran

# 1 INTRODUCTION

Most insecticides have harmful effects on non-target organisms, especially natural enemies (Croft, 1990). Biocontrol agents are commonly susceptible to insecticide applications. In recent years, integrated pest management systems attempt to use natural enemies in combination with lower doses of insecticides for pest control. The combined use of biocontrol agents and insecticides to manage pests requires adequate knowledge about the aspects and selective effects of insecticides on natural enemies (Croft, 1990; Dent, 1995; Banks & Stark, 1998; Bajc et al., 2017; Laznik & Trdan, 2014). Several natural enemies can be used for biological control of lepidopteran pests. Among these, *Habrobracon* spp. are used as effective parasitoids of different pests specially in stored products and crop plants (Navaei et al., 2002).

The bollworms, *Helicoverpa* spp. (Lepidoptera: Noctuidae) moths have a variety of key pests in the world that are polyphagous and have a wide range of host plants for the crops, vegetables and etc. in the world and Iran. Three species of *Helicoverpa armigera* (Hubner, (1808)), *Heliothis viriplaca* (Hufnagel, 1766) and *Heliothis peltigera* (Denis & Schiffermüller, 1775) have been reported in chickpea fields of Iran. The dominant species, *H. viriplaca* is reported in the chickpea fields of most regions of Iran. Farmers every year spray one or two times to prevent damage to *Helicoverpa* spp. by chemical insecticides such as Carbaryl (Sevin), Malathion and Phosalone. However, some species of this genus, such as *H. viriplaca*, have shown resistance to the major types of organophosphate, carbamate, and pyrethroid insecticides (Kranthi et al., 2001; Murray et al., 2005).

*Habrobracon hebetor* Say is an ectoparasitoid and has been studied as a biocontrol agent of various lepidopteran pests in several countries (Gerling, 1971; Youm & Gilstrap, 1993; Magro & Parra, 2001). In recent years, the mass rearing program of *H. hebetor* has been initiated in Iran and it has been used to control *H. armigera*, *H. viriplaca* and *Ostrinia nubilalis* (Hübner, 1796) specially in different regions of Iran (Navaei et al., 2002; Rafiee-Dastjerdi, 2008; Sarmadi et al., 2010). Limited information is available on the sublethal effects of commonly used insecticides on this biocontrol agent (Rafiee-Dastjerdi et al., 2008 and 2012). Estimation of lethal and sublethal effects of pesticides on a natural enemy is necessary to recognize their effects of it (Walthal & Stark, 1997; Stapel et al., 2000; Stark & Banks, 2003). Demographic toxicology is usually considered to be the best way to evaluate total effects of pesticides on a target insect. So, stable population parameters have been recommended to evaluate the overall effects of pesticides, because it is based on both survivorship and fecundity parameters (Stark & Wennergren, 1995).

Considering the importance of recognizing the susceptibility of various growth stages of parasitoid to insecticides, and in order to determine the best time to release them in the field and to perform the spraying, we try to evaluate the *H. hebetor* wasp susceptibility in the adult stages in comparison with the recommended dosage. The main objective of this study was to evaluate the sublethal effects of the malathion, commonly used insecticide against *H. viriplaca* in the chickpea fields of most regions of Iran and to determine the possibility of integrating the application of this species with insecticides in IPM program of this key pest.

# 2 MATERIALS AND METHODS

## 2.1 Insects rearing

Insects *H. hebetor* was obtained in adult stage from the insectarium in Lorestan province in Iran. The colony of *H. hebetor* was reared on 5$^{th}$ instar larvae of the flour moth, *Ephestia kuehniella* Zeller, 1879 reared on wheat flour in semi-clear plastic boxes (40×25×15 cm) in the laboratory. Fifth instar larvae of *E. kuehniella* were used for both rearing the colony of wasp and applying experiments. Bioassay and rearing conditions were 26 ± 1$^{o}$C, 60 ± 5% RH and a photoperiod of 16 : 8 h (L: D).

## 2.2 Bioassay experiments

### 2.2.1 Preliminary bioassay

Malathion (EC 57 %, Partonar Co., Iran) was used in this experiment. Serial dilutions were prepared and concentrations (determined after preliminary tests) ranged from 5 to 200 µl $l^{-1}$ used immediately after preparation. Distilled water alone was used as a control. Contact toxicity of the insecticide was assessed on the adult, larvae and pupae of *H. hebetor*. Five concentrations of the insecticide were prepared to treat the insect. First, a number of parasitoid adult wasps were placed in a refrigerator at +5 °C for five minutes to freeze and do not move. Then for each concentration (treatment), 80-100 insects were used. Then using the

POLO PC software, the concentrations of $LC_{10}$, $LC_{25}$, $LC_{50}$, and $LC_{90}$ were obtained. The adult, larvae and pupae of parasitoid were exposed to sublethal concentrations ($LC_{10}$ and $LC_{25}$) and compared with the control.

### 2.2.2 Effect of sublethal concentrations on the adults of *H. hebetor*

For each concentration of malathion, three 1000 ml plastic glasses were considered separately, then the walls of the glasses were sprayed with ($LC_{10}$ = 10.5 and $LC_{25}$ = 20.2 $\mu l l^{-1}$) concentrations, after the treated glasses were completely dried, in each glass, about 80 to 100 adult insects were released. After 24 hours, twenty impregnated adults (12 female and 8 male) from each glass were transferred into new 150 ml plastic glasses, then each one was reversed on a piece of paper containing 30-days old host larvae. After 48 hours, the insects were removed and the parasitized larvae were subsequently transferred and kept in germinator at the same conditions as used for their respective parental adults. The number of parasitized larvae of the host and the percentage of parasitism was recorded. Upon emergence, the number and sex of the progeny and longevity of adults were recorded. This experiment was replicated three times for each concentration, separately. The control treatment was only with distilled water (Abedi et al., 2014).

### 2.2.3 Effect of sublethal concentrations on the larvae of *H. hebetor*

First, in order to obtain *H. hebetor* larvae, a large number of male and female wasps were randomly placed in ten clear cylindrical plastic containers (30 cm height and 14 cm diameter) with a valve at the bottom covered with fine mesh gauze for ventilation and with a 2.5 cm diameter hole for feeding with 10 % honey solution. Then, on five $A_4$ size papers, a large number of *E. kuehniella* larvae were placed and exposed to adult parasitoids in plastic containers. After 48 hours, adult parasitoids were removed and the parasitized host larvae were kept under controlled at the same conditions as used for their respective parental adults. After appearance of *H. hebetor* larvae on the host, they were exposed to sublethal concentrations of the insecticide, so that $A_4$ paper containing wasp larvae sticking to host larvae was cut to smaller size. On each paper cut, about 10 wasp larvae were attached to the host larvae, each cut piece was dipped separately into the insecticide solutions and immediately removed, and then the impregnated larvae were placed under rearing conditions to adult stage.

In this experiment, twenty adults (12 female and 8 male recognized by genitalia) emerged from impregnated larvae were transferred into new 150 ml plastic glasses, then each one was reversed on a piece of paper containing 30-days old host larvae. After 48 hours, the insects were removed and the host larvae were kept under the same conditions as used for their respective parental adults. The number of parasitized larvae of the host and the percentage of parasitism, number of emerged males and females and longevity of adults were calculated. This experiment was replicated three times for each concentration, separately. The control was treated only with distilled water (Mahdavi et al., 2011).

### 2.2.4 Effect of sublethal concentrations on the pupae of *H. hebetor*

The rearing of a large number of *H. hebetor* pupae was carried out similar to the previous experiment for larvae. After the pupae were formed, they were exposed to sublethal concentrations, so that the paper containing the pupae was cut to smaller sizes. On each paper cut, about 40 pupae were placed, each cut was individually dipped in containers containing different concentrations of the insecticide and quickly pulled out and then the impregnated pupae were placed under rearing conditions to adult stage (Rafiee-Dastjerdi et al., 2012).

In this experiment, twenty adults (12 female and 8 male) emerged from impregnated pupae were transferred into new 150 ml plastic glasses, then each one was reversed on a piece of paper containing 30-days old host larvae. After 48 hours, the insects were removed and the host larvae were kept under the same conditions as used for their respective parental adults. The number of parasitized larvae of the host and the percentage of parasitism, number of emerged males and females and longevity of adults were calculated. This experiment was replicated three times for each concentration, separately. The control was treated only with distilled water (Rafiee-Dastjerdi et al., 2012).

### 2.2.5 Data analysis

The biological data were submitted to analysis of variance and the means were compared by the Tukey test, using SPSS 14.0 software program (SPSS, 2004). The demographic parameters and their corresponding standard errors were estimated by the Jackknife technique (Meyer, 1986; Sokal & Rohlf, 1981), and the means were compared by the Tukey test, using SPSS 14.0 software program (SPSS, 2004).

## 3 RESULTS

### 3.1 Effect of sublethal concentrations on the adults of *H. hebetor*

According to analysis variance of the the data obtained from *H. hebetor* adult, rate of parasitism percentage was found to have a significant difference at 1 % level in the sublethal concentrations of malathion ($F_{2, 6} = 6.7$; $P < 0.01$). The results showed that the highest percentage of parasitism in the control treatment was 95.56 ± 1.11 % and the lowest in the $LC_{25}$ concentration was 87.88 ± 1.11 %. Also, results of *H. hebetor* male production were found to be significantly different between treatments ($F_{2, 6} = 5.26$; $P < 0.01$). The highest percentage of males was appeared in $LC_{25}$ concentration that was 38.56 ± 5.26 % and the lowest in control treatment was 22.73 ± 2.62 %. Also, based on the results of the analysis of variance, the adult longevity of *H. hebetor* under the influence of different concentrations of malathion was significant ($F_{2, 6} = 20.73$; $P < 0.01$). The results showed that the maximum adult longevity was 16.00 ± 0.58 days for the control treatment and the lowest for the $LC_{25}$ concentration for 10.67 ± 0.33 days (Table 1).

**Table 1**: Mean comparison (±SE) of percentage of parasitism, male production and longevity of *Habrobracon hebetor* adult wasps effected by sublethal concentrations of malathion

| Concentration | Parasitism (%) | Male production (%) | Longevity (day) |
|---|---|---|---|
| Control | 92.22 ± 1.11 a* | 30.94 ± 2.17 a | 16.67 ± 0.88 a |
| $LC_{10}$ | 92.22 ± 1.11 a | 29.33 ± 2.13 a | 12.23 ± 0.88 b |
| $LC_{25}$ | 87.78 ± 1.11 b | 39.63 ± 0.37 b | 11.67 ± 0.33 b |

Values within the columns followed by different letters are significantly different based on Duncan Multiple Range test.

### 3.2 Effect of sublethal concentrations on the larvae of *H. hebetor*

According to analysis variance of the data obtained from *H. hebetor* adults emerged from impregnated larvae, rate of parasitism percentage was found to have a significant difference at 1 % level in the sublethal concentrations of malathion ($F_{2, 6} = 9.8$; $P < 0.01$). The results showed that the highest percentage of parasitism in the control and $LC_{10}$ treatments was 92.22 ± 1.11 % and the lowest in the $LC_{25}$ concentration was 87.78 ± 1.11 %. Also, results of *H. hebetor* male produced from impregnated larvae were found to be significantly different between treatments ($F_{2, 6} = 9.83$; $P < 0.01$). The highest percentage of males was appeared in $LC_{25}$ concentration that was 39.63 ± 0.37 % and the lowest in $LC_{10}$ treatment was 29.33 ± 2.13 %. Also, based on the results of the analysis of variance, the longevity of *H. hebetor* adults emerged from impregnated larvae under the influence of different concentrations of malathion was significant ($F_{2, 6} = 13.27$; $P < 0.01$). The results showed that the maximum adult longevity was 16.67 ± 0.88 days for the control treatment and the lowest for the $LC_{25}$ concentration for 11.67 ± 0.33 days (Table 2).

**Table 2:** Mean comparison (±SE) of percentage of parasitism, male production and longevity of *Habrobracon hebetor* adult wasps emerged from impregnated larvae effected by sublethal concentrations of Malathion

| Concentration | Parasitism (%) | Male production (%) | Longevity (day) |
|---|---|---|---|
| Control | 92.22 ± 1.11 a* | 30.94 ± 2.17 a | 16.67 ± 0.88 a |
| $LC_{10}$ | 92.22 ± 1.11 a | 29.33 ± 2.13 a | 12.23 ± 0.88 b |
| $LC_{25}$ | 87.78 ± 1.11 b | 39.63 ± 0.37 b | 11.67 ± 0.33 b |

Values within the columns followed by different letters are significantly different based on Duncan Multiple Range test.

### 3.3 Effect of sublethal concentrations on the pupae of H. hebetor

According to analysis variance of the data obtained from *H. hebetor* adults emerged from impregnated pupae, rate of parasitism was found to have a significant difference at 1 % level in the sublethal concentrations of malathion ($F_{2, 6} = 5.6$; $P < 0.01$). The results showed that the highest percentage of parasitism in the control and $LC_{10}$ treatments was 95.56 ± 1.11 % and the lowest in the $LC_{25}$ concentration was 88.89 ± 1.11 %. Also, results of *H. hebetor* male produced from impregnated pupae were found to be significantly different between treatments ($F_{2, 6} = 34.68$; $P < 0.01$). The highest percentage of males was appeared in $LC_{25}$ concentration that was 37.12 ± 1.49 % and the lowest in control treatment was 22.98 ± 0.53 %. Also, based on the results of the analysis of variance, the longevity of *H. hebetor* adults emerged from impregnated pupae under the influence of different concentrations of malathion was significant ($F_{2, 6} = 11.55$; $P < 0.01$). The results showed that the maximum adult longevity was 16.33 ± 0.33 days for the control treatment and the lowest for the $LC_{25}$ concentration for 12 ± 0.58 days (Table 3).

**Table 3:** Mean comparison (±SE) of percentage of parasitism, male production and longevity of *Habrobracon hebetor* adult wasps emerged from impregnated pupae effected by sublethal concentrations of malathion

| Concentration | Parasitism (%) | Male production (%) | Longevity (day) |
|---|---|---|---|
| Control | 93.33 ± 1.92 b* | 22.98 ± 0.53 b | 16.33 ± 0.33 a |
| $LC_{10}$ | 95.56 ± 1.11 a | 23.91 ± 1.70 b | 14.33 ± 0.88 a |
| $LC_{25}$ | 88.89 ± 1.11 b | 37.12 ± 1.49 a | 12.00 ± 0.58 b |

Values within the columns followed by different letters are significantly different based on Duncan Multiple Range test.

## 4 DISCUSSION

Parasitism is a practice where the parasitoid needs to have searching efficiency, eggs in the ovary, and the ability to access the host. Therefore, reducing each of the above factors can lead to reduction in the percentage of parasitism. Chemical pesticides may be more or less able to do so. In natural condition, natural enemies first find their host habitat through kairomones that comes from the plant (Blassioli-Moraes et al., 2016). According to Cloyd (2012), the smell of the insecticide and the material produced from the plant at low doses may cause confusion of the natural enemy and reduce parasitism. Although it is easy to find the host in the laboratory condition, but the smell of insecticide may lessen the ability to search and access the host. Research has also shown that some insecticides reduce the number of eggs in the ovary (Daniel, 1975). The results of our experiments showed that there is a significant difference between the percentage of parasitism of adult wasps exposed to sublethal concentrations of malathion. Parasitism rate of *H. hebetor* has increased with decreasing sublethal concentrations. The percentage of parasitism in exposed wasps at the mentioned concentrations ($LC_{25}$, $LC_{10}$ and control) was 87.78, 92.22 and 95.56 %, respectively.

Rafiee-Dastjerdi et al. (2009) examined the susceptibility of the immature stages of *H. hebetor* parasitoid wasp to the pesticides in order to investigate the effect of the recommended dose of Spinosad, Profenofos, Thiodicarb and Hexaflumuron in two methods of spraying and placement on the immature stages of the wasp and the emergence of adults. Based on the International Organization for Biological Control, Profenofos, Spinosad and Thiodicarb, with a mean decrease of 73.62, 39 and 29.25 %, respectively, of the emergence of insects were placed in in the less toxic group and Hexaflumuron with a reduction of 87.8 % in the adult emergence in the harmless insecticide group. It seems that the difference between the results of this study and Rafiei Dastjerdi et al. (2009) is due to difference in the laboratory conditions, host as well as differences in the stage of life of the wasp. Because the susceptibility of the adult stages have been studied in this study. It seems that the larval growth stage of *H. hebetor* is less affected by the sublethal concentration of the insecticide, because until the adult stage most of insecticide decomposes inside the insect's body. It also seems that due to the fact that wasp has been fed with water, honey and vitamin C until death, less fluctuations has occurred over their lifetime (Soyelu, 2013).

Results of timed bioassay of Baker et al. (1995) showed that field strain of *B. hebetor* was significantly more tolerant of malathion compared to the laboratory strain. Also, triphenyl phosphate (TPP) substantially delayed the toxicity of malathion. The results of Mahdavi & Saber (2013) showed that malathion rather than diazinon had the lowest adverse effects on the functional response of *H. hebetor*. After conducting advanced field studies, it was found that malathion may be used as a compatible chemical material with biological control in IPM programs.

The results of this study showed that there was a significant difference between male production of *H. hebetor* adult wasps and sublethal concentrations of malathion. In the $LC_{25}$ concentration, male production was increased in adult wasps and was reduced by decreasing sublethal concentrations. Amir-Maafi et al. (2002) stated in their study on *Trissolcus grandis* (Thompson, 1861) that the female wasps in the first five days lay 26 % of their total eggs and 51 % of the hatched eggs of the female wasp is related to its first five days of activity. Also, 63.66 % of the total produced females were related to this period, but after 5 days from the evaluation period, the male production and mortality of the female wasps increased. In fact, in the male production of insecticide-exposed wasps, the age of the wasps is also important. Also, based on the growth characteristics of parasitoid wasps of the sunn pest, male parasitoids appear earlier than females and work around un-emerged parasitized eggs (Amir Majafi, 2000; Safavi, 1974; Asgari, 1996). For this reason, chemical insecticides appear to effect on male wasps and cause mating ability to reduce and inoculate the egg, resulting in the production of haploid (male) individuals.

Based on the results of the effect of sublethal concentrations of malathion on the longevity, the results were significantly different between treatments. According to the results, with the increase of sublethal dose of malathion, the longevity of *H. hebetor* progeny has decreased compared to control treatment. The results of Shishehbor and Faal-Mohammad-Ali (2013) showed that the effects of Flufenoxuron (Cascade) and Lufenuron insecticides on different stages of *H. hebetor* larvae were not significantly different in terms of longevity and egg oviposition of females. But different growth stages had a significant effect on the longevity of female parasitoid. According to their results, the highest longevity was related to the lufenuron-treated pupal stage. Interaction effects of insecticides and different growth stages did not have a significant effect on the longevity of the female. The results of our study were not similar to this study. It seems that the difference in the results is due to the type of insecticide used in the treatment. It may also be possible that reduction of longevity of the wasps at high concentrations of insecticide is due to reduction in wasp body water content. Wasps seem to need more water to eliminate toxic compounds in their body, resulting in more activity to eliminate poisonous compounds, lose their body water and die.

The results showed that the percentage of parasitism decreased with increasing sublethal concentration of malathion. The results of Faal-Mohammad-Ali et al. (2015) in evaluating the effects of chlorpyrifos and fenpropathrin in the sublethal concentration of $LC_{25}$ on the parameters of stable population of *H. hebetor* parasitoid wasp in the larval stage showed that fenpropathrin insecticide had the most effect on the parameters of stable population growth. There was a significant difference between the treatments and the control. There was no significant difference between chlorpyrifos and fenpropathrin except for the population growth rate. The results of these researchers are similar to the results of the present study, and the numerical differences in the data are related to the test conditions and insecticide concentrations.

Abedi et al. (2014) evaluated the sublethal effects of azadirachtin and cypermethrin insecticide and the adult wasps were exposed to the $LC_{30}$ concentration of the insecticides, and then demographic parameters of the surviving wasps were determined. Fecundity, fertility, and parameters including the intrinsic rate of increase ($r_m$) were affected negatively.

## 5 CONCLUSION

Even though results obtained in small laboratory arenas may not be realized under natural conditions (Kareiva, 1990), these kinds of investigations under the laboratory conditions can be helpful in selecting insecticides for additional studies under more natural conditions and for application of suitable insecticides along with natural enemies in the pest management programs.

## 6 ACKNOWLEDGEMENTS

The work received financial support from Postgraduate Education Bureau of the Shahed University, Iran, which is greatly appreciated.

## 7 REFERENCES


Abedi, Z., Saber, M., Gharekhani, G., Mehrvar, A., & Kamita, S.G. (2014). Lethal and sublethal effects of azadirachtin and cypermethrin on *Habrobracon hebetor* (Hymenoptera: Braconidae). *Journal of Economic Entomology, 107*(2), 638-645. doi:10.1603/EC13227

Amir-Maafi, M., Kharazi Pakdel, A., Rasoulian, Gh., & Sahragard, A. (2002). Efficiency and regulation mechanisms of sunn pest population, *Eurygaster integriceps* Put. (Het.: Scutelleridae), by egg parasitoids in Varamin. *Journal of Entomological Society of Iran, 22*(1), 29-47.

Bajc, N., Držaj, U., Trdan, S., Laznik, Ž. (2017). Compatibility of acaricides with entomopathogenic nematodes (*Steinernema* and *Heterorhabditis*). *Nematology, 19*, 891-898. doi:10.1163/15685411-00003095

Baker, J.E., Weaver, D.K., Throne, J.E., & Zettler, J.L. (1995). Resistance to Protectant Insecticides in Two Field Strains of the Stored-Product Insect Parasitoid *Bracon hebetor* (Hymenoptera: Braconidae). *Journal of Economic Entomology, 88*(3), 512-519. doi:10.1093/jee/88.3.512

Blassioli-Moraes, M.C., Borges, M., Michereff, M.F., Magalhães, D.M., & Laumann, R.A. (2016). Semiochemicals from plants and insects on the foraging behavior of Platygastridae egg parasitoids. *Pesquisa Agropecuária* Brasileira, *51*(5), 454-464. doi:10.1590/S0100-204X2016000500005

Banks, J.E., & Stark, J.D. (1998). What is ecotoxicology? An ad-hoc grab bag or an interdisciplinary science? *Integrative Biology*. Wiley-Liss, Inc., pp. 195-204.

Cloyd, R.A. (2012). Indirect effects of pesticides on natural enemies, pp. 127-150. In: Soundararajan, R.P. [ed.]. *Pesticides Advances in Chemical and Botanical Pesticides*. In Tech, Rijeka, Croatia. 382 pgs. ISBN no. 978-953-51-0680-7.

Croft, B.A. (1990). *Arthropod Biological Control Agents and Pesticides*. John Wiley and Sons, New York.

Daniel, J.C., & Amladi, S.R. (1975). The Nilgiri Wood Pigeon, *Columba elphinstonii* (Sykes) on Salsette Island, Bombay. *Journal of the Bombay Natural History Society, 71*, 304.

Dent, D. (1995). *Integrated Pest Management*. Chapman and Hall, London.

Faal-Mohammad-Ali, H., Allahyari, H., & Saber, M. (2015). Sublethal effect of chlorpyriphos and fenpropathrin on functional response of *Habrobracon hebetor* (Hym.: Braconidae). *Archives of Phytopathology and Plant Protection, 48*(4), 288-296. doi:10.1080/03235408.2014.886411

Gerling, D. (1971). Occurrence, abundance, and efficiency of some local parasitoids attacking *Spodoptera littoralis* (Lepidoptera: Noctuidae) in selected cotton fields in Israel. *Annales of Entomological Society of America, 64*, 492-499. doi:10.1093/aesa/64.2.492

Kareiva, P. (1989). The spatial dimension in pest-enemy interactions. In: Mackauer, Ehler and Roland (ed). *Critical Issues in Biological Control*. Intercept, Andover, Intercept Ltd., U. K., pp. 213-227.

Kranthi, K.R., Jadhav, D., Wanjari, L.R., & Russel, D. (2001). Pyrethroid resistance and mechanism of resistance in field strain of *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Journal of Economic Entomology, 94*, 1-6. doi:10.1603/0022-0493-94.1.253

Laznik, Ž., & Trdan, S. (2014). The influence of insecticides on the viability of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) under laboratory conditions. *Pest Management Science, 70*, 784-789. doi:10.1002/ps.3614

Mahdavi, V., & Saber, M. (2013). Functional response of *Habrobracon hebetor* Say (Hym.: Braconidae) to Mediterranean Flour Moth (*Anagasta Kuehniella*), in response to pesticides. *Journal of Plant Protection Research, 53*(4), 399-403. doi:10.2478/jppr-2013-0059

Mahdavi, M., Saber, M., Rafiee-Dastjerdi, H., & Mehrvar, A. (2011). Comparative study of the population level effects of carbaryl and abamectin on larval ectoparasitoid *Habrobracon hebetor* Say (Hymenoptera: Braconidae). *BioControl, 56*, 823-830. doi:10.1007/s10526-011-9356-8

Magro, S.R., & Parra, J.R.P. (2001). Biologia do ectoparasitoide *Bracon hebetor* Say, 1857 (Hymenoptera: Braconidae) em sete especies de lepidopteros. *Scientia Agricola, 58*, 693-698. doi:10.1590/S0103-90162001000400007

Meyer, J.S., Ingersoll, C.G., McDonald, L.L., & Boyce, M.S. (1988). Estimating uncertainty in population growth rates: Jack knife vs. bootstrap techniques. *Ecology, 67*, 1150-1166.

Murray, D.A.H., Lloyd, R.J., & Hopkinson, J.E. (2005). Effective of new insecticides for management of *Helicoverpa* spp. (Lepidoptera: Noctuidae) in

Australian grain crops. *Australian Journal of Entomology, 44*(1), 62-67. doi:10.1111/j.1440-6055.2005.00422.x

Navaei, A.N., Taghizadeh, M., Javanmoghaddam, H., Oskoo, T., & Attaran, M.R. (2002). Efficiency of parasitoid wasps, *Trichogramma pintoii* and *Habrobracon hebetor* against *Ostrinia nubilalis* and *Helicoverpa* sp. on maize in Moghan. In *Proceedings of the 15th Iranian Plant Protection Congress. September 7-11*, Razi University of Kermanshah, Iran. [In Persian]

Rafiee-Dastjerdi, H. (2008). *Studying lethal effects of thiodicarb, profenofos, spinosad and hexaflumuron on cotton bollworm and their lethal and sublethal effects on ectoparasitoid* Habrobracon hebetor *Say (Hymenoptera: Braconidae)*. Ph.D. dissertation. Tabriz University, Tabriz, Iran, 108 pp.

Rafiee-Dastjerdi, H., Hassanpour, M., Nouri-Ganbalani, G., Golizadeh, A., & Sarmadi, S. (2012). Sublethal effects of indoxacarb, imidacloprid and deltamethrin on life table parameters of *Habrobracon hebetor* (Hymenoptera: Braconidae) in pupal stage treatment. *Journal of Crop Protection*, *1*(3), 221-228.

Rafiee-Dastjerdi, H., Hejazi, M.J., Nouri-Ghanbalani, G., & Saber, M. (2008). Toxicity of some biorational and conventional insecticides to cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae) and its ectoparasitoid, *Habrobracon hebetor* (Hymenoptera: Braconidae). *Journal of Entomological Society of Iran, 28*, 27-37.

Rafiee-Dastjerdi, H., Hejazi, M.J., Nouri-Ghanbalani, G., & Saber M. 2009. Sublethal effects of some biorational and conventional insecticides on ectoparasitoid, *Habrobracon hebetor* Say (Hymenoptera: Braconidae). *Journal of Entomology, 6*, 82-89. doi:10.3923/je.2009.82.89

Sarmadi, S., Nouri-Ganbalani, G., Rafiee-Dastjerdi, H., Hassanpour, M., & Farshbaf-Pourabad, R. (2010). The effects of Imidacloprid, Indoxacarb and Deltamethrin on some biological and demographic parameters of *Habrobracon hebetor* Say (Hymenoptera: Braconidae) in adult stage treatment. *Munis Entomology & Zoology, 5*(2), 646-651.

Shishehbor, P., & Faal-Mohammad-Ali, H. (2013). Study of sublethal effects of Flufenoxuron and Lufenuron insecticides on the life table parameters of *Habrobracon* hebetor Say (Hym.: Braconidae). *Iranian Journal of Plant Protection Science, 43*(2), 242-233.

Sokal, R.R., & Rohlf, F.J. (1981). *Biometry, the principles and practice of statistics in biological research*. W.H. Freeman and Company, New York, 859 pp.

Soyelu, O.J. (2013). Suitability of a Novel Diet for a Parasitic Wasp, *Cotesia plutellae. Journal of Insect Science, 13*, 86. doi:10.1673/031.013.8601

SPSS (2004). *SPSS for windows*. SPSS INC., Chicago, Illinois.

Stapel, J.O., Cortesero, A.M., & Lewis, W.J. (2000). Disruptive sublethal effects of insecticides on biological control: altered foraging ability and life span of a parasitoid after feeding on extrafloral nectar of cotton treated with systemic insecticides. *Biological Control, 17*, 243-249. /doi:10.1006/bcon.1999.0795

Stark, J.D., & Banks, E. (2003). Population level effects of pesticides and other toxicants on arthropods. *Annual Review of Entomology, 48*, 505-519. doi:10.1146/annurev.ento.48.091801.112621

Stark, J.D., & Wennergren, U. (1995). Can population effects of pesticides be predicted from demographic toxicological studies? *Journal of Economic Entomology, 88*, 1089-1096. doi:10.1093/jee/88.5.1089

Walthall, W.K., & Stark, J.D. (1997). A comparison of acute mortality and population growth rate as endpoints of toxicological effect. *Ecotoxicology* and *Environmental Safety, 37*(1), 45-52. doi:10.1006/eesa.1997.1521

Youm, O., & Gilstrap, F.E. (1993). Life-fertility tables of *Bracon hebetor* Say (Hymenoptera: Braconidae) reared on *Heliocheilus albipunctella* de Joannis (Lepidoptera: Noctuidae). *Insect Science and its Application, 14*, 455-459. doi:10.1017/S1742758400014120

doi:10.14720/aas.2018.111.3.13 Original research article / izvirni znanstveni članek

# Obravnava vročinskih valov in primer toplotne obremenitve delavcev v kmetijstvu v času vročinskih valov 2017

Tjaša POGAČAR*[1], Lučka KAJFEŽ BOGATAJ[2], Zalika ČREPINŠEK[3]

Received July 09, 2018; accepted October 03, 2018.
Delo je prispelo 09. julija 2018, sprejeto 03. oktobra 2018.


## IZVLEČEK

Delavci v kmetijstvu so poleti v veliki meri izpostavljeni vročinskemu stresu, kar lahko vpliva na zmanjšano storilnost in izgubo dohodka. Temperature zraka v Sloveniji v zadnjih desetletjih naraščajo, projekcije podnebnih sprememb pa kažejo, da se bo ta trend nadaljeval hkrati s povečanjem števila dni z vročinskim stresom. Analizirali smo spremembe v številu vročinskih valov v osrednji in jugozahodni Sloveniji za obdobje 1961–2017 ter obremenitev delavcev z vročinskim stresom med vročinskimi valovi na primeru leta 2017. Vročinski val nastopi, če je temperaturni prag za povprečno dnevno temperaturo dosežen ali presežen vsaj tri zaporedne dni, pri čemer je prag za omiljeno celinsko podnebje (Ljubljana) 24 °C in za omiljeno sredozemsko podnebje (Bilje) 25 °C. Kazalnik za spremljanje toplotne obremenitve delavcev WBGT (Wet Bulb Globe Temperature) smo izračunali iz temperatur zraka in zračne vlage. Na obeh lokacijah se povečuje število dni v vročinskih valovih in njihova intenzivnost, povprečne dnevne temperature zraka dosegajo precej večje vrednosti kot v prvi polovici obravnavanega obdobja. Podaljšuje se tudi časovni razpon, v katerem se pojavljajo, saj se do leta 1990 vročinski valovi niso pojavljali zgodaj junija in pozno avgusta, tako kot v zadnjih letih. Izračunane vrednosti kazalnika WBGT kažejo, da je bila v večini dni v vročinskih valovih 2017 v Ljubljani in Biljah mejna vrednost WBGT 23 °C presežena praktično cel dan, kar za delavce z veliko fizično obremenitvijo pomeni visoko stopnjo tveganja zaradi obremenitve z vročinskim stresom. Pri preseženih mejnih vrednostih WBGT bi morali delodajalci oziroma kmetje sami uvesti preventivne ukrepe za zmanjšanje tveganja vročinskega stresa.


**Ključne besede:** vročinski val; vročinski stres; kazalnik WBGT; kmetijstvo; delavci; Slovenija


## ABSTRACT

### HEAT WAVES ANALYSIS AND THE HEAT LOAD OF AGRICULTURAL WORKERS DURING THE HEAT WAVES IN 2017 (USING INDEX WBGT)

Workers in agriculture are regularly exposed to heat stress during summer, which can affect reduced labour productivity and income losses. Air temperatures in Slovenia have been rising in recent decades, and climate change projections show that this trend will continue along with an increase in the number of days with heat stress risk. Changes in the number of heat waves in central and south-western Slovenia for the period 1961–2017 were analysed as well as the risk of the heat stress for workers during heat waves in the year 2017. The heat wave occurs if the temperature threshold for the average daily temperature is reached or exceeded on at least three consecutive days, with the threshold for the mild continental climate (Ljubljana) 24 °C and the mild Submediterranean climate (Bilje) 25 °C. The WBGT (Wet Bulb Globe Temperature) index, assessing the risk of heat stress, was calculated from relative humidity and air temperatures. At both locations, the number of days in heat waves increased as well as their intensity, average daily air temperatures were significantly higher than in the first half of the considered period. The time span, in which the heat waves occur, also extended, as until 1990 they did not appear in early June and late August, as in recent years. The calculated values of the WBGT show that for most days in the heat waves in 2017 in Ljubljana and Bilje, the WBGT 23 °C threshold was exceeded practically all day, which shows a high level of heat stress risk for physically intense work. In the case of exceeded WBGT reference values, employers or farmers themselves should take actions to reduce the risk of heat stress.


**Key words:** heat wave; heat stress; WBGT index; agriculture; workers; Slovenia

[1] dr., Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za agronomijo, Jamnikarjeva ulica 101, 1000 Ljubljana,Slovenija; *Corresponding author: tjasa.pogacar@bf.uni-lj.si

[2] prof. dr., Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za agronomijo, Jamnikarjeva ulica 101, 1000 Ljubljana, Slovenija

[3] doc. dr., Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za agronomijo, Jamnikarjeva ulica 101, 1000 Ljubljana, Slovenija

## 1 UVOD

Delo na prostem pomeni izpostavljenost okoljskim razmeram, ki se s podnebnim spremembami v zadnjih letih močno spreminjajo. V Sloveniji naraščajo povprečne temperature zraka še intenzivneje kot drugod po Evropi in svetu, v obdobju 1961–2011 je bil izmerjen časovni trend povprečne letne temperature zraka okoli 0,34 °C/desetletje, prav tako je pozitiven trend najvišje julijske temperature zraka, ki ga navaja Agencija RS za okolje (ARSO, 2013). Zmerno optimistični scenarij (RCP4.5) v obdobju 2011–2040 predvideva dvig povprečne temperature zraka za 0,5 do 1,0 °C, v obdobju 2041–2070 za 1,0 do 2,0 °C, v obdobju 2071–2100 pa za 1,3 do 2,6 °C (ARSO, 2017). Za povprečno poletno in najvišjo julijsko temperaturo zraka kažejo projekcije za konec stoletja po najbolj optimističnem scenariju (RCP2.6) povečanje za 1 °C in po najmanj optimističnem (RCP8.5) za 4.5 °C, pri vročih dneh (najvišja dnevna temperatura zraka enaka ali višja od 30 °C) to pomeni povečanje za od 2 do 35 dni (Pogačar in sod., 2018).

Po podatkih FAO (Staal Wästerlund, 2018) je 32 % svetovne delovne populacije zaposlene v kmetijskem sektorju, v katerem je izmed vseh panog najvišja stopnja smrtnosti zaradi vročine, saj delo večinoma poteka na prostem. Razmere se zaradi globalnega segrevanja še slabšajo, zato so raziskave na tem področju nujne, kar je prepoznala tudi Evropa, ki tem vprašanjem namenja dodatna finančna sredstva (projekt Heat-Shield: https://www.heat-shield.eu/).

Delavci v kmetijstvu so pri svojem delu poleti v veliki meri izpostavljeni vročinskemu stresu, pri čemer je obremenitev zaradi fizične zahtevnosti opravil, pri kateri telo proizvaja dodatno toploto, velika tudi v zmernejših razmerah (Staal Wästerlund, 2018). Dodatno težavo lahko predstavlja zaščitna obleka, kadar je potrebna zaradi narave dela, saj ne omogoča zadostnega odvajanja toplote v okolico in omejuje potenje. Delavci v velikem deležu poročajo o žeji, povečanem potenju, utrujenosti, glavobolih, v manjšem deležu tudi o hujših simptomih vročinskega stresa, oboje pa brez ukrepanja vodi v zmanjšano storilnost (Pogačar in sod., 2017). Vročinski stres zmanjša celotno produktivnost in posledično pomeni izgubo dohodka (Kjellstrom in sod., 2009). Dehidriranost vodi v počasnejše delo, večja je verjetnost napak, kar lahko povzroči delovne nesreče (Kjellstrom in sod., 2018), povečana je tudi možnost za vročinski udar (t.j. kap) (Staal Wästerlund, 2018). V raziskavi v ZDA so ugotovili, da je tveganje za smrt zaradi vročinskega udara pri delavcih v kmetijstvu 35-krat večja kot pri povprečni delovni populaciji (Gubernot in sod., 2015).

Pomembno je, da spremljamo vročinski stres na delovnem mestu, a vendar v tej smeri predvsem v zmernih podnebjih, kjer do sedaj ni bilo večjih težav, še ni veliko raziskav. Nikjer nismo zasledili analize toplotne obremenitve evropskih delavcev v kmetijstvu, najbližje so s svojimi raziskavami Flouris in sod. (2018), ki so raziskovali občutljivost ljudi na vročinski stres pri vsakdanjih opravilih in pri težjem delu, in Ioannou in sod. (2017), ki so ob razvijanju metode določanja produktivnosti s pomočjo snemanja časovnega poteka dela spremljali tudi toplotno obremenitev delavcev. Na splošno se v toplotno obremenjenih okoljih za spremljanje obremenitve delavcev oz. spremljanje vročinskega stresa v največji meri uporablja kazalnik WBGT (Wet Bulb Globe Temperature), ki ga izračunamo iz temperature in vlažnosti zraka in ga kot kazalnik toplotne obremenitve na delovnem mestu priporoča ISO standard. V okviru EU projekta Heat-Shield je bil izbran WBGT, ker je najširše uporabljen kazalnik za določanje vročinskega stresa pri delavcih, saj ga lahko izračunamo iz standardnih meteoroloških meritev na klasičnih ali avtomatskih postajah in je enostaven za interpretacijo z uporabo mednarodnih standardov, kot sta ISO 1989 in ISO 2017. Nazorneje so ga v primerjavi z drugimi kazalniki predstavili Gao in sod. (2018), ki glede na d'Ambrosio Alfano in sod. (2016) opozarjajo, da kljub široki možnosti uporabe ni primeren pri veliki vlagi v zraku in zelo počasnemu gibanju zraka ter da ne upošteva tveganja za dehidracijo pri povečanem potenju. Za Slovenijo kažejo projekcije podnebnih sprememb podobno kot za poletne temperature povečanje povprečnih in največjih poletnih vrednosti WBGT za 1 do 3,5 °C ter povečanje števila dni z veliko obremenjenostjo z vročinskim stresom (WBGT večji od 27 °C) do 20 dni v osrednji Sloveniji in več kot 30 dni v Biljah po najmanj optimističnem scenariju (Pogačar in sod., 2018).

Namen prispevka je predstaviti, kako se povečuje število vročinskih valov v osrednji Sloveniji in na jugozahodu ter kakšna je obremenitev delavcev z vročinskim stresom med vročinskimi valovi, kar bomo prikazali na primerih podatkov iz leta 2017.

## 2 MATERIAL IN METODE DELA

### 2.1 Obravnava vročinskih valov v daljšem obdobju

Za dve lokaciji, Ljubljano (14°31', 46°4', 299 m n.m.v.) in Bilje (13°38', 45°54', 55 m n.m.v.), smo obravnavali vročinske valove v obdobju 1961–2017 (v Biljah od 1970, ko so dostopni podatki). Vročinski val nastopi, če je temperaturni prag za povprečno dnevno temperaturo dosežen ali presežen vsaj tri zaporedne dni, pri čemer je prag za vlažno in zmerno podnebje hribovitega sveta 22 °C, za omiljeno celinsko podnebje (Ljubljana) 24 °C in za omiljeno sredozemsko podnebje (Bilje) 25 °C (Ključevšek in sod., 2018). Grafično smo prikazali število dni v vročinskem valu po mesecih za vsako leto in povprečno dnevno temperaturo po dnevih za vsak vročinski val. Podatke o povprečnih dnevnih temperaturah zraka smo pridobili iz arhiva Agencije RS za okolje (ARSO).

### 2.2 Obravnava polurnih vrednosti toplotne obremenitve s kazalnikom WBGT

Polurne vrednosti kazalnika WBGT [°C] v senci smo na podlagi polurnih vrednosti temperature zraka ($T_a$ [°C]) in psihrometrične temperature mokrega termometra ($T_{pwb}$ [°C]), izračunali po enačbi (Lemke in Kjellstrom, 2012 po Bernard in Pourmoghani 1999):

$$WBGT = 0{,}67T_{pwb} + 0{,}33T_a,$$

pri čemer je $T_{pwb}$ je temperatura zraka pri umetno ustvarjenih razmerah, ko je hitrost vetra 3-5 m s$^{-1}$ in jo z iteracijo določimo iz $T_a$, temperature rosišča $T_d$ [°C], delnega parnega tlaka $e_d$ [hPa] in nasičenega parnega tlaka $e_w$ [hPa] (McPherson, 2008):

$$1556e_d - 1{,}484e_dT_{pwb} - 1556e_w + 1{,}484e_wT_{pwb} + 1010\left(T_a - T_{pwb}\right) = 0,$$

$$e_d = 6{,}106\exp\left(\frac{17{,}27T_d}{237{,}3 + T_d}\right),$$

$$e_w = 6{,}106\exp\left(\frac{17{,}27T_{pwb}}{237{,}3 + T_{pwb}}\right),$$

Za izračun $T_d$ in $e_d$ potrebujemo podatke o relativni zračni vlagi. Podatke o terminskih vrednostih $T_a$ in relativne vlage vsake pol ure v času vročinskih valov v letu 2017 smo pridobili iz meritev avtomatskih postaj, ki so shranjene v arhivu ARSO. Preglednica 1 predstavlja ISO standard mejnih vrednosti kazalnika WBGT za aklimatizirane in ne-aklimatizirane delavce, ki so določene tako, da pri uporabi lahkotnih oblačil (kratka majica in hlače) telesna temperatura ne preseže 38 °C, kar bi pomenilo preveliko toplotno obremenitev oziroma vročinski stres (Staal Wästerlund, 2018).

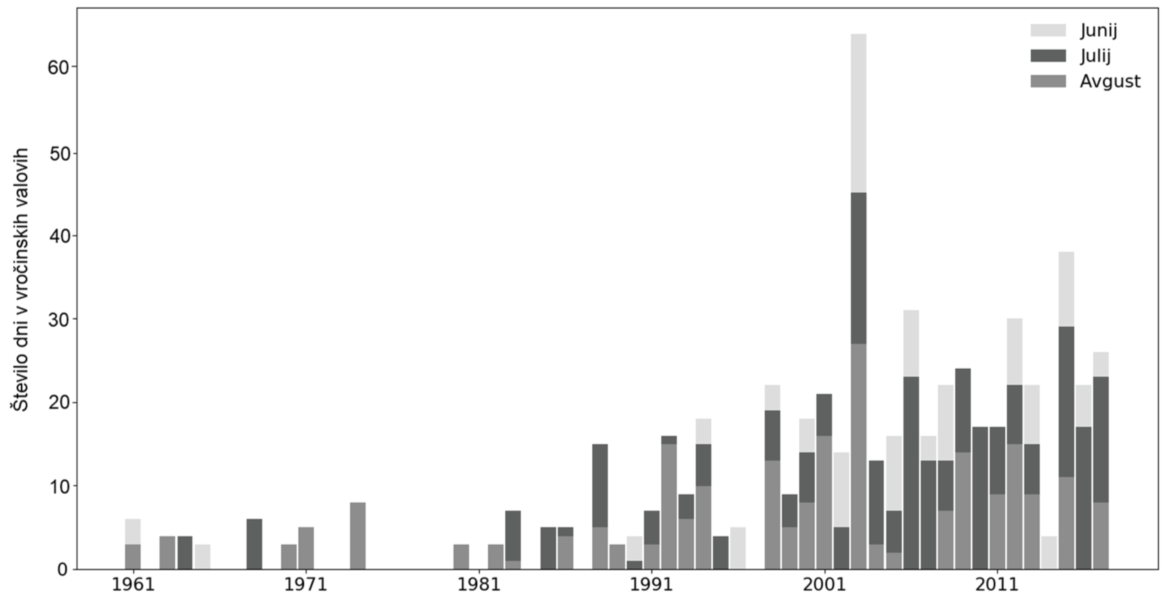
**Preglednica 1**: Mejne vrednosti kazalnika WBGT po ISO standardu 7243 (1989) (Staal Wästerlund, 2018) glede na stopnjo metabolizma
**Table 1**: Wet-bulb globe temperature index reference values, according to ISO 7243 (1989) (Staal Wästerlund, 2018)

| Stopnja metabolizma M [W/m$^2$] | Mejna vrednost kazalnika WBGT [°C] | | | |
|---|---|---|---|---|
| | Aklimatizirana oseba | | Ne-aklimatizirana oseba | |
| $M \leq 65$ | 33 | | 33 | |
| $65 < M \leq 130$ | 30 | | 30 | |
| $130 < M \leq 200$ | 28 | | 28 | |
| $200 < M \leq 260$ | Brez občutnega gibanja zraka 25 | Občutno gibanje zraka 26 | Brez občutnega gibanja zraka 22 | Občutno gibanje zraka 23 |
| $M > 260$ | 23 | 25 | 18 | 20 |

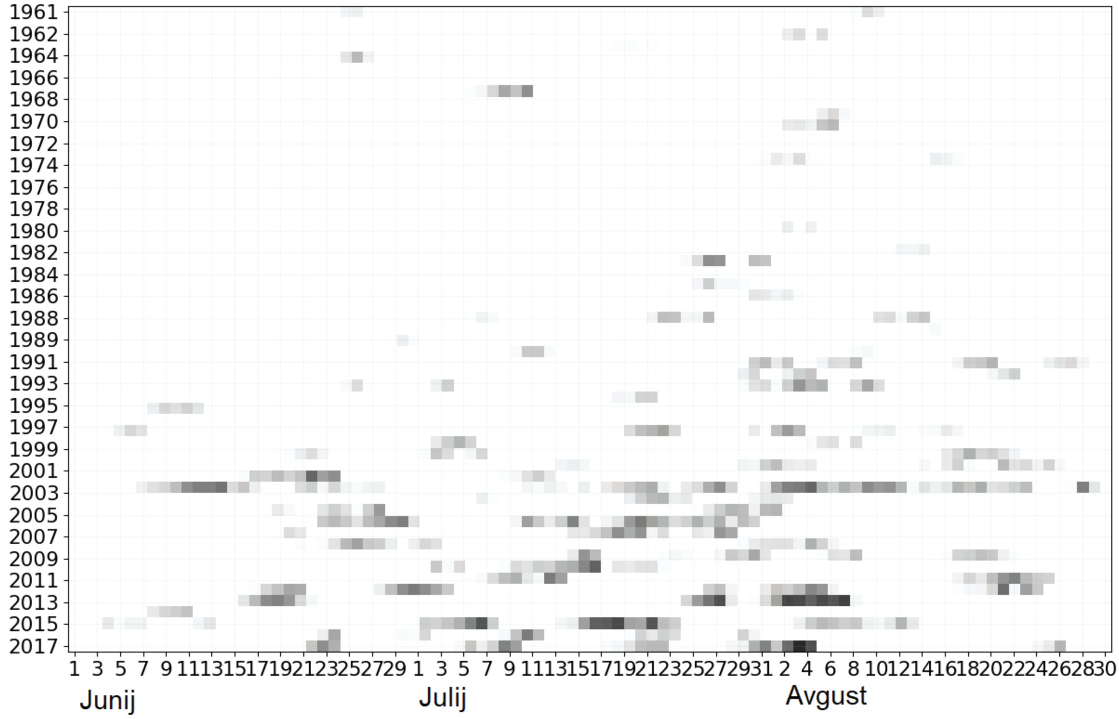
## 3 REZULTATI

### 3.1 Vročinski valovi v Ljubljani in Biljah

Število dni v vročinskih valovih je začelo po letu 1990 močno naraščati. V Ljubljani, ki ima omiljeno celinsko podnebje, so nastopili po letu 1988 vročinski valovi prav vsako leto, z izjemo leta 1997, okoli 20 do 30 dni v vročinskih valovih vsako poletje je postalo stalnica (Slika 1). V tem času so postali običajni tudi vročinski valovi v juniju (zgornji, svetel del stolpcev). Najbolj ekstremno po številu dni je bilo leto 2003, ko so se vročinski valovi vrstili en za drugim tekom celotnega poletja (Slika 2). Temnejše barve nakazujejo, da se ne povečuje le število dni v vročinskih valovih, temveč tudi njihova intenzivnost, saj povprečne dnevne temperature zraka dosegajo precej večje vrednosti kot v prvi polovici obravnavanega obdobja.



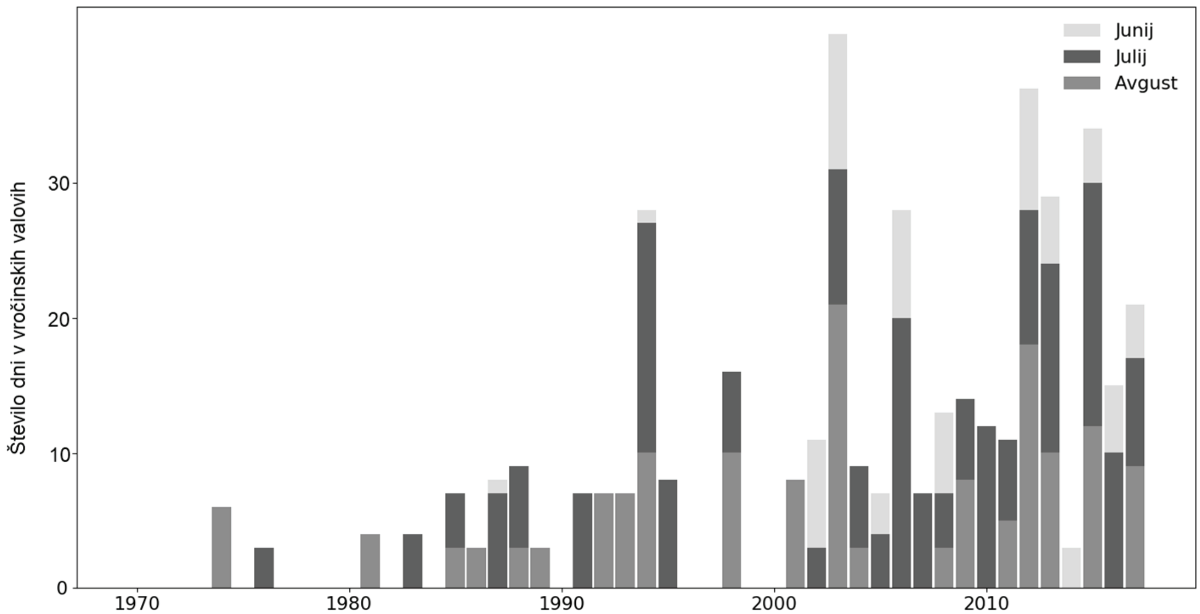

**Slika 1**: Število dni v vročinskih valovih po mesecih in letih v obdobju 1961–2017 v Ljubljani
**Figure 1**: Number of days within heat waves in months and years in the period 1961–2017 in Ljubljana



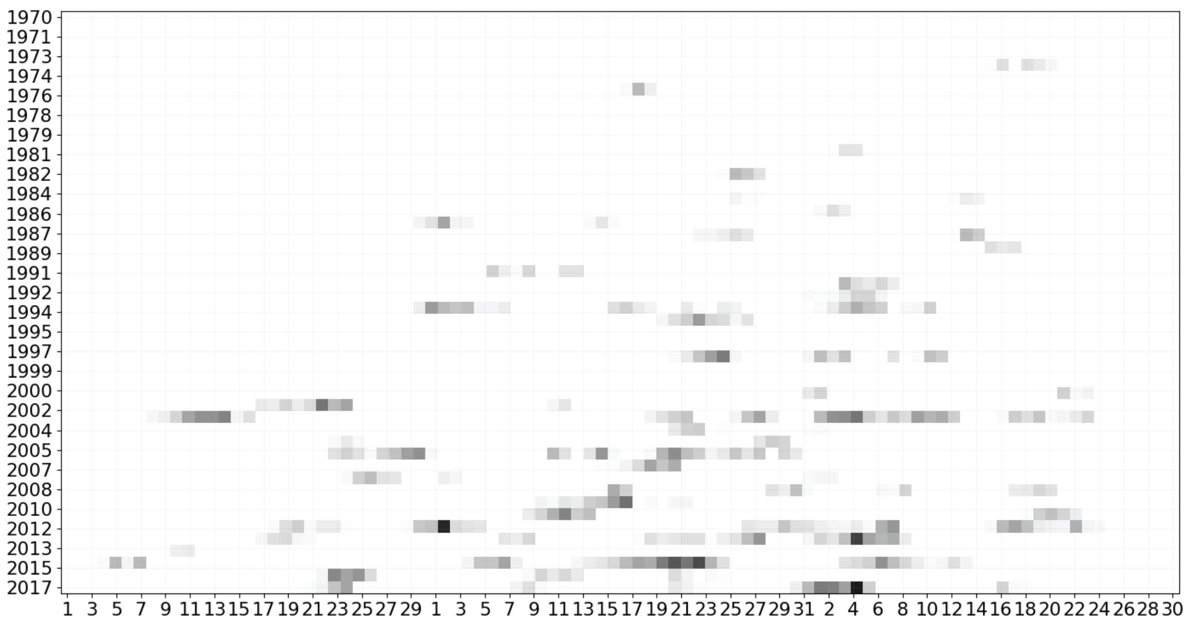

**Slika 2**: Ljubljana od 1. junija do 31. avgusta v obdobju 1961–2017: dnevi v vročinskem valu so obarvani sivo (višja povprečna dnevna temperatura zraka je predstavljena s temnejšo barvo) (različica do leta 2015 objavljena v Kajfež Bogataj in sod., 2018)

**Figure 2**: Ljubljana from June 1 to August 31 in the period 1961–2017: days in heat waves are colored grey (higher average daily air temperature is represented with darker color) (version until 2015 was published in Kajfež Bogataj et al., 2018)

Stanje je podobno v Biljah, ki imajo omiljeno sredozemsko podnebje, kjer je prag za nastop vročinskega vala 1 °C višji kot v omiljenem celinskem podnebju. Poleg leta 1997 so bila tu brez vročinskih valov še leta 1991, 1998, 2000 in 2001 (Slika 3). Tudi tu je bilo najbolj ekstremno leto 2003, vendar z manjšim številom dni v vročinskih valovih kot Ljubljana. Sledijo leta 2012, 2015 in 2013. Podobno kot v Ljubljani, so tudi v Biljah povprečne dnevne temperature zraka v vročinskih valovih v zadnjih letih višje. Na obeh lokacijah je jasno vidno, da se pred letom 1990 vročinski valovi niso pojavljali zgodaj junija in pozno avgusta, v zadnjih dveh desetletjih pa ni to nič nenavadnega. Razpon povprečnih dnevnih temperatur zraka v času vročinskih valov je bil v Ljubljani od 24 do 30 °C (doseženo v letih 2013 in 2017) in v Biljah od 25 do 30 °C (doseženo v letih 2012 in 2017).



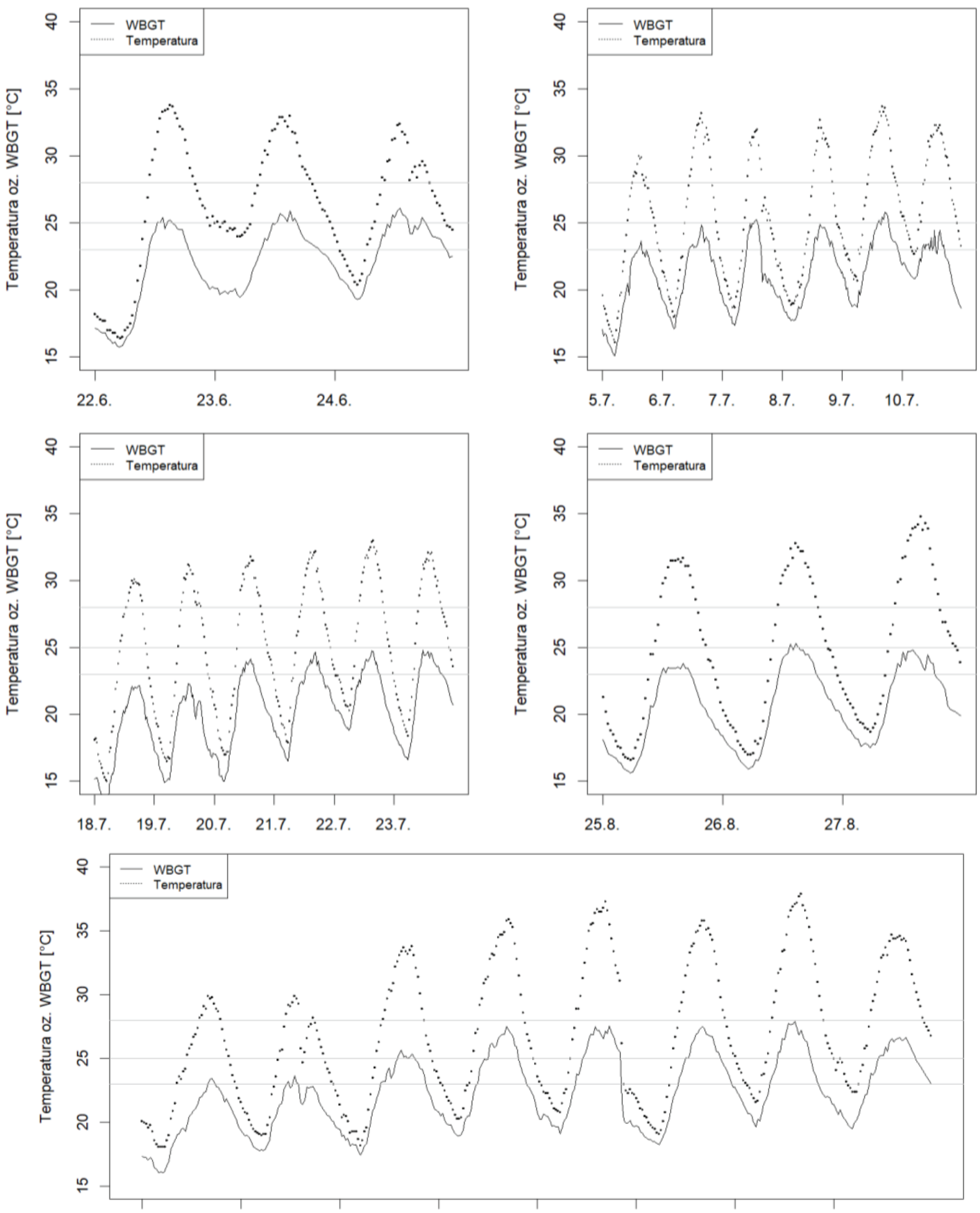

**Slika 3**: Število dni v vročinskih valovih po mesecih in letih v obdobju 1970–2017 v Biljah
**Figure 3**: Number of days within heat waves in months and years in the period 1970–2017 in Bilje




**Slika 4**.: Bilje od 1. junija do 31. avgusta v obdobju 1971–2017: dnevi v vročinskem valu obarvani sivo (višja povprečna dnevna temperatura zraka je predstavljena s temnejšo barvo)
**Figure 4**: Bilje from June 1 to August 31 in the period 1970–2017: days in heat waves are colored grey (higher average daily air temperature is represented with darker color)

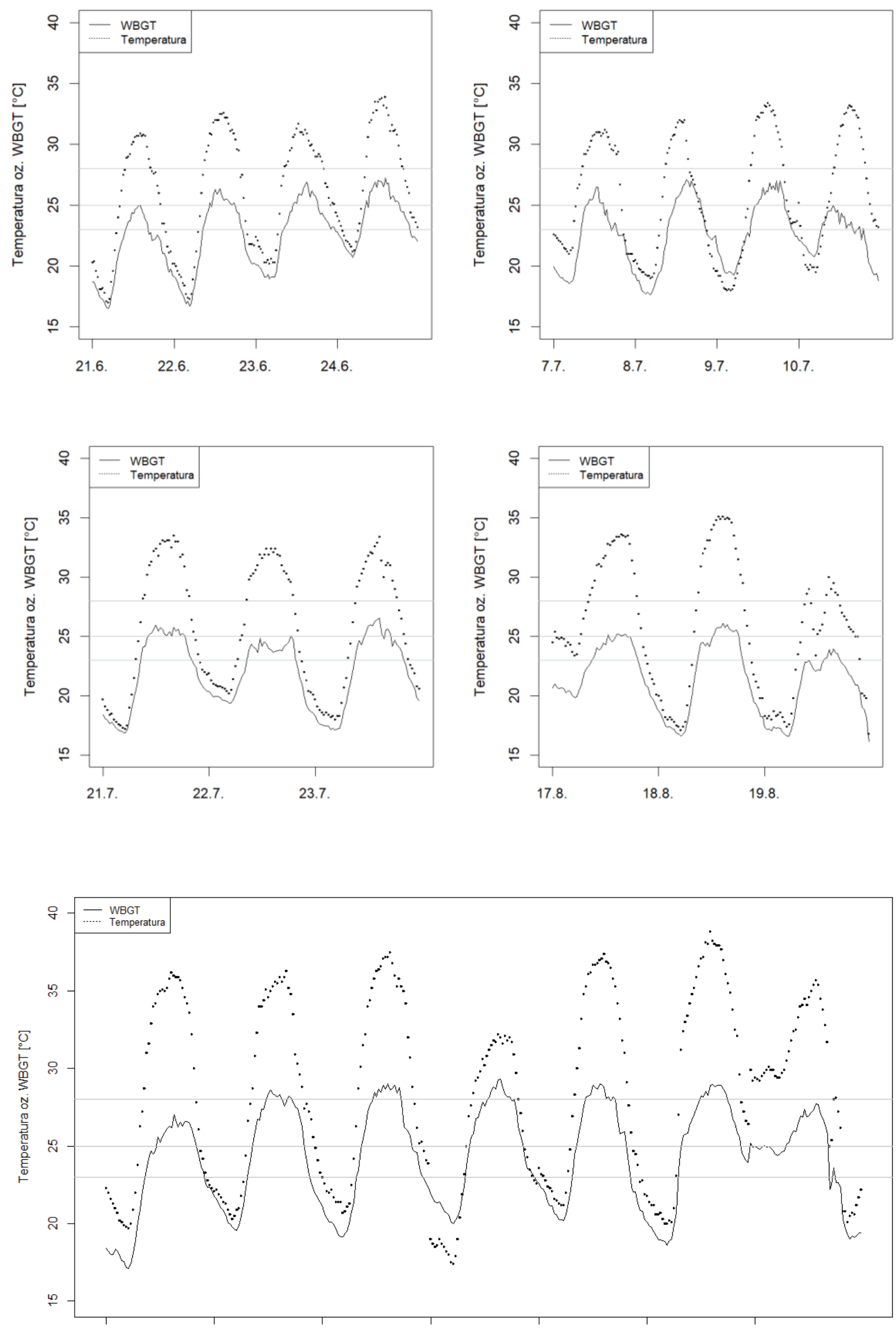
### 3.2 Polurne vrednosti toplotne obremenitve med vročinskim valovi v letu 2017

Leta 2017 je tako v Ljubljani kot v Biljah nastopilo 5 vročinskih valov, dolgih od 3 do 7 (Bilje) oz. 8 (Ljubljana) dni. Za vsak vročinski val smo predstavili izmerjene polurne terminske vrednosti temperature zraka in izračunane vrednosti kazalnika WBGT. Te predstavljajo toplotno obremenitev delavcev, ob preseženih mejnih vrednostih gre za vročinski stres, ki negativno vpliva na počutje, zdravje in produktivnost. Pri različni stopnji fizične obremenjenosti morajo delavci upoštevati različne mejne vrednosti (Preglednica 1). V urah, ko so mejne vrednosti presežene, bi se delavci morali poskušati izogniti delu ali uporabiti omilitvene ukrepe. V Ljubljani (Slika 5) vrednosti kazalnika WBGT niso nikoli presegle mejne vrednosti 28 °C, v najhujšem vročinskem valu (Slika 5 spodaj) so od tretjega dne dalje vsak dan presegle vrednost 25 °C za 6 do 9 ur (4 dni), v šestih dneh za 3 do 4 ure in v dveh dneh za dve uri, z začetkom nekje med 11. in 14. uro. Meja WBGT 23 °C je bila v vseh vročinskih valovih presežena vsaj za nekaj ur, večinoma od 7 do 9 ur, v najhujšem vročinskem valu pa 10 do 15 ur, z začetkom običajno med 9. in 11. uro. V celotnem poletju niso vrednosti WBGT nikoli izven vročinskih valov presegle vrednosti 25 °C.




**Slika 5:** Polurne vrednosti temperature zraka in WBGT med vročinskimi valovi leta 2017 v Ljubljani, z vodoravnimi linijami so označene nekatere mejne vrednosti za kazalnik WBGT iz Preglednice 1 (23 °C, 25 °C in 28 °C, ki za aklimatizirano osebo predstavljajo prag za stopnjo metabolizma več kot 260 W $m^{-2}$, 200 do 260 W $m^{-2}$ in 130 do 200 W $m^{-2}$)

**Figure 5:** Air temperatures and WBGT values every half hour during heat waves in the year 2017 in Ljubljana, reference WBGT values (see Table 1) are marked with horizontal lines (23 °C, 25 °C and 28 °C, for acclimatized person representing threshold for metabolic rates more than 260 W $m^{-2}$, 200 to 260 W $m^{-2}$ and 130 to 200 W $m^{-2}$, respectively

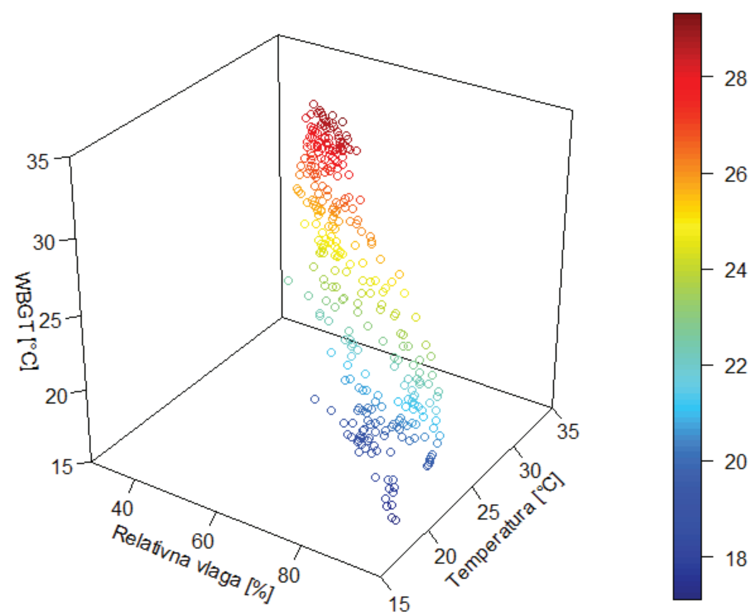



**Slika 6**: Polurne vrednosti temperature zraka in WBGT med vročinskimi valovi leta 2017 v Biljah, z vodoravnimi linijami so označene nekatere mejne vrednosti za kazalnik WBGT iz Preglednice 1 (23 °C, 25 °C in 28 °C, ki za aklimatizirano osebo predstavljajo prag za stopnjo metabolizma več kot 260 W $m^{-2}$, 200 do 260 W $m^{-2}$ in 130 do 200 W $m^{-2}$)

**Figure 6:** Air temperatures and WBGT values every half hour during heat waves in the year 2017 in Bilje, reference WBGT values (see Table 1) are marked with horizontal lines (23 °C, 25 °C and 28 °C, for acclimatized person representing threshold for metabolic rates more than 260 W $m^{-2}$, 200 to 260 W $m^{-2}$ and 130 to 200 W $m^{-2}$, respectively)

Temperature zraka so v času vročinskih valov dnevno močno nihale, tudi za več kot 15 °C, najvišje so segale v Ljubljani od 30 do 38 °C, najnižje pa od 15 do skoraj 24 °C. V Biljah (Slika 6) najnižje temperature zraka na splošno niso bile bistveno višje, morda le kakšno stopinjo, gibale so se v podobnem intervalu, najvišja dosežena temperatura zraka pa je bila 39 °C. Temperatura zraka in vlaga sta bili v Biljah glede na Ljubljano toliko bolj obremenilni, da je bila v vseh dneh v vročinskih valovih razen dveh presežena tudi mejna vrednost WBGT 25 °C. Mejna vrednost 23 °C je bila v času vročinskih valov v Biljah presežena večinoma nekje od 8. oziroma 9. ure dalje od 10 do 11 ur dnevno (tudi do 14, izjemoma po en dan 4 in 6 ur), enkrat pa se je celo zgodilo, da se ni prekinila od 7. ure zjutraj do 16. ure naslednjega dne. Meja 25 °C je bila večinoma presežena nekje od 9. do 12. ure dalje, največkrat za več kot 8 ur. Poleg tega pa je bila v Biljah v najhujšem vročinskem valu v začetku avgusta v petih dneh za vsaj pet ur med 12. in 17. uro (izjemoma med 10:30 in 17:30) presežena tudi mejna vrednost 28 °C.

Za lažjo predstavo je na primeru četrtega (najdaljšega) vročinskega vala v Biljah predstavljena odvisnost kazalnika WBGT od temperature zraka in relativne vlage (Slika 7). Največje vrednosti je kazalnik dosegal ob visokih temperaturah zraka in majhni vlagi, za manjše vrednosti WBGT pa se snop zlagoma širi proti večjim vrednostim vlage in manjšim vrednostim temperature zraka. V času vročinskih valov je bil v vseh primerih precej tipičen potek, da se je relativna vlaga z dviganjem temperature zraka tekom dneva zmanjševala in obratno.




**Slika 7**: Polurne vrednosti WBGT v odvisnosti od temperature zraka in relativne vlage med 4. vročinskim valom leta 2017 v Biljah
**Figure 7**: WBGT values vs. air temperature and relative humidity every half hour during 4th heat wave in the year 2017 in Bilje

## 4 DISKUSIJA

Z naraščanjem temperature zraka se opazno povečuje število dni v vročinskih valovih in njihova intenzivnost, podaljšuje pa se tudi časovni razpon, v katerem se pojavljajo, kar potrjuje mnogo različnih raziskav kljub različnim definicijam vročinskih valov (npr. Morabito et al., 2017; Russo et al., 2015; Bittner et al., 2013; Kuglitsch in sod., 2010). Povprečna dnevna temperatura zraka je v Sloveniji uporabljena kot prag za določanje nastopa vročinskega vala, saj njena vrednost odraža informacijo o najnižji in najvišji temperaturi zraka ter relativni vlagi. Je dobra mera za jakost toplotne obremenitve, saj bo približno enaka za dolgotrajno obremenitev pri nekoliko nižjih temperaturah (majhna amplituda dnevnega temperaturnega hoda v vlažni zračni masi) ali kratkotrajno obremenitev pri višjih temperaturah z vmesno osvežitvijo (velika amplituda dnevnega temperaturnega hoda v suhi zračni masi) (Ključevšek in sod., 2018). Tako nam pove več kot le najvišje dnevne temperature zraka, kljub vsemu pa ljudje po naravi nimamo občutka za povprečje in v običajnem poletnem dnevu težko ocenimo, kakšna je bila povprečna dnevna temperatura zraka. Svojo ogroženost dojemamo na podlagi najvišjih dnevnih temperatur zraka in občutka, da nam je vroče. Pri rezultatih lahko približno ocenimo, da WBGT preseže 23 °C pri temperaturah zraka okoli 30 °C, medtem ko preseže 25 °C običajno pri temperaturah zraka okoli 32 °C, če ostaja vlaga v normalnih okvirih.

Za kmetijska opravila se glede na težavnost dela stopnja metabolizma giblje od 150 do 500 W $m^{-2}$ (Preglednica 2). Lundgren in sod. (2014) so iz srčnega utripa in opazovanja štirih delavcev v Indiji za pripravo zemlje za obdelavo, sejanje, zalivanje, pletje, kontrolo škodljivcev, gnojenje, vzdrževanje rastlin, žetev ter vmesno hojo in sklanjanje ocenili stopnjo metabolizma na 190 W $m^{-2}$. Pri lažjih delih, kot je oranje s traktorjem ali zgoraj našteto delo, lahko za mejo nevarne ogroženosti z vročinskim stresom upoštevamo mejo pri vrednosti WBGT 28 °C (Preglednica 1), ki v Ljubljani v letu 2017 ni bila presežena, v Biljah pa le v najhujšem vročinskem valu po vsaj 5 ur dnevno, vedno vsaj med 12. in 17. uro. Pri nekoliko bolj zahtevnih delih, kot je na primer skobljanje lesa ali podiranje drevesa z motorno žago, je meja za kazalnik WBGT nižja – v mirnem ozračju 25 °C (z vetrom 26 °C) za aklimatizirane delavce in še nižja 22 °C (oz. 23 °C) za ne-aklimatizirane delavce. V večini dni v vročinskih valovih je bila leta 2017 tako v Ljubljani kot v Biljah meja 23 °C presežena praktično cel dan, kar za delavce pomeni visoko stopnjo tveganja zaradi obremenitve z vročinskim stresom. Pri zelo napornem fizičnem delu, kot je kopanje z lopato ali sekanje, je stanje v mirnem ozračju, ko kazalnik WBGT preseže 23 °C, nevarno že za aklimatizirane delavce (z vetrom pri 25 °C), za ne-aklimatizirane pa se lahko težave pojavijo že pri preseženih vrednostih 18 oz. 20 °C. Pri preseženih mejnih vrednostih WBGT bi morali delodajalci oz. kmetje sami uvesti preventivne ukrepe, kot so reorganizacija dela, več odmorov na hladnem, več zaužite tekočine ipd., da zmanjšajo tveganje vročinskega stresa (Staal Wästerlund, 2018).

**Preglednica 2**: Ocene stopnje metabolizma za nekaj kmetijskih opravil po ISO standardu (2004) (Staal Wästerlund, 2018)
**Table 2**: Estimates of metabolic rate for agricultural activities, according to ISO (2004) (Staal Wästerlund, 2018)

| Opravilo | Stopnja metabolizma M [W $m^{-2}$] |
|---|---|
| Sekanje (2 kg težka sekira, 33 udarcev na minuto) | 500 |
| Kopanje z lopato (24 dvigov na minuto) | 380 |
| Podiranje drevesa z motorno žago | 235 |
| Skobljanje lesa | 225 |
| Obrezovanje sadnega drevja | 205 |
| Prenašanje tovora (10 kg pri hitrosti hoje 10 km/h) | 185 |
| Grabljenje listja | 170 |
| Oranje s traktorjem | 170 |

Za primerjavo, delavci na poljih sladkornega trsa v Kostariki v precej bolj vročem podnebju so v letih 2010 in 2011 delali pri vrednostih WBGT med 20 °C in 34 °C (Crowe in sod., 2013). Sahu in sod. (2013) so ugotovili, da v prvi uri pobiranja riža pri vrednostih WBGT 27 °C delavci v povprečju poberejo 86 svežnjev, pri vrednostih 31 °C pa le še 65. V raziskavi na Cipru so delavci v vinogradu tekom poletja 87 % časa delali pri vrednostih WBGT, višjih od 25 °C (Ioannou in sod., 2017).

Za zagotavljanje normalnega delovanja organov moramo telesno temperaturo ohranjati na 37 °C, najbolj običajen način hlajenja je potenje. Raziskave so pokazale, da se ženske potijo manj kot moški, potiti pa se začnejo pri višji telesni temperaturi, zato je pri njih večje tveganje za simptome vročinskega stresa ali z vročino izzvane bolezni (Staal Wästerlund, 2018). To se je potrdilo tudi v raziskavi med slovenskimi delavci v kmetijstvu, kjer so ženske v večjem deležu navajale težave zaradi vročinskega stresa (Pogačar in sod., 2017). Razpon potrebne količine popite tekočine je zelo velik – od dveh litrov na dan pri lahkem delu in vrednostih WBGT okoli 10 °C do 15 litrov pri izredno napornem delu in vrednostih WBGT okoli 30 °C (Staal Wästerlund, 2018).

Dodatno toplotno obremenitev, ki je kazalnik WBGT ne prikaže, pomenijo pretople, t.i. tropske noči, ko se tudi ponoči temperatura zraka ne spusti pod 20 °C. Takrat se delavci med nočnim počitkom večinoma slabo odpočijejo in naslednji dan že začnejo utrujeni. Zanimivo je, da se je v Ljubljani to zgodilo že po prvem dnevu prvega vročinskega vala leta 2017, ko je bila najnižja 24-urna temperatura zraka 23,8 °C. Dve tropski noči sta bili še ob koncu drugega vročinskega vala, ena predzadnji dan tretjega ter štiri z vmesno prekinitvijo ob koncu četrtega vala. V Biljah sta bili tropski zadnji dve noči prvega vročinskega vala, zadnja noč drugega, druga noč tretjega in prva noč petega vročinskega vala. V najhujšem, četrtem vročinskem valu je bilo kar pet tropskih noči. Številne raziskave so pokazale, da nočne temperature zraka naraščajo hitreje kot dnevne, prav tako modeli za prihodnost napovedujejo, da se bo ta trend nadaljeval (Peng in sod., 2013). Tudi za Slovenijo velja, da se zadnja leta pojavljajo rekordno visoke nočne temperature (Sušnik in Pogačar, 2011; Vertačnik, 2014), kar za delavce pomeni še dodatno toplotno obremenitev, ki jo bo v nadaljnjih raziskavah potrebno upoštevati.

## 5 SKLEPI

Analiza vročinskih valov za obdobje 1961–2017 za Ljubljano in Bilje je pokazala, da je število dni v vročinskih valovih začelo močno naraščati po letu 1990 na obeh lokacijah. Prav tako se je povečala intenziteta vročinskih valov, saj povprečne dnevne temperature zraka dosegajo precej večje vrednosti kot v prvi polovici obravnavanega obdobja. Podaljšuje se časovni razpon, v katerem se pojavljajo, na obeh lokacijah je jasno vidno, da se pred letom 1990 vročinski valovi niso pojavljali zgodaj junija in pozno avgusta, v zadnjih dveh desetletjih je takšna časovna razporeditev postala običajna. Leta 2017 je tako v Ljubljani kot v Biljah nastopilo 5 vročinskih valov, dolgih od 3 do 7 (Bilje) oziroma 8 (Ljubljana) dni, za katere smo izračunali kazalnik WGBT za oceno tveganja vročinskega stresa pri delavcih v kmetijstvu.

Nekatere mejne vrednosti so redno presežene tudi v Sloveniji, torej je primerno, da kazalnik WBGT uporabimo za prikaz toplotne obremenitve delavcev. V Ljubljani vrednosti kazalnika WBGT sicer niso nikoli presegle mejne vrednosti 28 °C (meja za običajno kmetijsko delo), v najhujšem vročinskem valu so presegle vrednost 25 °C (težje delo), v vseh vročinskih valovih pa vsaj za nekaj ur 23 °C (fizično izredno naporno delo). Temperatura zraka in vlaga sta bili v Biljah glede na Ljubljano toliko bolj obremenilni, da je bila skoraj v vseh dneh v vročinskih valovih presežena tudi mejna vrednost WBGT 25 °C. Poleg tega je bila v Biljah v najhujšem vročinskem valu v začetku avgusta v petih dneh za vsaj pet ur (med 12. in 17. uro) presežena tudi mejna vrednost 28 °C. Pri preseženih mejnih vrednostih WBGT bi morali delodajalci oziroma kmetje sami uvesti preventivne ukrepe za zmanjšanje tveganja vročinskega stresa, na primer reorganizirati delo, uvesti več rednih odmorov na hladnem, piti več tekočine in nositi primerna oblačila.

## 6 ZAHVALA

Raziskava je bila finančno podprta s strani okvirnega programa EU za razvoj in inovacije Obzorje 2020 s pogodbo št. 668786. Zahvaljujemo se prof. Larsu Nybu, Andreasu Flourisu in Tordu Kjellstromu za vodenje projekta.

## 7 VIRI

ARSO. (2013). *Podnebna spremenljivost Slovenije: Glavne značilnosti gibanja temperature zraka v obdobju 1961-2011.* Dostopno na: http://meteo.arso.gov.si/uploads/probase/www/climate/text/sl/publications/PSS-Glavne-znacilnosti-gibanja-temperature-zraka-1961-2011.pdf

ARSO. (2017). *Ocena podnebnih sprememb v Sloveniji do konca 21. stoletja: Povzetek temperaturnih in padavinskih povprečij*. Dostopno na: http://meteo.arso.gov.si/uploads/probase/www/climate/text/sl/publications/povzetek-podnebnih-sprememb-temp-pad.pdf

Bernard, T. E., Pourmoghani, M. 1999. Prediction of Workplace Wet Bulb Global Temperature. *Applied Occupational and Environmental Hygiene, 14*, 126-134. doi:10.1080/104732299303296

Bittner, M.I., Matthies, E.F., Dalbokova, D., Menne, B. (2013) Are European countries prepared for the next big heat-wave? *European Journal of Public Health, 24*(4), 615-619. doi:10.1093/eurpub/ckt121

Crowe, J., Wesseling, C., Roman Solano, B., Umana, M.P., Ramírez, A.R., Kjellstrom, T., Morales, D., Nilsson, M. (2013). Heat exposure in sugarcane harvesters in Costa Rica. *American Journal of industrial Medicine*, *56*(10), 1157-1164. doi:10.1002/ajim.22204

d'Ambrosio Alfano, F.R., Palella, B.I., Riccio, G., Malchaire, J. (2016). On the Effect of Thermophysical Properties of Clothing on the Heat Strain Predicted by PHS Model. *The Annals of Occupational Hygiene*, *60*(2), 231-251. doi:10.1093/annhyg/mev070

Flouris, A. D., McGinn, R., Poirier, M. P., Louie, J. C., Ioannou, L. G., Tsoutsoubi, L., Sigal, R. J., Boulay, P., Hardcastle, S. G., Kenny, G. P. (2018). Screening criteria for increased susceptibility to heat stress during work or leisure in hot environments in healthy individuals aged 31–70 years. *Temperature, 5,* 86-99. doi:10.1080/23328940.2017.1381800

Gao, C., Kuklane, K., Östergren, P. O., Kjellstrom, T. (2018). Occupational heat stress assessment and protective strategies in the context of climate change. *International Journal of Biometeorology, 62,* 359-371. doi:10.1007/s00484-017-1352-y

Gubernot, D.M., Andersson, G.B., Hunting, K.L. (2015). Characterizing occupational heatrelated mortality in the United States, 2000-2010: An analysis using the census of fatal occupational injuries database. *American Journal of Industrial Medicine, 58*(2)*,* 203-211. doi:10.1002/ajim.22381

Ioannou, L. G., Tsoutsoubi, L., Samoutis, G., Kajfez Bogataj, L., Kenny, G.P., Nybo, L., Kjellstrom, T., Flouris, A. D. (2017). Time-motion analysis as a novel approach for evaluating the impact of environmental heat exposure on labor loss in agriculture workers. *Temperature, 4,* 330-340. doi:10.1080/23328940.2017.1338210

Kajfež Bogataj, L., Katkić, V., Pogačar, T. (2018). Vpliv podnebnih sprememb na povečano rabo energije za klimatizacijo. V: Lipič, K. (ur.), Rižnar, K. (ur.). *Nacionalni program varstva okolja in njegov dialog z lokalnimi skupnostmi : strokovno posvetovanje 2018, Moravske Toplice, Hotel Ajda, 5. in 6. april 2018*. Ljubljana: Zveza ekoloških gibanj Slovenije, str. 145-152.

Kjellstrom, T., Holmer, I., Lemke, B. (2009). Workplace heat stress, health and productivity - an increasing challenge for low and middle income countries during climate change. *Global Health Action, 2*(1)*,* 2047. doi:10.3402/gha.v2i0.2047

Kjellstrom, T., Freyberg, C., Lemke, B., Otto, M., Briggs, D. (2018). Estimating population heat exposure and impacts on working people in conjunction with climate change. *International Journal of Biometeorology, 62*(3)*,* 291-306. doi:10.1007/s00484-017-1407-0

Ključevšek, N., Hrabar, A., Dolinar, M. (2018). Podnebne podlage za definicijo vročinskega vala. *Vetrnica,* 10, 44-53.

Kuglitsch, F.G., Toreti, A., Xoplaki, E., Della-Marta, P.M., Zerefos, C.S., Türkeş, M., Luterbacher, J. (2010). Heat wave changes in the eastern Mediterranean since 1960. *Geophysical Research Letters, 37*. doi:10.1029/2009GL041841

Lemke, B., Kjellstrom, T. (2012). Calculating Workplace WBGT from Meteorological Data: A Tool for Climate Change Assessment. *Industrial Health, 50*(4)*,* 267-278. doi:10.2486/indhealth.MS1352

Lundgren, K., Kuklane, K., Venugopal, V. (2014). Occupational heat stress and associated productivity loss estimation using the PHS model (ISO 7933): a case study from workplaces in Chennai, India. *Global Health Action, 2014* (7). doi:10.3402/gha.v7.25283

McPherson, M. J. (2008). Subsurface Ventilation and Environmental Engineering, 2nd Ed., Ch. 17. *Physiological reactions to climatic conditions*. Mine Ventilation Services Inc., Clovis. http://www.mvsengineering.com/index.php?cPath=25

Morabito, M., Crisci, A., Messeri, A., Messeri, G., Betti, G., Orlandini, S., Raschi, A., Maracchi, G. (2017). Increasing Heatwave Hazards in the Southeastern European Union Capitals. *Atmosphere, 8*(7)*,* 115. doi:10.3390/atmos8070115

Peng S., Piao S., Ciais P., Myneni R.B., Chen A., Chevallier F., Dolman A.J., Janssens I.A., Penuelas J., Zhang G., Vicca S., Wan S., Wang S., Zeng H. 2013. Asymmetric effects of daytime and night-time warming on Northern Hemisphere vegetation. Nature, 501, 88-92. doi:10.1038/nature12434

Pogačar, T., Črepinšek, Z., Kajfež Bogataj, L., Nybo, L. (2017). Comprehension of climatic and occupational heat stress amongst agricultural advisers and workers in Slovenia. *Acta Agriculturae Slovenica, 109*(3)*,* 545-554. doi:10.14720/aas.2017.109.3.06

Pogačar, T., Casanueva, A., Kozjek, K.,Ciuha, U., Mekjavić, I., Kajfež Bogataj, L., Črepinšek, Z. (2018). The effect of hot days on occupational heat stress in the manufacturing industry: implications for workers' well-being and productivity. *International Journal of Biometeorology.* doi:10.1007/s00484-018-1530-6

Russo, S., Sillman, J., Fischer, E.M. (2015). Top ten European heatwaves since 1950 and their occurrence in the coming decades. *Environmental Research Letters, 10*(12)*,* 124003. doi:10.1088/1748-9326/10/12/124003

Sahu, S., Sett, M., Kjellstrom, T. (2013). Heat exposure, cardiovascular stress and work productivity in rice harvesters in India: implications for a climate change future. *Industrial Health*, *51*(4)*,* 424-431. doi:10.2486/indhealth.2013-0006

Staal Wästerlund, D. (2018). Managing heat in agricultural work: increasing worker safety and productivity by controlling heat exposure. *Forestry Working Paper No. 1*. Rome, FAO. 53 str.

Sušnik, A., Pogačar, T. 2011. Vremensko povzročeni stresi kmetijskih rastlin v letu 2011. Ujma, 25, 81-92.

Vertačnik, M. (2014) *Ekstremne temperature in njihova spremenljivost v Sloveniji v obdobju 1961-2013*. Diplomska naloga. Ljubljana, Biotehniška fakulteta, oddelek za agronomijo: 36 str.

doi:10.14720/aas.2018.111.3.14 Original research article / izvirni znanstveni članek

# Correlation and path coefficient analysis among seed yield and yield related traits of Ethiopian chickpea (*Cicer arietinum* L.) landraces

Awol MOHAMMED*, Asnake FIKRE[1]

Received July 13, 2018; accepted November 23, 2018.
Delo je prispelo 13. julija 2018, sprejeto 23. novembra 2018.

## ABSTRACT

The experiment was done on 202 new chickpea (*Cicer arietinum* L.) landraces with 2 checks to assess the association, direct and indirect effect of different characters on yield. The experiment was planted at Sirinka and Jari, Ethiopia, under rain fed condition in 2016 using alpha lattice design with three replications. Data were collected on yield and yield related traits. Analysis of variance showed highly significant differences among genotypes. The correlation of grain yield with biomass and with harvest index was positive and highly significant both at genotypic and phenotypic levels. In addition, its association with pod filling period, plant height, secondary branches and hundred seed mass was positive but unsignificant both at genotypic and phenotypic levels. Path coefficient analysis at genotypic level showed that among the 15 causal (independent) traits; biomass, harvest index, pod length, days to pod setting, pod filing period, canopy width, primary branches, secondary branches, and number of pods per plant had positive and directly influence on grain yield. Although the days to flowering, plant height and hundred seed mass had positive genotypic correlation with grain yield. In general correlation coupled with path coefficient analysis revealed that biomass and harvest index had a direct relationship with seed yield.

**Key words:** chickpea; correlation; path coefficient analysis; seed yield; yield related traits

## IZVLEČEK

### ANALIZA ODVISNOSTI PRIDELKA SEMENA ETIOPSKIH LOKALNIH ZVRSTI ČIČERKE (*Cicer arietinum* L.) OD S PRIDELKOM POVEZANIH LASTNOSTI

Poskus je potekal na 202 novih lokalnih zvrsteh čičerke (*Cicer arietinum* L.) z dvema preiskusoma za ovrednotenje neposredne in posredne povezave učinkov različnih lastnosti na pridelek. Poskus je bil izveden leta 2016 v krajih Sirinka in Jari v Etiopiji v razmerah brez namakanja kot nepopolni bločni poskus s tremi ponovitvami. Zbrani so bili podatki o pridelku in z njim povezanimi lastnostmi. Analiza variance je pokazala zelo značilne razlike med genotipi. Povezava med pridelkom z biomaso in žetvenim indeksom je bila pozitivna in zelo značilna na genotipski in fenotipski ravni. Dodatno je bila povezava pridelka pozitivna z lastnostmi kot so obdobje polnjenja strokov, višina rastlin, število sekundarnih poganjkov in masa stotih semen a nestatistično značina, ne na genotipski niti na fenotipski ravni. Analiza povezav na genotipski ravni je pokazala, da ima 15 znakov (lastnosti) kot so biomasa, žetveni indeks, dolžina stroka, število dni do nastavka strokov, obdobje polnjena strokov, širina nadzemnega dela rastlin, število primarnih in sekundarnih stranskih pogankov in število strokov na rastlino pozitivni in neposredni vpliv na pridelek semena. Tudi lastnosti kot so število dni do cvetenja, višina rastlin in masa stotih semen so imele pozitivne genetske povezave s pridelkom zrnja. Nasplošno je povezava med znaki povezana s povezavo med posameznimi znaki odkrila, da sta imela biomasa in žetveni indeks neposredno povezavo s pridelkom semena.

**Ključne besede:** čičerka; korelacija; analiza odvisnosti posameznih znakov; pridelek semena; s pridelkom povezani znaki

[1] Sirinka Agricultural Research Center, P. O.Box 74, Woldia, Ethiopia; *Corresponding author: mawol50@yahoo.com

## 1 INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most important pulse crop in the world, after dry common bean and field pea (Padmavathiv et al., 2013). Southeast Turkey and Syria are considered as the two primary centers of origin of chickpea (Singh et al., 1997). The world chickpea production reached 13.3 million tons in 2013 and 14.2 million tons in 2014 (FAO, 2014). India is the largest chickpea producing country accounting for 72 % of the global chickpea production (Ojiewo, 2016). Ethiopia is considered as one of the secondary centers of diversity for chickpea (van der Maesen, 1987). In Ethiopia, chickpea is the third largest legume crop in area and production (CSA, 2015).

There are two types of chickpea depending on seed color, shape, and size. The Kabuli type has large, round or ram head and cream-colored seeds, and is grown in temperate regions (ICRISAT, 2010). The Desi type chickpea is grown in the semi-arid tropics and is characterized by relatively small angular shaped seeds with light brown, yellowish or black colour.

Chickpea is very important due to its good nutritional value having an average of 4.5 % fat, 8 % crude fiber, 22 % protein, 63 % carbohydrate and 2.7 % ash (Shafiqueet al., 2016). Besides being an important source of human food and animal feed, it is also an important contributor to soil fertility as it provides nitrogen to soil through fixation of atmospheric nitrogen (Gul et al., 2011).

Many international agreements proclaim food security which implies the conservation of plant genetic resources for food and agriculture. The landraces or farmer varieties are important source of the genetic diversity and potential material that could be used to broaden the base for plant breeding. An indigenousness landrace is a variety with a high capacity to tolerate biotic and abiotic stress, resulting in high yield stability and an intermediate yield level under a low input agricultural system (FAO, 1998). Ethiopia has a large number of chickpea landraces cultivated by the farmers through traditional method of selection over a long time. This provides the basic material for developing any variety or hybrid.

Study of yield and yield components provide a basic framework for selecting useful characters in chickpea improvement programs. Seed yield is an important character that is polygenic in nature and significantly influenced by environmental conditions (Singh et al., 2014). Most of plant breeders are interested in maximizing selection efficiency that supports the identification of best genotypes. Estimation of correlation coefficient is useful in planning future breeding and provides a measure of association among traits, which could be useful as a selection guide. The path coefficient analysis enables to determine the direct and indirect contribution of various traits toward yield. Correlation analysis provides information of associations among yield components. Path coefficient analysis permits the separation of the correlation coefficient into component of direct and indirect effects and to measure the relative importance of each (Singh and Chaudhary, 1977; Sharma, 1998). Therefore the objective of this study was to determine the association of different characters with seed yield, direct and indirect influence of characters towards yield and yield contributing traits and assess magnitude to define seed yield.

## 2 MATERIALS AND METHODS

### 2.1 Description of Experimental Sites

The experiment was carried out under rain fed condition at Sirinka and Jari Agricultural stations, Ethiopia. The former one is located at $11^0$ 45' North latitude and $39^0$ 36' 36'' East longitudes. Its altitude is 1850 meter above sea level located in North Wollo Zone. The annual rainfall of this site is 1006.3mm with 13.6 $^0$C minimum and 26.7 $^0$C maximum temperature. Jari, is located at $11^0$ 21' North latitude and $39^0$ 47' East longitudes and at an altitude of 1680 meter above sea level in South Wollo Zone. The annual rainfall of this site is 987.3 mm with 14.2 $^0$C minimum and 28.7 $^0$C maximum temperature. According to Sirinka Agricultural Research Center soil classification (unpublished), the soils of the sites are classified advertises.

### 2.2 Experimental Materials and Design

A total of 202 newly collected Desi type landraces from Amhara, Oromiya and SNNP Regional States were used for this study. The collecting expedition was done on elevations ranging from 1174 to 2660meter above sea level. The collections were made in 2013 (N = 42) and 2016 (N = 160). This puts 90 landraces from Amhara region, 91 from Oromiyaand 24 from SNNP. A total of 202 collected landraces with two released varieties as checks, 'Fetenech' (early maturing) and 'Minjar' (high yielder) were tested and characterized for morphological traits. The experiment was planted on 02 September 2016 by using alpha lattice designwith three replications. Each landrace was sown in two rows at 60 cm, 30 cm, and 10 cm spacing between plots, rows, and plants, respectively; with 1 m row length. All agronomic practices were done uniformly for all accessions as required. There was no fertilizer application. For controlling pod borer Karatewas sprayed at the rate of 200 ml 300 liter$^{-1}$ha$^{-1}$ and picked by hand.

### 2.3 Data Collection

The data of morphological, phenological, and agronomical traits were collected during the growth period of the crop, depending on the descriptors for chickpea (IBPGR, ICRISAT and ICARDA, 1993). The data of plant height, stem colour, number of leaflets per leaf, plant canopy width, number of primary branches, number of secondary branches, pod length, number of pods per plant, number of seeds per pod, days to 50 % flowering, days to 50 % pod setting, pod filling period, days to 75 % maturity, total biomass, hundred seed mass, seed yield, harvest index, seed coat colour, seed shape, diseases and insect damage score (1-9) and seed testa texture were collected.

### 2.4 Analysis of variance

Analysis of variance (ANOVA) was performed for the quantitative data using SAS computer software (SAS, 2004) as per the following linear model for alpha lattice design.

$Y_{ijk} = \mu + R_i + B_{ij} + T_k + e_{ijk}$:where, μ = the grand mean of trait Y; $R_i$ = the effect of replicate I; $B_{ij}$ = effect of block j within replicate I; $T_k$ = effect of treatment k

### 2.5 Phenotypic and genotypic correlation coefficients

Phenotypic and genotypic correlation were estimated using the formula suggested by Miller et al. (1958)

Phenotypic correlation was computed as:

$$r_{pxy} = \frac{\sigma^2 pxy}{\sqrt{(\sigma^2 px)(\sigma^2 py)}}$$

Genotypic correlation was computed as:

$$r_{gxy} = \frac{\sigma^2 gxy}{\sqrt{(\sigma^2 gx)(\sigma^2 gy)}}$$

Where $r_{pxy}$ is phenotypic correlation coefficient and $r_{gxy}$ is genotypic correlation coefficient between characters x and y; $\sigma^2$ pxy and $\sigma^2$ gxy are phenotypic covariance and genotypic covariance between characters x and y, respectively. $\sigma^2$ px and $\sigma^2$ gx are phenotypic and genotypic variances for character x and $\sigma^2$ py and $\sigma^2$ gy are phenotypic and genotypic variances for character y.

The coefficient of correlation at phenotypic level was tested for its significance with table for simple correlation coefficient using n-2 df as suggested by Gomez and Gomez (1984) or using 't' table, with observed t expressed as

$$t = \frac{r_{pxy}\sqrt{n-2}}{\sqrt{1-r^2{}_{pxy}}}$$

The calculated 't' value was compared with the tabulated 't' value at n-2 degree of freedom, (n = 204) at 5 % and 1 % level of significance (where n is the number of genotypes).

The coefficient of correlation at genotypic level was tested according to Robertson (1959);

$$t = \frac{r_{gxy}}{SEr_{gxy}}$$

where, $r_{gxy}$ = genotypic correlation coefficient,

$SEr_{gxy}$ = standard error of genotypic correlation coefficient

$$SEr_{gxy} = \sqrt{\frac{(1-r^2 gxy)^2}{2h_1{}^2 h^2{}_2}}$$

Where $h^2{}_1$ and $h^2{}_2$ are broad sense heritability for character 1 and 2

The calculated 't' value was compare with the 't' tabulated value at n-2 (df = 202) at the 5 % and 1 % level of significance (where n is the number of accessions).

### 2.6 Path coefficient analysis

Associations of yield with its components were determined by the application of correlation and path analysis. The use of path analysis requires a cause and effect situation among the variables. Path coefficient analysis was calculated using the formula suggested by Dewey and Lu (1959) to assess direct and indirect effects of different traits on grain yield as:

$$r_{ij} = p_{ij} + \sum r_{ik}\ p_{kj}$$

Where $r_{ij}$ is mutual association between the independent traits (i) and the dependent trait (j) as measured by the correlation coefficient, $p_{ij}$ is component of direct effect of the independent trait (i) on the dependent variable (j); and $r_{ik}$ $p_{kj}$ is the components of indirect effect of a given independent trait (i) on the dependent traits (j) via all other independent traits (k). The residual effect (U) which is the unexplained variation of the trait that is not accounted for by path coefficient and is calculated using the formula of Dewey and LU (1959) as:

$$U = \sqrt{1-R^2}\text{ , where } R^2 = \sum r_{ik}\, p_{kj}$$

## 3 RESULTS AND DISCUSSION

### 3.1 Analysis of variance

Analysis of variance revealed highly significant differences (P < 0.001) among genotypes for most of the studied traits indicating genetic variability in the characters studied (Table 1).The magnitude of mean squares due to genotypes was high for grain yield, biomass yield, harvest index and number of pods per plant; while low genotype mean square values exhibited for number of seed per pod, primary branches per plant, pod length, and number of leaflets per leaf (Table 1).Similarly, previous studies on chickpea landraces also reported by (Tesfamickael et al., 2014; Uday et al., 2012).

**Table 1:** Mean squares, significance and CV % of morpho - agronomic characters of chickpea germplasm

| Traits | Mean square (CV %) | | |
|---|---|---|---|
| | Sirinka | Jari | Combined |
| DF | 24.25**(6.56) | 23.73**(5.04) | 4.24 $^{ns}$(8.2) |
| DP | 31.23**(6.46) | 15.06**(4.89) | 9.73 $^{ns}$(4.98) |
| PFP | 18.70**(6.07) | 24.54**(5.02) | 5.99 $^{ns}$(6.23) |
| DM | 42.32**(4.28) | 22.08**(5.68) | 5.39 $^{ns}$(4.52) |
| CW | 38.83**(8.15) | 122.25**(11.5) | 14.03*(22.31) |
| NLtL | 0.87**(5.97) | 3.04*(15.78) | 0.79*(16.23) |
| PH | 31.12**(9.85) | 96.27**(17.60) | 8.61**(20.13) |
| PB | 0.72**(19.65) | 0.05$^{ns}$(21.5) | 0.11$^{ns}$(28.9) |
| SB | 11.11**(17.16) | 0.62$^{ns}$(20.42) | 1.88*(25.61) |
| NPP | 520.46**(21.02**)** | 267.32*(28.6) | 50.90**(30.2) |
| NSP | 0.08 $^{ns}$ (12.06) | 0.02$^{ns}$(15.23) | 0.029 $^{ns}$(11.6) |
| PL | 0.24**(26.5) | 0.65*(29.42) | 0.25*(31.2) |
| HSW | 8.34**(13.23) | 6.27**(12.52) | 0.43$^{ns}$(8.96) |
| GYKH | 441140.22**(28.66) | 393086.47**(24.56) | 23558.56$^{ns}$ (30.2) |
| BMKH | 1936174.5**(25.97) | 1734469.2*(29.62) | 123242.6$^{ns}$(32.21) |
| HI | 240.00**(21.48) | 189.23* (18.96) | 1.42$^{ns}$(28.9) |

DF = Days to flowering, DP = Days to pod setting, PFP = Pod filling period, DM = Days to maturity, CW = Canopy width, NLtL = Number of leaflets per leaf, PH = Plant height, PB = Primary branches, SB = Secondary branches, NPP = Number of pods per plant, NSP = Number of seeds per pod, PL = Pod length, HSW = Hundred seed mass, GYKH = Grain yield kilo gram per hectare, BM = Biomass kilo gram per hectare, HI = Harvest index, $^{ns}$ = non – significant and *, ** significant at 5 % and 1 % probability level, respectively.

This low magnitude of mean squares indicated the traits were relatively sensitive to environmental effects. These results are also confirmed similarly by reports of Zerihun (2011), Feven (2002) and Melese (2005).

### 3.2 Correlation studies

Studies on correlations among agronomic traits and seed yield can supply reliable information on the nature and level of their inter relationships. Identification and exploitation of traits positively attributing to seed yield is essential as it enhances breeding efficiency of chickpea. In this study, genotypic and phenotypic correlation coefficients for all possible combinations among 16 traits are presented in Table 2.

#### 3.2.1 Association of grain yield with other traits

The linear correlation coefficient analysis determines the magnitude and degree of relationship between two traits. Association between traits could be due to genotypic correlation, which is attributed to linkage between genes or pleiotropic gene effect (Shafique et al., 2016), or due to environmental effect, or both (Falconer and Mackey 1996).The correlation of grain yield with biomass ($r_g$ = 0.71 and $r_{ph}$ = 0.72) and with harvest index ($r_g$ = 0.52 and $r_{ph}$ = 0.54) was positive and highly significant both at genotypic and phenotypic levels. The correlation of grain yield with pod filling period, plant height, secondary branches and hundred seed mass was positive but non-significant both at genotypic and phenotypic levels. Muhammad et al. (2005) reported similar results. On the other hand, the correlation of grain yield with days to maturity, primary branches, and number of seeds per pod was negative but no significant at both levels.

The genotypic correlations of grain yield with days to flowering, days to pod setting, canopy width and number of pods per plant were positive but not significant. On the other hand, grain yield was negatively and non- significantly correlated with number of leaflets per leaf. However, genotypic correlation between grain yield and biomass yield was positive and highly significant, which is in agreement with the reports of Zerihun (2011), Melese (2005), and Tesfamickael et al. (2014).That means breeding for better biomass cultivars could be an indirect selection on chickpea improvement or landrace promotion.

3.2.2 Associations among other traits

Days to flowering was positively and significantly correlated with days to maturity, days to pod setting, number of leaflets per leaf, secondary branches, number of pods per plant, and hundred seed mass, but negatively correlated with primary branches at phenotypic and genotypic level (Table 2).Moreover, the genotypic correlation coefficients of days to flowering with these traits were greater than the corresponding phenotypic correlation coefficients, indicating genetic causes for the observed association. As a result, late flowering varieties are expected to take long duration to develop pods and mature late. According to Thakur and Sirohi (2009), genotypic correlation coefficients higher than that of phenotypic correlation coefficients indicate strong inherent association between the traits and the possibility of effective phenotypic selection.

Days to maturity showed significant and positive correlations with days to flowering, days to pod setting, number of leaflets per leaf, canopy width, secondary branches, number of pods per plant, and hundred seed mass at both phenotypic and genotypic levels. This is in agreement with Zerihun (2011). However, the correlation of days to maturity with primary branches and number of seeds per pod was negative at both phenotypic and genotypic levels (Table 2).

Plant height correlated positively and strongly with days to pod setting, number of leaflets per leaf, canopy width, and pod filing period; but negatively associated with number of seeds per pod both at genotypic and phenotypic levels. Primary branches had strong negative correlation with days to pod setting, pod filing period, days to maturity, and secondary branches at genotypic level; and with harvest index at phenotypic level. On the other hand,it had positive and significant correlation with number of pods per plant and number of seeds per pod at phenotypic level.

Secondary branches had high positive correlation with days to flowering, days to pod setting, pod filing period, canopy width, plant height, number of pods per plant, and hundred seed mass at both phenotypic and genotypic levels. However, it showed negative correlation with primary branches at both levels.

Hundred seed mass showed high positive correlation with days to flowering, days to pod setting, days to maturity, plant height, and secondary branches both at genotypic and phenotypic level; but negatively associated with the number of seeds per pod at both levels. Biomass yield had high positive correlation with grain yield at genotypic and phenotypic levels, but negatively correlated with harvest index at both levels (Table 2).Generally, among the 16 traits in this study, biomass and harvest index correlated positively and significantly with grain yield at both genotypic and phenotypic levels.

In general, all positive correlation between grain yield with traits of pod filling period, plant height, secondary branches, hundred seed mass and biomass both at genotypic and phenotypic levels are best traits which are important to direct selection process.

**Table 2:** Genotypic (above diagonal) and phenotypic (below diagonal) correlation coefficients among 16 traits of 204 chickpea genotypes

| Trait | DF | DP | PFP | DM | CW | NLtL | PH | PB | SB | NPP | NSP | HSW | GY | BY | HI | PL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DF | | 0.94** | 0.03 | 0.48** | 0.24** | 0.17* | 0.44 | -0.23** | 0.32** | 0.17* | -0.18 | 0.25** | 0.04 | -0.07 | 0.1 | -0.10 |
| DP | 0.89** | | 0.09 | 0.44** | 0.25** | 0.17* | 0.43** | -0.25 | 0.32** | 0.16* | -0.16* | 0.27** | 0.02 | -0.05 | 0.09 | -0.09 |
| PFP | -0.1* | 0 | | 0.35** | 0.06 | 0.2* | 0.32** | -0.2* | 0.19* | 0.16* | -0.17* | 0.2* | 0.06 | 0.01 | 0.06 | 0.1 |
| DM | 0.39** | 0.35** | 0.33** | | 0.24* | 0.27** | 0.44** | -0.18* | 0.24* | 0.15* | -0.15* | 0.10 | -0.01 | -0.03 | 0.01 | -0.07 |
| CW | 0.16 | 0.16** | 0.12* | 0.18** | | 0.04 | 0.55** | -0.05 | 0.42** | 0.29** | 0.01 | -0.02 | 0.05 | 0.05 | 0.01 | -0.01 |
| NLtL | 0.11* | 0.11* | 0.09* | 0.16** | 0 | | 0.18* | -0.11 | 0.09 | -0.02 | 0.04 | 0.05 | -0.06 | -0.07 | 0.01 | -0.03 |
| PH | 0.34** | 0.33** | 0.29** | 0.37** | 0.54** | 0.10* | | -0.11 | 0.51** | 0.5** | -0.23* | 0.17* | 0.1 | 0.04 | 0.1 | 0.03 |
| PB | -0.18** | -0.2** | -0.17** | -0.15** | 0.01 | -0.06 | -0.06 | | -0.14* | 0.07 | 0.06 | 0.06 | -0.05 | 0.03 | -0.12 | -0.03 |
| SB | 0.20** | 0.22** | 0.13* | 0.19** | 0.39** | 0.05 | 0.42** | -0.06 | | 0.57** | 0.02 | 0.14* | 0.05 | -0.02 | 0.06 | -0.08 |
| NPP | 0.12* | 0.12* | 0.16** | 0.15** | 0.32** | -0.03 | 0.46** | 0.08* | 0.55** | | 0.04 | 0.05 | 0.02 | 0.01 | -0.03 | 0.01 |
| NSP | -0.13 | -0.13* | -0.11* | -0.1* | 0.06 | 0.07 | -0.12* | 0.08* | 0.06 | 0.08 | | -0.2* | -0.08 | -0.04 | -0.06 | -0.07 |
| HSW | 0.18** | 0.18** | 0.13 | 0.11* | -0.01 | 0.01 | 0.12* | 0.04 | 0.09* | 0.05 | -0.14* | | 0.07 | 0.09 | -0.02 | -0.04 |
| GY | -0.04 | -0.02 | 0.06 | -0.02 | -0.02 | 0.04 | 0.05 | -0.05 | 0.02 | -0.01 | -0.06 | 0.04 | | 0.72** | 0.54** | -0.02 |
| BY | -0.1* | -0.07 | 0.06 | -0.01 | 0.01 | -0.03 | 0.02 | 0.02 | 0.03 | -0.03 | -0.03 | 0.05 | 0.71** | | -0.18* | -0.04 |
| HI | 0.06 | 0.06 | 0.01 | -0.01 | -0.05 | 0.07 | 0.04 | -0.10* | 0.03* | -0.01 | -0.05 | -0.04 | 0.52** | -0.22** | | 0.02 |
| PL | -0.06 | -0.07 | 0.04 | -0.03* | 0.01 | -0.04 | 0.01 | -0.01 | -0.06 | -0.04 | 0.03 | -0.03 | 0.04 | -0.03 | 0.07 | |

DF = Days to flowering, DP = Days to podding, DP = Pod filling period, DM = Days to maturity, CW = Canopy width, Number of leaflets per leaf, PH = Plant height, PB = Primary branches, SB = Secondary branches, NPP = Number of pods per plant, NSP = Number of seeds per pod, HSW = Hundred seed weight, GY = Grain yield, BY = Biomass, HI = Harvest index, PL = Pod length, and *, ** significant at 5 % and 1 % probability level, respectively

### 3.3 Path coefficient analysis

Grain yield is a resultant trait of many component traits, known as yield components. Associations between yield and yield components as determined by correlation coefficients do not indicate the relative importance of the direct and indirect effect of associated trait on grain yield. Hence, path coefficient analysis would give the opportunity to see grain yield as the dependent variable and other traits as casual factors (independent variables). The results of genotypic path coefficient analysis are given in Table 3.

Path coefficient analysis at genotypic level showed that among the fifteen traits; biomass (0.84), harvest index (0.69), pod length (9.73), days to pod setting (0.024), pod filing period (0.020), canopy width (0.003), primary branches (0.007), secondary branches (0.003), and number of pods per plant (0.019) had positive directly influence on grain yield.

Other traits such as days to flowering (-0.016), days to maturity (-0.015), number of leaflets per leaf (-0.004), plant height (-0.019), number of seeds per pod (-0.002), and hundred seed mass (-0.002) had negative direct effects on grain yield. These study findings are also in line with the reports of Yucel et al. (2006), Yucel and Anlarsal (2010), Ali et al. (2011) and Jadhav et al. (2014) in chickpea. Padmavathi et al., (2013) also reported high positive direct effects of biological yield, number of pods per plant, and harvest index on grain yield signifying the importance of these traits in improvement of grain yield.

Pod length exerted the highest direct effect (9.73), and also positive indirect effects on grain yield via pod filing period, plant height, number of pods per plant, and harvest index. However, the positive direct effect of pod length on grain yield was counter balanced by relatively high negative indirect effects via days to flowering, days to pod setting, days to maturity, canopy width, number of leaflets per leaf, primary branches, secondary branches, number of seeds per pod, hundred seed mass and biomass; which resulted in negative correlation with grain yield ($r_g$ = -1.44) (Table 3). Biomass yield exerted the second highest positive direct effect (0.84) on grain yield. It also exhibited positive indirect effects on grain yield through pod filing period, canopy width, plant height, primary branches, number of pods per plant and hundred seed mass. However, it had high negative indirect effects on grain yield through days to flowering, days to pod setting, days to maturity, number of leaflets per leaf, secondary branches, and number of seeds per pod, harvest index, and pod length.

Although the days to flowering, plant height and hundred seed mass had positive genotypic correlation with grain yield, their direct effects on grain yield were negative. This imply that the observed positive correlations of these traits with grain yield were due to their indirect positive effects on grain yield through primary branches, number of seeds per pod, biomass, pod length, canopy width and harvest index. This indicates the importance of these traits in the breeding program to identify high yielding genotypes through indirect selection for these traits. According to Singh and Chaudhary (1977), whenever a character has positive association and high positive indirect effects but negative direct effect on economic trait like grain yield, emphasis should be given to the indirect effects.

The estimated residual effect of path analysis was low (0.156), which indicated that about 85 % of the variability in grain yield was contributed by the traits studied. This residual effect towards grain yield in the present study might be due to other characters or environmental factors and, or sampling errors (Sengupta and Kataria, 1971).

According to the path coefficient analysis at phenotypic level biomass (0.86), harvest index (0.71), pod length (0.01), days to pod setting (0.021), primary branches (0.004) hundred seed mass (0.003) and number of pods per plant (0.011) had positive directly influence on grain yield and signifying the importance of these traits in improvement of grain yield (Table 4).

Other traits such as days to flowering (-0.02), days to maturity (-0.004), number of leaflets per leaf (-0.02), plant height (-0.04), canopy width (-0.01),), secondary branches (-0.04), and pod filing period (-0.01) had negative direct effects on grain yield (Table 4).

The estimated residual effect of path analysis was low (0.168), which indicated that about 84 % of the variability in grain yield was contributed by the traits studied. This residual effect towards grain yield in the present study might be due to other characters or environmental factors and, or sampling errors (Sengupta and Kataria, 1971).

**Table 3:** Genotypic direct (bold, underlined and diagonal) and indirect effects of 15 traits on grain yield for 204 chickpea genotypes

| Traits | DF | DP | PFP | DM | CW | NLtL | PH | PB | SB | NPP | NSP | HSM | BM | HI | PL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DF | **-0.016** | 0.023 | 0.001 | -0.07 | 0.001 | -0.02 | -0.09 | -0.02 | 0.001 | 0.003 | 0.001 | -0.01 | -0.06 | 0.069 | -9.82 |
| DP | -0.015 | **0.024** | 0.002 | -0.06 | 0.001 | -0.01 | -0.08 | -0.02 | 0.001 | 0.003 | 0.001 | -0.01 | -0.04 | 0.061 | -8.33 |
| PFP | -0.001 | 0.002 | **0.020** | -0.05 | 0.002 | -0.01 | -0.06 | -0.05 | 0.001 | 0.003 | 0.001 | -0.01 | 0.010 | 0.04 | 9.60 |
| DM | -0.007 | 0.010 | 0.006 | **-0.02** | 0.001 | -0.01 | -0.08 | -0.01 | 0.001 | 0.003 | 0.001 | -0.01 | -0.02 | 0.009 | -6.25 |
| CW | -0.004 | 0.006 | 0.001 | -0.03 | **0.003** | -0.01 | -0.01 | -0.01 | 0.001 | 0.005 | -1.3 | 3.49 | 0.044 | 0.004 | -6.72 |
| NLtL | -0.003 | 0.004 | 0.003 | -0.03 | 0.001 | **-0.04** | -0.04 | -0.01 | 0.001 | -0.01 | -6.2 | -0.01 | -0.06 | 0.007 | -3.04 |
| PH | -0.007 | 0.010 | 0.006 | -0.06 | 0.002 | -0.01 | **-0.02** | -0.01 | 0.002 | 0.009 | 0.001 | -0.01 | 0.035 | 0.067 | 3.31 |
| PB | 0.004 | -0.06 | -0.04 | 0.03 | -0.01 | 0.001 | 0.002 | **0.01** | -0.01 | 0.001 | -0.01 | -0.01 | 0.024 | -0.08 | -2.77 |
| SB | -0.005 | 0.007 | 0.003 | -0.03 | 0.001 | -0.01 | -0.01 | -0.01 | **0.003** | 0.011 | -2.7 | -0.01 | -0.02 | 0.041 | -7.56 |
| NPP | -0.003 | 0.003 | 0.003 | -0.02 | 0.001 | 6.48 | -0.09 | 0.001 | 0.001 | **0.019** | -7.4 | -0.01 | 0.007 | -0.02 | 1.30 |
| NSP | 0.003 | -0.04 | -0.03 | 0.02 | 2.42 | -0.01 | 0.004 | 0.001 | 5.22 | 0.001 | **-0.02** | 0.001 | -0.03 | -0.04 | -6.99 |
| HSW | -0.004 | 0.007 | 0.004 | -0.02 | -4.8 | -0.02 | -0.03 | 0.005 | 0.001 | 0.001 | 0.004 | **-0.02** | 0.08 | -0.01 | -3.90 |
| BY | 0.001 | -0.01 | 0.001 | 4.82 | 0.002 | 0.001 | -0.01 | 0.001 | -6.1 | 0.001 | 6.9 | -0.01 | **0.84** | -0.12 | -3.71 |
| HI | -0.002 | 0.002 | 0.001 | -0.01 | 2.02 | -3.7 | -0.01 | -0.01 | 0.001 | -6.2 | 0.001 | 4.37 | -0.15 | **0.69** | 2.18 |
| PL | 0.001 | -0.01 | 0.002 | 0.01 | -2.2 | 1.11 | -0.01 | -0.01 | -0.01 | 0.001 | 0.001 | 9.37 | -0.03 | 0.02 | **9.73** |

DF = Days to flowering, DP = Days to podding, DP = Pod filling period, DM = Days to maturity, CW = Canopy width,NLtL = Number of leaflets per leaf, PH = Plant height, PB = Primary branches, SB = Secondary branches, NPP = Number of pods per plant, NSP = Number of seeds per pod, HSM = Hundred seed mass, BM = Biomass, HI = Harvest index, PL = Pod length; **Residual = 0.156**

In general the information obtained from path analysis revealed that number of pods per plant, biomass, harvest index, secondary branches per plant, canopy width, days to podding and pod filing period had positive direct effect on grain yield at both genotypic and phenotypic levels. Thus, these traits may be used as effective selection parameters for obtaining high yield in breeding programme for yield enhancement in chickpea.

## 4 CONCLUSSION

The correlation of grain yield with biomass and harvest index was positive and highly significant both at genotypic and phenotypic levels. In addition, its association with pod filling period, plant height, secondary branches and hundred seed mass was positive both at genotypic and phenotypic levels.In general, all positive correlation between grain yield with traits of pod filling period, plant height, secondary branches, hundred seed mass and biomass both at genotypic and phenotypic levels are best traits which are important to direct selection process. Path coefficient analysis at genotypic level showed that among the fifteen causal (independent) traits; biomass, harvest index, pod length, days to pod setting, pod filing period, canopy width, primary branches, secondary branches and number of pods per plant had positive direct effects on grain yield per plot.In generalthe information obtained from path analysis revealed that number of pods per plant, biomass, harvest index, secondary branches per plant, canopy width, days to podding and pod filing period had positive direct effect on grain yield at both genotypic and phenotypic levels. Thus, these traits may be used as effective selection parameters for obtaining high yield in breeding programme for yield enhancement in chickpea.

## 5 ACKNOWLEDGEMENTS

First of all, the authors deepest gratitude and acknowledge goes to Amhaera Agricultural Research Institute and/or Sirinka Agricultural Research Center for providing research budget and facilitate the process. We would like also to express sincere thanks to Sirinka Agricultural Research Center pulse case team members for contributing their great effort this successful accomplishment of the experiment.

## 6 REFERENCES

Ali Q, Tahir MHN, Sadaqat HA, Arshad S, Farooq J, Ahsan M, Waseem M, Iqbal A (2011). Genetic variability and correlation analysis for quantitative traits in chickpea genotypes (*Cicer arietinum* L.). *Jornal of Bacteria Research*, *3*, 6-9.

Central Statistical Authority (2014/2015). Agricultural Sample Survey, Volume I:Report on Area and Production for major crops (*Meher* Season) *Statistical Bulletin No*. 578 Addis Ababa, Ethiopia

Dewey, J.R and K.H. Lu (1959). A correlation and path coefficient analysis of yield components of crested wheat seed production.*AgronomyJournal,51*, 515-518.doi:10.2134/agronj1959.00021962005100090002x

Falconer, D.S. and T.F.C. Mackay (1996). *Introduction to Quantitative Genetics*, 4th, ed. Longma Group Limited, Malaysia. 438 p

FAO.(1998). *The state of ex situ conservation*. Page 90 *in* the state of world's plant genetic resources for food and agriculture. Rome, Italy: Food and Agriculture Organization of the United Nations.

Feven W. (2002). *Morphological and biochemical diversity analysis in chickpea (Cicer arietinum L.) landraces of Ethiopia*. M.Sc. Thesis submitted to Addis Ababa University,Addis Ababa

Gomez, A.K. and Gomez A.A. (1984).*Statistical procedure for agricultural research*. John Wiley and Sons 680 p.

Gul R., Khan H., Sattar S, Farhatullah, Munsif F, Shadman, Khan BSA, Khattak SH, Arif M, Ali A. (2011). Comparison among nodulated and non nodulated chickpea genotypes.*Sarhad Journal of Agric*ulture,*27*(4), 577-581.

IBPGR, ICRISAT and ICARDA. (1993). *Descriptor for chickpea (Cicer arietinum* L*.)*. International Board for Plant Genetic Resources, Italy; International Crop Research Institute for the Semi-Arid Tropics, Patancheru, India and International Center for Agricultural Research in Dry Areas, Aleppo, Syria.

ICRISAT (International crop research institute for semi arid tropics) (2010).

Pooran M. Gaur, Shailesh Tripathi, CL Laxmipathi Gowda, GV Ranga Rao, HC Sharma, Suresh Pande and Mamta Sharma, Patancheru 502 324 Andhra Pradesh, India 2010. *Chickpea Seed Production Manual.*

Melese D., (2005). *Morphological and Random Amplified Polymorphic DNA (RAPD) marker variation analysis in some drought tolerance and susceptible chickpea (Cicer arietinum L.) genotypes*; Thesis submitted to University of Addis Ababa, Ethiopia.

Ojiewo Chris (2016). Presentation of Chickpea Production, *Technology Adoption and Market Linkages in Ethiopia on Pan-African Grain Legume and World Cowpea Conference Livingstone* - Zambia Feb 28 – Mar 4, 2016

Padmavathi P.V, Sreemannarayana S, Murthy, V. Satyanarayana Rao and Lal Ahamed M. (2013). Correlation and Path Coefficient Analysis in *Kabuli* Chickpea (*Cicer arietinum* L.) on *International Journal of Applied Biology and Pharmaceutical Technology,*

Robertson, G.E. (1959). The sampling variance of genetic correlation coefficient. Biometrics, *15*, 469-485.

SAS Institute 2004 SAS/STAT guide for personal computers, version 9.0 edition, SAS Institute Inc. Cary, NC.

Sengupta, K. and Kataria A.S. 1971.Path coefficient analysis for some characters in soybean. *Indian Jornal of Genetics*, *31*, 290-295.

Shafique MS, Ahsan M, Mehmood Z, Abdullah M, Shakoor A, Ahmad MI. (2016). Genetic variability and interrelationship of various agronomic traits using correlation and path analysis in Chickpea (*Cicer arietinum* L.). *Acadamic Journal of Agricultural Res*earch, *4*(2), 082-085.

Sharma J.R. (1998). *Statistical and biometrical techniques in plant breeding*. New Age International (P) Limited Publishers. New Delhi. 432 p.

Singh, R.K and Chaudhary. (1977). *Biometrical methods in quantitative genetic analysis*. Kalyani Publisher, New Delhi, Ludhiana, India.300 p.

Singh, K. B., M. Omar, M. C. Saxena, and C. Johansen (1997). Screening for drought resistance in spring chickpea in the Mediterranean region. *Journal of Agronomy & Crop Science*, *178*, 22-235. doi:10.1111/j.1439-037X.1997.tb00495.x

Singh JL, Prasad C, Madakemohekar AH, Bornare SS (2014). Genetic variability and character association in diverse genotypes of barley (*Hordeum vulgare* L.). *The Bioscan (Supplement on Genetics and Plant Breeding)*, *9*(2),759-761.

Tesfamichael Semere Mullu, Stephen Githiri Mwangi, Aggrey Bernard Nyende, N. V. P. R Ganga Rao, Damaris Achieng Odeny, Abhishek Rathore and Anil Kumars (2014). Assessment of genetic variation and heritability of agronomic traits in chickpea (*Cicer arietinum* L). *International Journal of Agronomy and Agricultural Research. (IJAAR)* ISSN: 2223-7054 (Print) 2225-3610 (Online) http://www.innspub.net Vol. 5, No. 4, p. 76-88, 2014.

Thakur, S.K. and Sirohi, A. (2009). Correlation and path coffient analysis in chickpea (*Cicer arietinum* L.) under different seasons. *Legume Research, 32*, 1-6.

Uday Chand Jha1, Singh D.P. and Roopa Lavanya (2012). Assessment of genetic variability and correlation of important yield related traits in chickpea (*Cicer arietinum* L.). Department of Genetics and Plant Breeding G.B. Pant University of Agriculture & Technology, Pantnagar-263 145 India agricultural research communication centre *www.arccjournals.com /. Indian journals.comLegume Research*, *35* (4), 341 – 344.

Van der Maesen LJG (1987). Origin, history and taxonomy of chickpea. In: Saxena MC, Singh KB (eds) *The Chickpea*. Wallingford: *C.A.B International*, pp. 11-34.

Yucel DO, Anlarsal AE, Yucel C (2006). Genatic Variabilty, Correalation and Path Analysis of Yield and Yield Components in Chickpea (*Cicer arietinum* L.). *Turky Journal of Agric*ulture, *30*, 182-188.

Yucel DO and Anlarsal AE. (2010). Determination of selection criteria with path coefficient analysis in chickpea (*Cicer arietinum* L.) breeding. *Bulgarian Journal of Agricultural Science*, *16*(1), 42-48.

Zerihun J. B. (2011). *Genetic diversity of elite chickpea (Cicer arietinum L.) varieties using morphological and inter simple sequence repeat markers*. Thesis Submitted to the College of Natural and Computational Sciences, Department f Biology, School of Graduate Studies Haramaya University, Haramaya 2011.

doi:10.14720/aas.2018.111.3.15 Original research article / izvirni znanstveni članek

# Vpliv globine obdelave tal z vrtavkasto brano na porabo energije in pripravo setvenega sloja pred setvijo koruze

Rajko BERNIK[1], Filip VUČAJNK[1]

Received July 30, 2018; accepted November 23, 2018.
Delo je prispelo 30. julija 2018, sprejeto 23. novembra 2018.

## IZVLEČEK

Na Laboratorijskem polju Biotehniške fakultete smo v letu 2012 izvedli poljski poskus, v katerem smo želeli ugotoviti vpliv obdelave tal na porabo energije, na fizikalno mehanske lastnosti tal v setveni posteljici in na vznik koruze. Za predsetveno pripravo tal smo uporabili vrtavkasto brano, s katero smo nastavili globino obdelave tal na 5 cm, 10 cm in 15 cm (dejansko dosežene globine tal 7,3 cm, 8,7 cm in 11,2 cm). Poskusna zasnova so bili slučajni bloki. V poskusu smo uporabili traktor z brezstopenjskim menjalnikom z imensko močjo 73 kW in vrtavkasto brano z delovno širino 2,5 m. Hitrost obdelave tal na traktometru je bila 5,0 km $h^{-1}$ in vrtilna frekvenca motorja 1900 $min^{-1}$. Poraba goriva na uro, poraba goriva na hektar ter poraba energije na hektar so naraščali s povečanjem nastavljene globine obdelave tal z vrtavkasto brano od 5 do 15 cm. Pri nastavljenih globinah obdelave tal 10 in 15 cm je bila vertikalna upornost tal na globinah med 8 in 13 cm manjša kot pri globini obdelave tal 5 cm. Med tremi nastavljenimi globinami obdelave tal z vrtavkasto brano ni bilo značilnih razlik v fizikalnih lastnosti tal v setveni posteljici in vzniku koruze. Globina obdelave tal 5 cm je bila najprimernejša, tako glede porabe goriva in porabe energije, kot tudi fizikalno mehanskih lastnosti tal v setvenem sloju in poljskega vznika koruze.

**Ključne besede:** obdelava tal; vrtavkasta brana; poraba energije; fizikalno-mehanske lastnosti tal; koruza

## ABSTRACT

### INFLUENCE OF SOIL CULTIVATION DEPTH ON ENERGY CONSUMPTION AND ON PREPARATION OF SEED BED USING ROTARY HARROW BEFORE MAIZE PLANTING

In 2012 on the Laboratory Field of Biotechnical Faculty the field trial was carried out, trying to establish the influence of the soil cultivation on the fuel consumption, on the physical-mechanical soil properties of the seed bed and at the end on the field emergence of maize. A rotary harrow was used for soil preparation just before maize planting and it was adjusted to the soil cultivation depths of 5 cm, 10 cm and 15 cm. The trial was designed as random blocks. A tractor with stepless transmission and nominal power of 73 kW and a rotary harrow with working width of 2.5 m were used. The speed of soil cultivation was 5.0 km $h^{-1}$ on the tractometer and the engine rotational frequency was 1900 rpm. The fuel consumption per hour, the fuel consumption per hectare and the energy consumption per hectare increased by increasing the adjusted soil depth cultivation from 5 cm to 15 cm using the rotary harrow. At the adjusted soil cultivation depth of 10 and 15 cm cm, the vertical soil resistance at depths between 8 and 13 cm was lower than at the adjusted soil depths of 5 cm. No significant differences were found regarding the soil physical properties in the seed bed and the field emergence of maize among three adjusted soil cultivation depths. The soil cultivation depth of 10 cm proved to be the most appropriate in view of the fuel consumption, energy consumption as well as the physical-mechanical soil properties of the seed bed and plant emergence.

**Key words:** soil cultivation; rotary harrow; energy consumption; physical-mechanical soil properties; maize

[1] University of Ljubljana, Biotechnical Faculty, Agronomy Department, Jamnikarjeva ulica 101, SI-1111 Ljubljana, Slovenia, *corresponding author: filip.vucajnk@bf.uni-lj.si

Prispevek je del magistrskega dela Luke Dularja. Mentor: prof. dr. Rajko Bernik

## 1 UVOD

Z dopolnilno obdelavo tal poskrbimo, da je velikost talnih delcev v setveni plasti ustrezna in so s tem tla optimalno pripravljena za setev (Bernik, 2005). Obdelava tal je eden najpomembnejših, energetsko potratnih in dragih tehnoloških postopkov pri pridelavi rastlin. Ustrezen način obdelave tal je odvisen od rastlinske vrste, vremenskih razmer, fizikalno mehanskih lastnosti tal, obstoječih strojev za obdelavo tal in ostalih dejavnikov. Vsak način obdelave tal ima določene prednosti in slabosti. Pri konvencionalni obdelavi tal je večja možnost za doseganje večjih pridelkov in boljše kakovosti kot pri drugih načinih obdelave tal. Po drugi strani je zaradi slabe storilnosti strojev in potrebe po močnejših traktorjih konvencionalna obdelava tal dražja (Šarauskis in sod., 2014). Isti avtorji so v poskusu primerjali globoko in plitvo oranje, globoko in plitko rahljanje tal ter direktno setev pri pridelavi koruze. Pri reducirani obdelavi tal brez oranja je bila poraba goriva 12-58 % manjša kot pri konvencionalni obdelavi tal, ki je vključevala oranje. Zaradi manjše porabe goriva so bili pri reducirani obdelavi tal manjši stroški in manjši izpusti $CO_2$. Pri uporabi kmetijskih strojev pri reducirani obdelavi tal znašajo izpusti $CO_2$ v okolje 107-223 kg ha$^{-1}$, medtem ko pri globokem oranju 253 kg ha$^{-1}$ $CO_2$.

Mileusnić in sod. (2010) so primerjali različne načine obdelave tal glede na porabo goriva. V poskusu so primerjali 4 različne traktorje s priključenimi stroji za konvencionalno ter konzervirajočo obdelavo tal ter direktno setev. Pri konvencionalni obdelavi tal je pri vseh uporabljenih traktorjih znašala energijska poraba goriva od 412 do 740 MJ ha$^{-1}$. V primeru konzervirajoče obdelave tal je le-ta segala od 183 do 266 MJ ha$^{-1}$, medtem ko je bila pri direktni setvi med 80 do 284 MJ ha$^{-1}$. Energijska poraba goriva (MJ ha$^{-1}$) se izračuna kot produkt porabljene količine nafte (kg ha$^{-1}$) in kurilne vrednosti plinskega olja (42 MJ kg$^{-1}$).

Deperon Júnior in sod. (2016) so analizirali stroje za obdelavo tal pri pridelavi koruze, in sicer diskasto brano, plug in rahljalnik ter 4 stopnje zbitosti tal. Prva stopnja pomeni, da ni bilo prehoda traktorja, druga stopnja pomeni 3 prehode traktorja, tretja stopnja pomeni 6 prehodov traktorja in četrta stopnja pomeni 9 prehodov traktorja. Rezultati kažejo, da izbira stroja za obdelavo tal vpliva na volumsko gostoto tal in poroznost tal na globini 0-10 cm. Zaradi večje zbitosti tal se je povečala vertikalna upornost tal na globini 0-30 cm. Pri vertikalni upornosti tal nad 153 N cm$^{-2}$ se je linearno zmanjšal pridelek suhe snovi in pridelek zrnja. Če je bila vertikalna upornost tal večja kot 218 N cm$^{-2}$, se je zmanjšala tudi suha snov korenin.

Z uporabo ustreznih strojev za obdelavo tal je mogoče zmanjšati stroške energije pri rastlinski pridelavi (Mani in sod., 2007; Ozkan in sod., 2007; Tabatabaeefar in sod., 2009). Način obdelave tal vpliva na lastnosti tal, kot so volumska gostota tal, poroznost in zbitost tal (Strudley in sod., 2015). Vertikalna upornost tal služi kot indikator vpliva obdelave tal in števila prehodov tal s kmetijskimi stroji na talne lastnosti. Veliko raziskav je bilo narejenih o vplivu števila prehodov tal s kmetijskimi stroji na vertikalno upornost tal (Carrara in sod., 2007; Koch in sod., 2008). Ugotovljeno je bilo, da vertikalna upornost tal narašča s povečanjem števila prehodov (Barik in sod., 2014). Botta in sod. (2004) so ugotovili, da je zaradi večje vertikalne upornosti tal prišlo do zmanjšanja pridelka soje od 10 do 38 %. Pri konvencionalni obdelavi tal, ki vključuje oranje s plugom, je v zgornji plasti tal manjša vertikalna upornost tal za 230 N cm$^{-2}$ kot pri konzervirajoči obdelavi tal in tudi direktni setvi (Kuhwald in sod., 2016). Pri slednjih dveh obdelavah tal znaša vertikalna upornost tal 380 N cm$^{-2}$ v zgornjih 30 cm tal, medtem ko pri konvencionalni obdelavi tal le 150 N cm$^{-2}$. Na drugi strani nastane pri konzervirajoči obdelavi tal večje število por in večja hidravlična prevodnost na globini 30-35 cm kot pri konvencionalni obdelavi tal. Zaradi obračanja na koncu njive se tam pojavi največja vertikalna upornost tal, in sicer 600 N cm$^{-2}$.

Pred setvijo koruze je pomembna pravilna delovna globina predsetvene priprave tal, ki je odvisna tudi od priporočene globine setve koruze. Kot splošno priporočilo naj bi globina setve koruze znašala 5 cm, nikakor pa ne sme biti manjša kot 3,8 cm (Crop Focus, 2015). V setveni posteljici in tudi na površini tal ne sme biti prevelikih talnih delcev, ki bi lahko ovirali vznik rastlin.

V Sloveniji za predsetveno pripravo tal v mnogih primerih uporabljajo vrtavkasto brano. Njena uporaba je smiselna na srednje težkih do težkih tleh, pri katerih samo z rotirajočimi elementi dosežemo zadovoljivo drobljenje talnih delcev in optimalno pripravo setvene posteljice. Zaradi zgoraj naštetih dejavnikov smo se odločili za poskus, v katerem smo uporabili tri različne nastavitve globine obdelave tal, in sicer 5, 10 in 15 cm pri predsetveni pripravi tal z vrtavkasto brano za kasnejšo setev koruze. Namen dela je bil ugotoviti primerno delovno globino pri predsetveni pripravi tal za setev koruze za zrnje na težkih tleh. Pri tem nas je zanimala poraba energije, fizikalno-mehanske lastnosti tal v setvenem sloju, dejanska globina obdelave tal in na koncu tudi vznik koruze.

## 2 MATERIALI IN METODE

Poskusna zasnova so bili naključni bloki s štirimi ponovitvami. Obravnavali smo tri različne nastavljene globine obdelave tal pri delu z vrtavkasto brano pred setvijo koruze, in sicer 5 cm, 10 cm in 15 cm. Posamezna parcela je bila dolga 100 m in široka 2,5 m. V poskusu smo uporabili traktor imenske moči 73 kW in vrtavkasto brano delovne širine 2,5 m. Vrtilna frekvenca priključne gredi traktorja pri delu z vrtavkasto brano je bila 540 $min^{-1}$, medtem ko je bila vrtilna frekvenca nožev 284 $min^{-1}$. Nastavljena delovna hitrost na tempomatu je znašala 5,0 km $h^{-1}$. Za doseganje želene globine obdelave tal 5, 10 in 15 cm smo postavili sornik na nosilcu stroja v 3 različne izvrtine. Dolžina nožev na vrtavkasti brani je znašala 280 mm. Porabo goriva na uro smo odčitali na traktometru vsakih 20 m na posamezni poskusni parceli. Na podlagi porabe goriva na uro in površinske storilnosti smo izračunali porabo goriva na hektar. Nato smo iz porabe goriva na hektar in kurilne vrednosti plinskega olja (42 MJ $kg^{-1}$) določili porabo energije na hektar.

Globino obdelave tal smo izmerili z merilnim trakom na petih naključnih mestih pri vsakem obravnavanju. Vertikalno upornost tal smo merili z vertikalnim hidravličnim penetrometrom.

Hitrost pomikanja konice potenciometra v tla je 1,8 cm $s^{-1}$, frekvenca meritev pa 10 Hz. Podatki meritev so se shranjevali v .txt datoteko. Podatki meritev se obdelajo v programu LabView. Program izračuna vertikalno upornost tal za vsak centimeter globine in tudi povprečno vertikalno upornost tal do globine 25 cm. Meritev velikosti talnih delcev smo opravili do globine obdelave tal, in sicer po en vzorec za vsako obravnavanje. Vzorec tal smo vzeli na naključnem mestu s posebno lopato in ga stresli na sita, ki so bila zložena eden na drugega. Mase s sit s talnimi delci smo stehtali na elektronski tehtnici Kern. Sita so imela različne velikosti mrež, in sicer 50, 30, 10, 5, 3, 1 in 0,5 mm. Na podlagi mase posameznih frakcij talnih delcev in skupne mase talnih delcev smo izračunali povprečni masni premer talnih delcev.

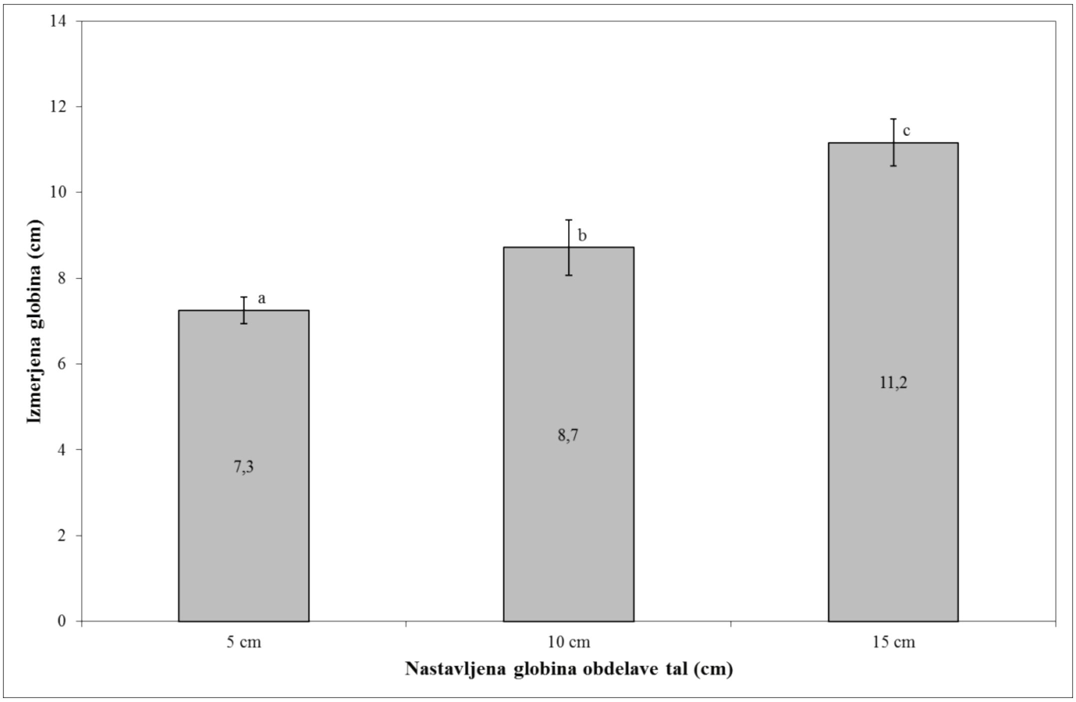
S Kopeckijevimi cilindri smo vzeli vzorce tal do globine obdelave tal z vrtavkasto brano. Vzorce smo po odvzemu najprej stehtali, nato pa smo jih 24 ur sušili na 105 °C. Po koncu sušenja smo jih ponovno stehtali in na izračunali volumsko gostoto tal, poroznost in masni odstotek vode.

Koruzo smo posejali 12.05. 2012 s pnevmatsko nadtlačno sejalnico za presledno setev Becker Aeromat 2. Na sejalnici smo najprej nastavili medvrstno razdaljo na 70 cm. Sejalnico smo s potisnim kolesom nastavili na želeno globino setve 5 cm. V poskusu smo uporabili seme koruze proizvajalca Pioneer du Pont hibrid PR39B29, zrelostni razred FAO 180. Proizvajalec priporoča gostoto setve od 90000 do 95000 zrn na hektar. Sejalnico smo nastavili na razdaljo med semeni v vrsti 15,5 cm, kar je pomenilo gostoto 92166 semena na hektar. Vznik koruze smo določili 13 dni po setvi tako, da smo na naključnem mestu prešteli rastline na dolžini 15,5 m. Povprečna dnevna temperatura zraka je v obdobju od priprave tal do vznika znašala 14,4 °C, medtem ko je bila količina padavin v tem obdobju 84,9 mm (Meteo, 2018).

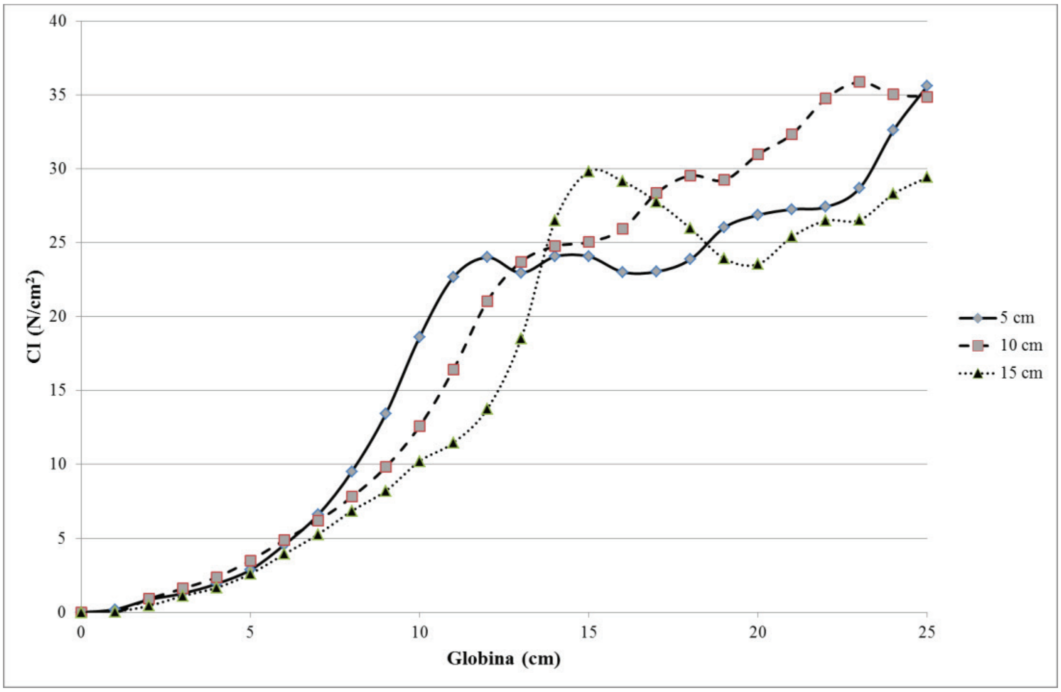
V programu Statgraphics Centurion 16 smo opravili analizo variance po postopku za slučajne bloke. Za ugotavljanje statističnih razlik med obravnavanji smo uporabili Duncanov test mnogoterih primerjav pri 5 % tveganju. Razlike med obravnavanji smo označili z različnimi črkami. Z regresijsko analizo smo ugotavljali odvisnost porabe energije na hektar od dejanske globine obdelave tal z vrtavkasto brano. Najprej smo preverili, kateri model je najprimernejši in nato izračunali ocene za posamezne parametre modela. Kasneje smo naredili analizo variance in izračunali F-statistiko, koeficient determinacije, koeficient korelacije in standardno napako regresije.

## 3 REZULTATI

Pri nastavljeni globini obdelave tal z vrtavkasto brano 5 cm je bila dejanska izmerjena globina nekoliko večja, in sicer 7,3 cm. Pri nastavljeni globini obdelave tal 10 cm in 15 cm je bila dejanska globina obdelave tal manjša, in sicer je znašala 8,7 cm oz. 11,2 cm (slika 1).




**Slika1:** Izmerjena globina tal pri treh nastavljenih globinah obdelave tal z vrtavkasto brano
**Figure 1:** Measured values of soil depth at three adjusted soil depths using rotary harrow




**Slika 2**: Vertikalna upornost tal pri treh globinah obdelave tal z vrtavkasto brano
**Figure 2**: Vertical soil resistance at three soil cultivation depths using rotary harrow

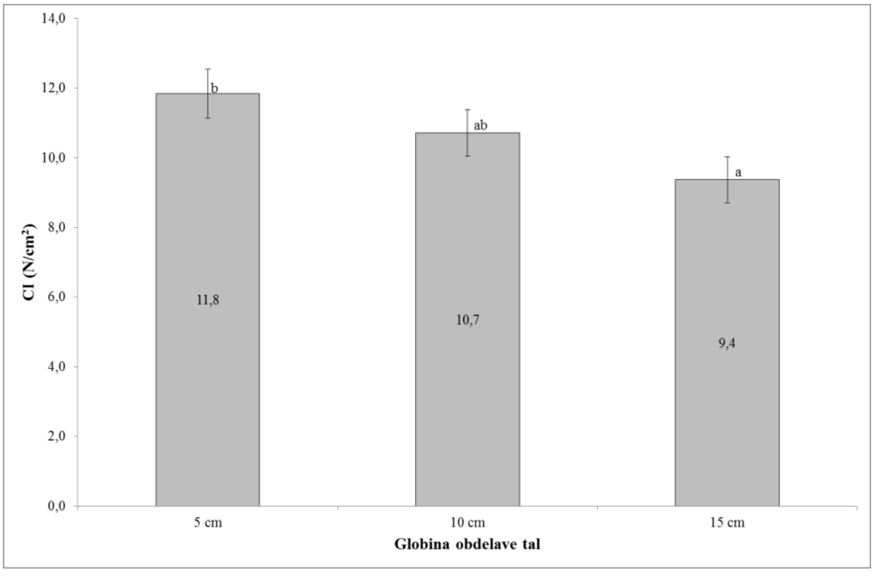
Na sliki 2 je prikazana vertikalna upornost tal pri treh globinah obdelave tal z vrtavkasto brano. Do globine obdelave tal 7 cm ni bilo značilnih razlik v vertikalni upornosti tal med tremi nastavljenimi globinami obdelave tal (Pregl. 1). Vertikalna upornost tal je na območju globine 0-7 cm znašala do 6,6 N $cm^{-2}$. Na 8 cm globine je bila pri nastavljeni globini 5 cm vertikalna upornost tal (9,5 N $cm^{-2}$) značilno večja kot pri nastavljeni globini obdelave 15 cm (6,9 N $cm^{-2}$). Na globinah 9 in 10 cm je bila vertikalna upornost tal pri nastavljeni globini obdelave 5 cm značilno večja kot pri ostalih dveh nastavljenih globinah 10 in 15 cm. Na globini meritve 11 cm so bile med vsemi tremi nastavljenimi globinami značilne razlike. Pri nastavljeni globini obdelave tal 5 cm je bila največja vertikalna upornost tal (22,7 N $cm^{-2}$), medtem ko je bila najmanjša vertikalna upornost tal pri nastavljeni globini 15 cm (11,4 N $cm^{-2}$). Pri nastavljeni globini obdelave tal 10 cm je bila vertikalna upornost vmes med obema globinama obdelave tal 5 in 15 cm. Na globini meritve 12 cm je bila vertikalna upornost tal pri nastavljenih globinah 5 in 10 cm značilno večja kot pri globini meritve 15 cm. Na globinah meritve 13-15 cm ni bilo značilnih razlik med tremi nastavljenimi globinami obdelave tal v vertikalni upornosti tal.

**Preglednica 1**: Razlike v vertikalni upornosti tal (CI) po globini meritev med posameznimi obravnavanji
**Table 1**: Differences in vertical soil resistance (CI) at measurement depths between individual treatments

| Globina (cm) | Značilnost razlik | CI (N $cm^{-2}$) | | |
|---|---|---|---|---|
| | | 5 cm | 10 cm | 15 cm |
| 1 | p = 0,0783 | 0,2 a* | -0,02 a | 0,19 a |
| 2 | p = 0,4034 | 0,9 a | 0,9 a | 0,4 a |
| 3 | p = 0,5015 | 1,3 a | 1,6 a | 1,1 a |
| 4 | p = 0,2764 | 1,9 a | 2,4 a | 1,7 a |
| 5 | p = 0,2525 | 2,9 a | 3,5 a | 2,6 a |
| 6 | p = 0,3669 | 4,6 a | 4,9 a | 3,9 a |
| 7 | p = 0,1935 | 6,6 a | 6,2 a | 5,3 a |
| 8 | p = 0,0236 | 9,5 b | 7,8 ab | 6,9 a |
| 9 | p = 0,0002 | 13,4 b | 9,8 a | 8,2 a |
| 10 | p = 0,0001 | 18,6 b | 12,6 a | 10,2 a |
| 11 | p = 0,0001 | 22,7 c | 16,4 b | 11,4 a |
| 12 | p = 0,0001 | 24,0 b | 21,1 b | 13,8 a |
| 13 | p = 0,0915 | 23,0 a | 23,7 a | 18,5 a |
| 14 | p = 0,7019 | 24,1 a | 24,8 a | 26,5 a |
| 15 | p = 0,1015 | 24,1 a | 25,0 a | 29,8 a |

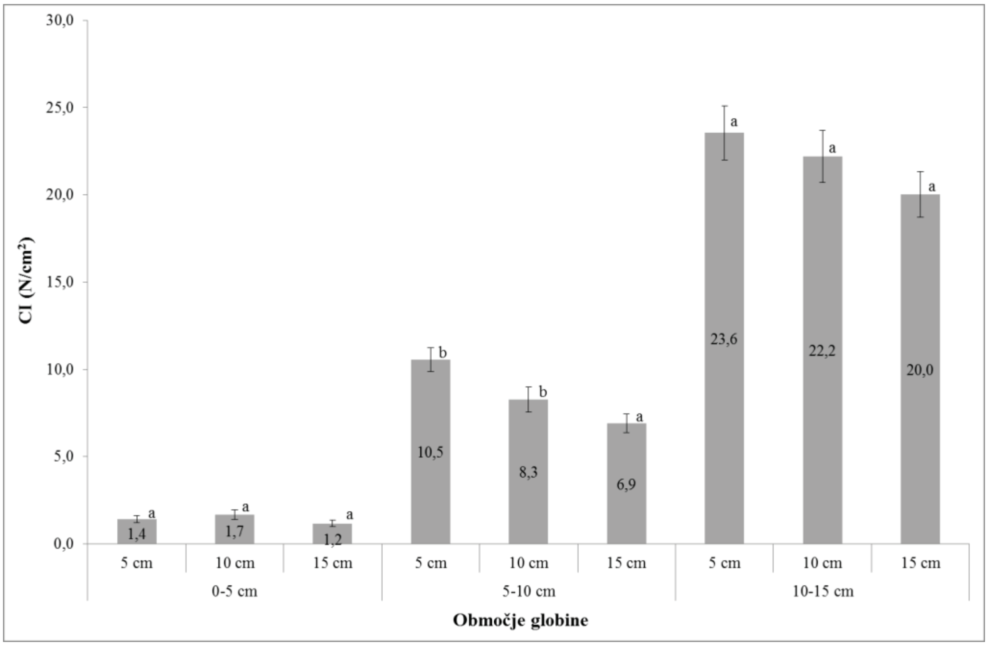
* različne črke v isti vrsti pomenijo statistično značilno razliko po Duncanov testu ($\alpha = 0{,}05$)

Izračunali smo tudi povprečno vertikalno upornost tal na območju globine 0-15 cm pri vseh treh nastavljenih globinah obdelave tal z vrtavkasto brano (Slika 3). Pri nastavljeni globini obdelave tal 5 cm je bila vertikalna upornost tal značilno večja kot pri globini obdelave tal 15 cm (9,4 N $cm^{-2}$).



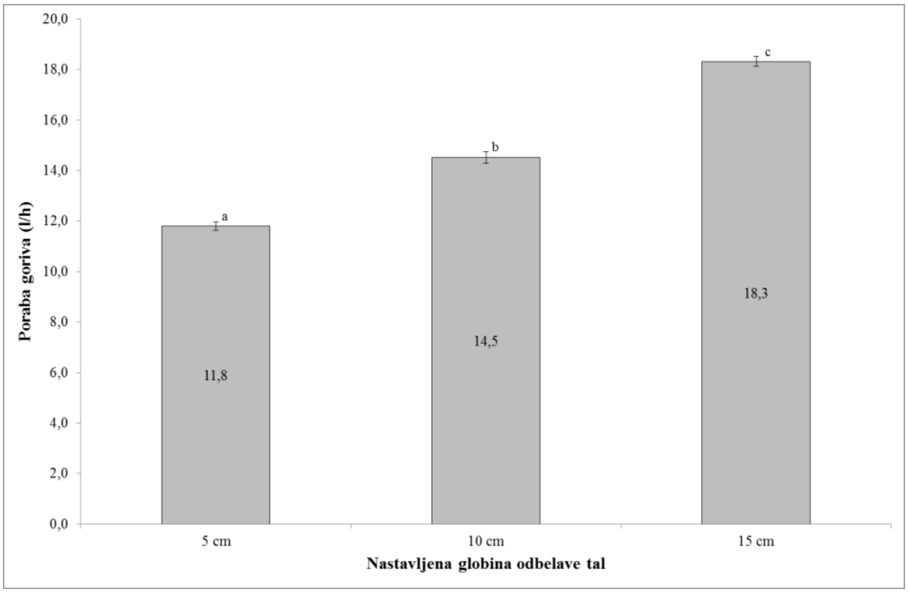

**Slika 3:** Povprečna vertikalna upornost tal za območje globine 0-15 cm
**Figure 3:** Mean vertical soil resistance at the depth area of 0 to 15 cm

Na območju globine 0-5 cm ni bilo značilnih razlik v povprečni vertikalni upornosti tal med tremi nastavljenimi globinami (slika 4). Pri nastavljeni globini obdelave tal 5 in 10 cm je bila vertikalna upornost tal na območju globine 5-10 cm značilno večja kot pri nastavljeni globini obdelave tal 15 cm. Na območju globine 10-15 cm ni bilo značilnih razlik med tremi nastavljenimi globinami obdelave tal.



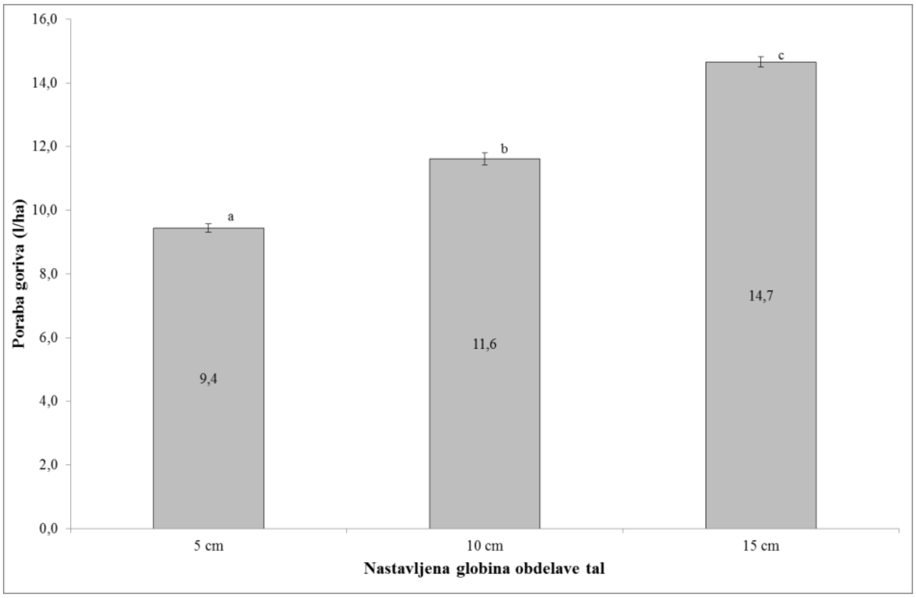

**Slika 4**: Povprečna vertikalna upornost tal po globinah 0-5 cm, 5-10 cm in 10-15 cm
**Figure 4**: Mean vertical soil resistance at depths of 0-5 cm, 5-10 cm and 10-15 cm

Poraba goriva na uro je naraščala s povečanjem nastavljene globine tal (Slika 5). Najmanjša poraba goriva na uro je bila pri nastavljeni globini obdelave tal 5 cm, in sicer 11,8 l $h^{-1}$. Pri nastavljeni globini obdelave tal 15 cm smo dosegli največjo porabo goriva uro (18,3 l $h^{-1}$). Pri nastavljeni globini obdelave tal 10 cm je znašala poraba goriva 14,5 l $h^{-1}$, kar je bilo vmes med porabo goriva pri 5 in 15 cm nastavljene globine.




**Slika 5:** Poraba goriva na uro pri treh nastavljenih globinah obdelave tal
**Figure 5:** Fuel consumption per hour at three adjusted soil cultivation depths

Izračunali smo tudi porabo goriva na hektar (Slika 6). Tudi poraba goriva na hektar je naraščala s povečanjem nastavljene globine obdelave tal z vrtavkasto brano. Najmanjša poraba goriva na hektar je znašala 9,4 l $ha^{-1}$ pri nastavljeni globini obdelave 5 cm, medtem ko je bila največja poraba 14,7 l $ha^{-1}$ pri nastavljeni globini obdelave 15 cm. Pri globini obdelave 10 cm je znašala 11,6 l $ha^{-1}$ in je bila vmes med obema globinama 5 in 15 cm.

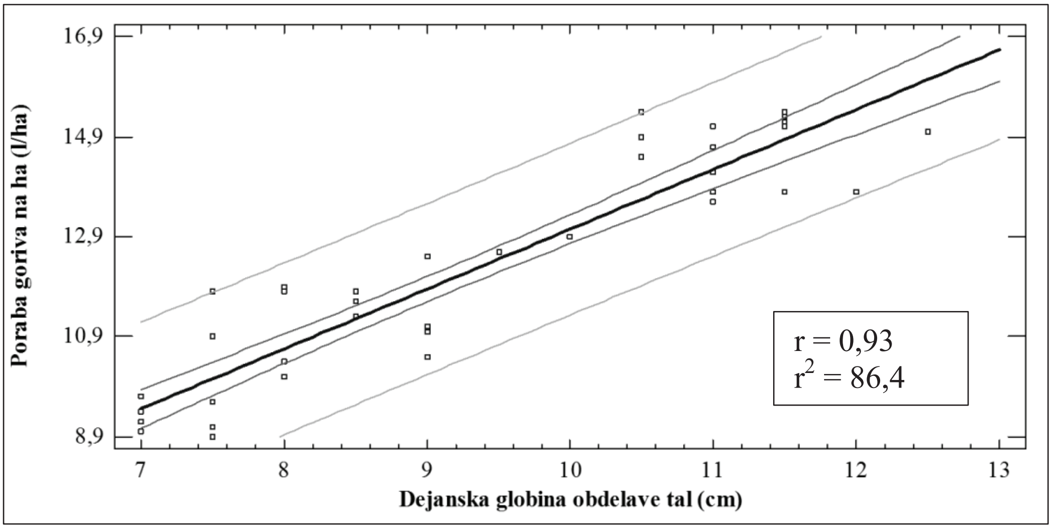



**Slika 6**: Poraba goriva na hektar pri treh globinah obdelave tal
**Figure 6**: Fuel consumption per hectare at three soil cultivation depths

Na sliki 7 je prikazana odvisnost porabe goriva na hektar od dejanske globine obdelave tal z vrtavkasto brano.

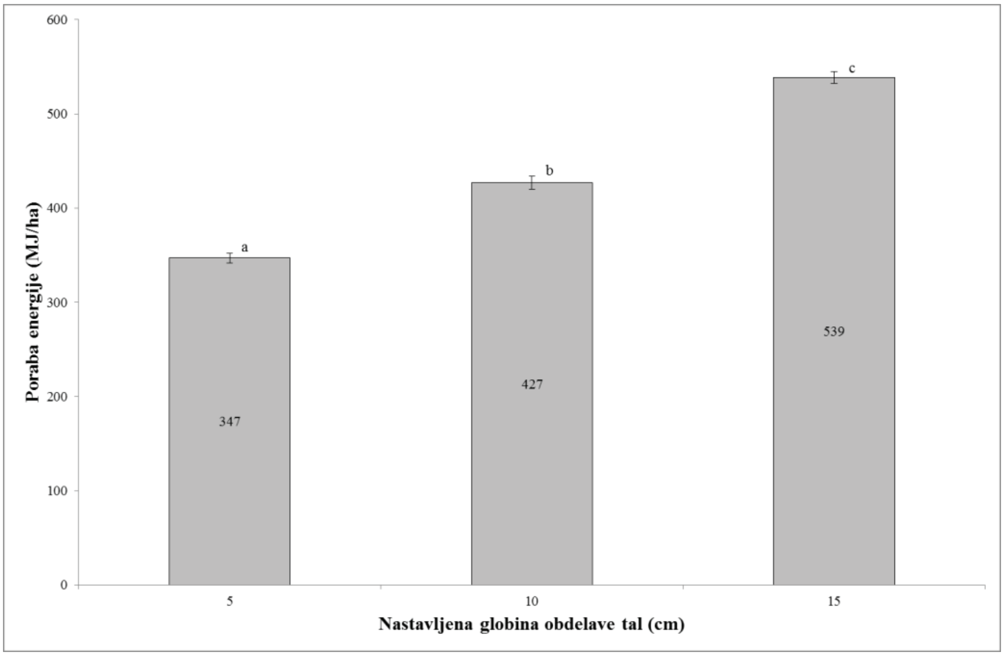
Model: Poraba na ha (l $ha^{-1}$) = 1,06815 + 1,19812*izmerjena globina (cm)

Če znaša globina obdelave tal z vrtavkasto brano 5,0 cm, bo predvidena poraba goriva znašala 7,1 l $ha^{-1}$. V kolikor bi bila globina obdelave tal 10 cm, bo napovedana poraba goriva znašala 13,0 l $ha^{-1}$. Za vsak cm v globini obdelave tal se poraba goriva poveča za 1,2 l $ha^{-1}$.




**Slika 7**: Linearni regresijski model za odvisnost porabe goriva na hektar od dejanske globine obdelave tal z intervali zaupanja za povprečno (notranji hiperboli) in posamezno napoved (zunanji hiperboli)
**Figure 7**: Linear regression model for the relationship between the fuel consumption per hectare and the actual soil cultivation depth with confidence intervals for the mean prediction (i.e. the inner bounds)

Poraba energije pri obdelavi tal z vrtavkasto brano na globini 5 cm je znašala 347 MJ $ha^{-1}$ in je bila najmanjša med vsemi tremi nastavljenimi globinami obdelave tal z vrtavkasto brano. Pri nastavljeni globini obdelave tal 15 cm je bila poraba energije največja in je znašala 539 MJ $ha^{-1}$ (Slika 8), medtem ko je pri nastavljeni globini obdelave tal 10 cm znašala 427 MJ $ha^{-1}$.




**Slika 8**: Poraba energije pri treh nastavljenih globinah obdelave tal z vrtavkasto brano
**Figure 8**: Energy consumption at three adjusted soil cultivation depths using rotary harrow

Med obravnavanji ni bilo ugotovljenih razlik v volumski gostoti tal, poroznosti, masnem odstotku vode in povprečnem masnem premeru talnih delcev. Volumska gostota tal je bila med 1,12 in 1,16 g $cm^{-3}$, medtem ko je poroznost znašala med 56,4 in 57,8 %. Masni odstotek vode se je gibal med 31,7 in 33,3 %, povprečni masni premer talnih delcev pa med 7,7 in 9,1 mm (Pregl. 2).

**Preglednica 2**: Volumska gostota tal, poroznost, masni odstotek vode v tleh in povprečni premer talnih delcev
**Table 2**: Soil density, soil porosity, soil water content and mean diameter of soil particles

| Nastavljena globina | Volumska gostota tal (g $cm^{-3}$) | Poroznost (%) | Masni odstotek vode (%) | Povprečni premer talnih delcev (mm) |
|---|---|---|---|---|
| 0 cm | 1,12 a | 57,8 a | 33,3 a | 7,7 a |
| 5 cm | 1,16 a | 56,4 a | 31,7 a | 8,1 a |
| 10 cm | 1,15 a | 56,4 a | 32,5 a | 9,1 a |

Med nastavljenimi globinami setve ni bilo razlik v poljskem vzniku koruze. Le ta je znašal od 96,0 do 97,7 %.

**Preglednica 3**: Vznik koruze pri treh nastavljenih globinah obdelave tal
**Table 3**: Maize emergence at three adjusted soil cultivation depths

| Nastavljena globina | Vznik (%) |
|---|---|
| 0 cm | 97,7 a |
| 5 cm | 96,3 a |
| 10 cm | 96,0 a |

## 4 RAZPRAVA

Dejanska globina obdelave tal je bila za 2,3 cm večja od nastavljene globine 5 cm. V tem primeru bi morali sornik na vrtavkasti brani prestaviti za 1 izvrtino nižje na nosilcu in s tem bi zmanjšali globino obdelave tal. Nastavljena globina 10 cm je bila za 1,3 cm manjša, kar pomeni, da bi morali sornik prestaviti za 1 izvrtino višje in s tem bi globino obdelave približali 10 cm. Pri nastavljeni globini 15 cm je bila dejanska globina za 3,8 cm manjša, kar pomeni, da bi morali sornik prestaviti za 2 luknji višje na nosilcu, s tem bi povečali delovno globino. Rezultati kažejo, da je dejansko globino obdelave tal težko natančno nastaviti predvsem pri večjih globinah.

Značilne razlike v vertikalni upornosti tal so se med tremi nastavljenimi globinami obdelave tal z vrtavkasto brano 5 cm, 10 cm in 15 cm pojavile na globinah od 8-12 cm. Na globinah 8 cm, 9 cm in 10 cm je bila pri nastavljeni globini obdelave tal 5 cm večja vertikalna upornost tal, kot je bila pri nastavljenih globinah 10 cm in 15 cm. Ti rezultati so bili pričakovani, saj so noži pri nastavljenih globinah 10 cm in 15 cm rahljali zemljo na globinah od 8-10 cm, medtem ko se pri nastavljeni globini 5 cm to ni zgodilo. Na globinah 11 in 12 cm je bila pri nastavljeni globini obdelave tal 15 cm manjša vertikalna upornost tal kot pri ostalih dveh nastavljenih globinah obdelave tal 5 in 10 cm, kar je bilo pričakovano. Podobne razlike smo pričakovali tudi na globinah 14 in 15 cm, vendar je bila dejanska globina delovanja nožev pri nastavljeni globini 15 cm manjša. V poskusu se je izkazalo, da je izredno težko nastaviti natančno dejansko globino delovanja nožev, kljub temu da smo pred izvedbo poskusa te nastavitve izvedli na sosednji parceli. Neposrednih primerjav iz tujih raziskav ni. Večina raziskav glede vertikalne upornosti tal pri pridelavi koruze se nanaša na različne načine obdelave tal. Zeyada in sod. (2017) pišejo, da je v tleh z majhno do srednje veliko vertikalno upornostjo tal (od 60 do 165 N $cm^{-2}$) najprimernejša globina obdelave tal z diskasto brano 10 cm. Na tleh z veliko vertikalno upornostjo tal (od 165 do 230 N $cm^{-2}$) pa je potrebno tla obdelati do globine 20 cm. Podatki iz njihove raziskave se navezujejo na vertikalno upornost tal pred dopolnilno obdelavo tal, kar pa ni direktno primerljivo z našim poskusom, saj mi nismo merili vertikalne upornosti pred dopolnilno obdelavo tal. V našem poskusu je bila izmerjena vertikalna upornost tal bistveno manjša, in sicer ni presegla 30 N $cm^{-2}$ na globini 15 cm, kolikor je znašala globina obdelave tal. Tako lahko trdimo, da je bila setvena posteljica zelo rahla in praktično nič zbita. Povprečna vertikalna upornost na globini 0-15 cm ni presegala 12 N $cm^{-2}$. Kuhwald in sod. (2016) so ugotovili manjšo vertikalno upornost tal v zgornji plasti tal pri konvencionalni obdelavi tal v primerjavi s konzervirajočo obdelavo tal in neposredno setvijo. Rezultate drugih raziskav o vertikalni upornosti tal ne moremo neposredno primerjati z našimi, saj gre za različne globine meritev vertikalne upornosti tal, različni čas meritev, različne načine obdelave tal, različne traktorje, stroje za obdelavo tal, različne vremenske razmere, itd.. Zaradi navedenega so vrednosti vertikalne upornosti v teh raziskavah precej večje od naših. Ker je neposrednih primerjav zelo malo, smo navedli nekatere rezultate raziskav. Deperon in sod. (2016) so ugotovili zmanjšanje pridelka koruznega zrnja in pridelka suhe snovi, če je znašala vertikalna upornost tal več kot 153 N $cm^{-2}$ pred dopolnilno obdelavo tal. Leghari in sod. (2016) trdijo, da je bila pri direktni setvi ugotovljena največja vertikalna upornost tal v primerjavi z konvencionalno in konzervirajočo obdelavo tal, in sicer je znašala 80 N $cm^{-2}$. Veliko je bilo raziskav o vplivu števila prehodov s kmetijskimi stroji, v katerih so ugotovili povečanje vertikalne upornosti tal s povečanjem števila prehodov po polju (Barik in sod., 2014; Koch in sod., 2008). Te rezultate ne moremo neposredno primerjati z našimi, saj mi števila prehodov v poskusu nismo obravnavali.

Poraba goriva na uro je naraščala s povečanjem globine obdelave tal z vrtavkasto brano, kar je bilo pričakovano. Bolj poglobljeno sliko glede porabe goriva nam pokaže poraba goriva na hektar. S povečanjem nastavljene delovne globine je značilno naraščala poraba goriva na hektar in to od 9,4 l $ha^{-1}$ pri nastavljeni globini 5 cm do 11,6 l $ha^{-1}$ pri nastavljeni globini 10 cm in na koncu do 14,7 l $ha^{-1}$ pri nastavljeni globini 15 cm. Naši rezultati glede porabe goriva na hektar pri delu z vrtavkasto brano so primerljivi z rezultati, ki jih navaja KTBL (2012), kjer znaša poraba goriva 10,4 l $ha^{-1}$, le da je v njihovem primeru delovna širina vrtavkaste brane znašala 3 m. Tudi Brehm (2010) je pri preizkušanju 3 m vrtavkaste brane na delovni globini 8 cm ugotovil porabo goriva 11,0 l $ha^{-1}$, kar je popolnoma primerljivo z našimi rezultati. Poraba goriva (l $h^{-1}$) je odvisna od imenske moči traktorja (kW) in specifične porabe goriva (g $kWh^{-1}$). Če primerjamo traktorja z isto imensko močjo, ki poganjata vrtavkasto brano iste delovne širine pri isti vozni hitrosti, bo poraba goriva na hektar manjša pri traktorju z manjšo specifično porabo goriva. V kolikor bi uporabili traktor z večjo imensko močjo in večjo specifično porabo goriva od našega v poskusu pri isti vozni hitrosti, bi bila poraba goriva na hektar večja kot je bila v našem poskusu. Traktor Fendt 210 Vario, ki je bil uporabljen v poskusu, ima pri imenski moči 70 kW specifično porabo goriva 210 g $kWh^{-1}$, kar pomeni zelo majhno specifično porabo goriva. Odvisnost porabe goriva na hektar od dejanske globine obdelave tal z vrtavkasto brano smo potrdili z linearnim regresijskim modelom. 86,4 % variabilnosti porabe goriva na hektar pojasni dejanska globina obdelave tal, ostali del ostane nepojasnjen. Koeficient korelacije znaša 0,99, kar pomeni močno povezavo med porabo goriva na hektar in dejansko globino obdelave tal.

Mileusnić in sod. (2010) so izračunali energijsko porabo goriva pri različnih načinih obdelave tal (MJ $ha^{-1}$) kot produkt porabljene količine plinskega olja (kg $ha^{-1}$) in kurilne vrednosti plinskega olja (42 MJ $kg^{-1}$). Če naše podatke o porabi goriva v litrih na hektar pretvorimo v kg $ha^{-1}$ in pomnožimo s 42 MJ $kg^{-1}$, dobimo energijsko porabo goriva na hektar. Pri nastavljeni globini obdelave tal 5 cm z vrtavkasto brano znaša poraba energije 347 MJ $ha^{-1}$, pri nastavljeni globini 10 cm znaša 427 MJ $ha^{-1}$ in pri nastavljeni globini 15 cm znaša 539 MJ $ha^{-1}$. Rezultati kažejo, da poraba energije na hektar značilno narašča z nastavljeno globino obdelave tal. Direktno primerljivih rezultatov z našo raziskavo ni. Po ugotovitvah Mileusnića in sod. (2010) je znašala poraba energije pri konvencionalni obdelavi tal med 412 in 740 MJ $ha^{-1}$. V tej energiji je zajeta energija za oranje, pripravo tal in setev, česar mi v našem poskusu nismo merili. V poskusu smo mi analizirali le porabo energije pri predsetveni pripravi tal, nič pa porabo energije za samo oranje in setev. Vsekakor naši podatki kažejo, da je pri nastavljeni globini 15 cm poraba energije na hektar nekoliko prevelika za predsetveno pripravo tal za koruzo. Veliko avtorjev navaja, da se pri konzervirajoči obdelavi tal in pri direktni setvi poraba energije na hektar zmanjša (Šarauskis in sod., 2014; Stajnko, 2017), a tega mi v poskusu nismo ugotavljali.

Med fizikalnimi lastnostmi tal ni bilo ugotovljenih razlik pri različnih nastavljenih globinah obdelave tal z vrtavkasto brano pred setvijo koruze, kar ni bilo v skladu s postavljeno hipotezo. Predvidevamo, da se razlike niso pojavile, ker je bila razlika v globini odvzema vzorcev tal S Kopeckijevimi cilindri zelo majhna (od 5 do 10 cm). Pri večjih globinah obdelave tal se fizikalne lastnosti tal na tako ozkem območju globine niso spremenile. Če bi bile te razlike med globinami obdelave tal večje, se bi pojavile tudi razlike v fizikalnih lastnostih tal. Volumska gostota tal je znašala od 1,12 do 1,16 g $cm^{-3}$. Tla, ki imajo volumsko gostoto manjšo od 1,40 g $cm^{-3}$, kot je bilo v našem poskusu, veljajo po Mrharju (1995) za malo zbita. Vsekakor je bila v našem poskusu precej manjša volumska gostota tal, kot navajajo Leghari in sod. (2016). V njihovem poskusu z različnimi načini obdelave tal pri koruzi je volumska gostota tal pri direktni setvi znašala med 1,4 in 1,5 g $cm^{-3}$. Pri konvencionalni in konzervirajoči obdelavi tal je bila volumska gostota tal manjša kot pri neposredni setvi. Tudi v poroznosti tal in masnem odstotku vode ni bilo značilnih razlik med tremi nastavljenimi globinami obdelave tal z vrtavkasto brano. Poroznost tal je v neposredni povezavi z volumsko gostoto tal, saj se s povečanjem zbitosti tal zmanjša poroznost tal zaradi zmanjšanega deleža makropor (Sommer, 1974). Po navedbah Sommerja in Zacha (1986) znaša poroznost za poljska tla med 40 in 50 %. V našem poskusu je bila poroznost nad 56 %, kar pomeni, da je bil delež makropor v tleh velik, zato so nastale ugodne razmere za izmenjavo zraka in vode v setveni posteljici ter kasneje tudi za rast korenin. Prav tako nismo ugotovili razlik v povprečnem premeru talnih delcev (MWD) in odstotku talnih delcev po frakcijah med tremi nastavljenimi globinami obdelave tal z vrtavkasto brano. MWD je znašal med 7,7 in 9,1 mm. Direktnih primerjav s tujimi raziskavami nimamo. Brehm (2010) navaja, da je po obdelavi tal z vrtavkasto brano povprečni premer na ilovnatem pesku znašal 9,6 mm, kar je nekoliko več kot v našem poskusu. Bernik (2005) navaja, da naj bi v srednji Evropi povprečni premer talnih delcev v setveni posteljici znašal med 8 in 10 mm, kar smo mi v našem poskusu potrdili.

Med tremi nastavljenimi globinami obdelave tal ni bilo razlik v poljskem vzniku koruze. Ta je znašal od 96,0 do 97,7 %. Rezultati kažejo, da je bila setvena posteljica pri vseh treh nastavljenih globinah dobro pripravljena, kar je omogočilo dober vznik rastlin. Lütke Entrup in sod. (2013) navajajo, da lahko pride do zaskorjenja površine tal, kar privede do zmanjšanega vznika rastlin. Do zaskorjenja lahko pride, če takoj po dopolnilni obdelavi tal pade večja količina dežja, nato pa nastopi daljše sušno obdobje brez padavin. Takrat se tla zaskorjijo še posebej, če so bila preveč intenzivno obdelana in ni bilo na površini tal večjih talnih agregatov. Ti bi povzročili, da bi bila površina bolj hrapava in ne bi prišlo do zaskorjenja tal. Predvidevamo, da bi lahko tudi v našem poskusu v primeru ekstremnih vremenskih razmer prišlo do zaskorjenja.

Glede na dobre fizikalno-mehanske lastnosti tal pri vseh treh nastavljenih globinah obdelave tal z vrtavkasto brano predvidevamo, da bi lahko izbrali večjo delovno hitrost od 5 km $h^{-1}$, ki je bila v poskusu (npr. 8 km $h^{-1}$). Tako bi dosegli manjšo porabo goriva na hektar in prav tako tudi manjšo porabo energije na hektar pri pripravi tal z vrtavkasto brano. Ta dejavnik hitrosti obdelave tal bo potrebno vključiti v nadaljnje poskuse

## 5 ZAKLJUČEK

Rezultati kažejo, da se je pri predsetveni pripravi tal z vrtavkasto brano za setev koruze, tako glede porabe goriva kot tudi fizikalno mehanskih lastnosti tal, kot najprimernejša izkazala nastavljena globina obdelave tal 5 cm.

## 6 VIRI


Barik K., Aksakal E. L., Islam K. R., Sari S., Angin I. (2014). Spatial veriability in soil compaction properites associated with field traffic operations. *Catena*, *120*, 122-133. doi:10.1016/j.catena.2014.04.013

Bernik R. (2005). *Tehnika v kmetijstvu: obdelava tal, setev, gnojenje*. Predavanja za študente agronomije in zootehnike. Biotehniška fakulteta, Oddelek za agronomijo: 138 str.

Botta G.P., Jorajuria D., Balbuena R., Rosatto H. (2004). Mechanical and cropping behavior of direct drilled soil under different traffic intensities: effect on soybean (*Glycine max* L.) yields. *Soil & Tillage Research*, *78*, 53-58. doi:10.1016/j.still.2004.01.004

Brehm D. (2010). Amazone KE 3000 Super. DLG-Prüfbericht 5897 F. Groß-Umstadt, DLG e.V. *Teestzentrum Tachnik und Betriebsmittel*: 6 str. http://www.dlg-test.de/tests/5897F.pdf (19. 3. 2018)

Carrara M., Castrignanò A., Comparetti A., Febo P., Orlando S. (2007). Mapping of penetrometer resistance in relation to tractor traffic using multivariate geostatistics. *Geoderma*, *142*, 294-307. doi:10.1016/j.geoderma.2007.08.020

Crop Focus. (2015). *Planting depth and spacing.* https://growersunited.files.wordpress.com/2014/03/c_depth-considerations.pdf (22.1.2018)

Deperon Júnior A. M., Nagahama H. J., Olszevski N., Cortez J. W., De Souza E. B. (2016). Tillage machinery and compaction level influence on soil physical properties and corn agronomic aspects. *Journal of the Brazilian Association of Agricultural Engineeing*, *36*, 367-376.

Dyer J.A., Desjardins R. L. (2003). The impact of farm machinery management on the greenhouse gas emission from Canading agriculture. *Journal of Sustainable Agriculture*, *22*(3), 59-47. doi:10.1300/J064v22n03_07

Filipović D., Košutić S., Gospodarić Z., Zimmer R., Banaj D. (2006). The possibilities of fuel savings and the reduction of $CO_2$ emissions in the soil tillage in Croatia. *Agriculture, Ecosysistems and Environment*, *115,* 290-294. doi:10.1016/j.agee.2005.12.013

Koch H. J., Heuer H., Tomanová O., Märländer B. (2008). Cumulative effect of annually repeated passes of heavy agricultural two tillage systems. *Soil and Tillage Research*, *101*, 69-77. doi:10.1016/j.still.2008.07.008

KTBL (2012). *Betriebsplanung Landwirtschaft, Kuratorium für Technik und Bauwesen in der Landwirtschaft*, Darmstadt, 824 str.

Kuhwald M., Blaschek M., Minkler R., Nazemtseva Y., Schwanebeck M., Winter J., Duttmann R. (2016). Spatial analysis of long-term effects of different tillage practices based on penetration resistance. *Soil Use and Managment*, *32*, 240-249. doi:10.1111/sum.12254

Leghari N., Mughal A. Q., Leghari K. Q., Farhad W., Mohkum Hammad M. and H. (2016). Efect of various tillage practices on soil properties and maize growth. *Pakistan Journal Botany*, *48*(3), 1173-1182.

Lütke Eintrup N., Schwarz F.J., Heilmann H. (2013). *Handbuch Mais*. Frankurt am Main, DLG Verlag: 442 str.

Meteo (2018). http://meteo.arso.gov.si/met/sl/app/webmet/#webmet==8Sdwx2bhR2cv0WZ0V2bvEGcw9ydlJWblR3LwVnaz9SYtVmYh9iclFGbt9SaulGdugXbsx3cs9mdl5WahxXYyNGapZXZ8tHZv1WYp5mOnMHbvZXZulWYnwCchJXYtVGdlJnOn0UQQdSf;

Mileusnić Z.I., Petrović D.V., Đević M.S. 2010. Comparison of tillage systems according to fuel consumption. *Energy*, *35*, 221-228. doi:10.1016/j.energy.2009.09.012

Mrhar M. (1995). *Racionalna obdelava tal*. Ljubljana, Kmetijski inštitut Slovenije: 109 str.

Ozkan B., Fert C., Karadeniz C. F. (2007). Energy and cost analysis for greenhouse and open-field grape production. *Energy*, *32*, 1500-1504.

Sommer C. (1974). *Die Verdichtungsempfindlichkeit zweier Ackerböden*. Dissertation. Braunschweig, Technische Universität: 158 str.

Sommer C., Zach M. (1986). Bodenverdichtungen und deren Auswirkungen auf die Pflanzenentwicklung und den Ertrag. V: *Bodenverdichtungen beim Schlepper- und Maschineneinsatz und Möglichkeiten zu ihrer Verminderung. KTBL-Schrift*, *308,* 73-88.

Stajnko D. (2017). *Obdelovanje tal in protierozijska zaščita na vodovarstvenih območjih*. Maribor, Univerzitetna založba Univerze v Mariboru: 100 str. doi:10.18690/978-961-286-066-0

Strudley M. W., Green T. R., Ascough I. I., James C. (2008). Tillage effects on soil hydraulic properties in space and time: state of the science. *Soil and Tillage Research*, *99*, 4-48. doi:10.1016/j.still.2008.01.007

Šarauskis E., Buragiene S., Masilionytė L., Romaneckas K., Avižienytė D., Sakalauskas D. (2014). Energy balance, costs and $CO_2$ analysis of tillage technologies in maize cultivation. *Energy*, *69*, 220-235. doi:10.1016/j.energy.2014.02.090

Tabatabaeefar A., Emamzadeh H., Gasemi Varnamkhasti M., Rahimizadeh R., Karimi M. (2009). Comparison of energy of tillage systems in wheat production. *Energy*, *34*, 41-45. doi:10.1016/j.energy.2008.09.023

Zeyada A. M., Al-Gaadi K. A., Tola E., Madugundu R., Kayad A. G. (2017). Impact of soil firmness and tillage depth on irrigated maize silage performance. *Applied Engineering in Agriculture*, *33*, 491-498. doi:10.13031/aea.11641

doi:10.14720/aas.2018.111.3.16 Original research article / izvirni znanstveni članek

# The influence of cultivation method on nitrate content in some lettuce samples

Veronika KMECL[1], Dragan ŽNIDARČIČ[2]

Received August 22, 2018; accepted November 13, 2018.
Delo je prispelo 22. avgusta 2018, sprejeto 13. novembra 2018.

**ABSTRACT**

The use of nitrogen fertilizers is one of the main effects for the accumulation of nitrates in plants. Conventional agriculture, in comparison to integrated and organic farming, causes greater environmental pollution and poorer quality of crops. Within the framework of the research, we studied the influence of the method of cultivation on the content of nitrates in the samples of lettuce (*Lactuca sativa* L.). The samples were received directly from growing areas from different parts of Slovenia and analysed in the laboratory for the nitrate content ($NO_3^-$) according to the accredited method. The samples from conventional cultivation showed the highest sample representation (51 %), with values in the highest concentration range (1000 - 2500 mg of $NO_3^-$ $kg^{-1}$, one sample exceeded 2500 mg $kg^{-1}$ fresh mass). Within the framework of integrated cultivation, there were less such samples (34 %), and among the samples from organic cultivation no sample exceeded 1000 mg of $NO_3^-$ $kg^{-1}$. Of 88 analysed samples, one sample exceeded the statutory limit value applicable to lettuce of the type Iceberg ('Ljubljanska ledenka'). On average, the Iceberg samples contained more nitrates than other types of lettuce.

**Key words:** organic; conventional; integrated cultivation; nitrates; lettuce

**IZVLEČEK**

**VPLIV NAČINA PRIDELAVE NA VSEBNOST NITRATOV V VZORCIH VRTNE SOLATE**

Raba dušikovih gnojil je eden glavnih vplivov kopičenja nitratov v rastlinah. Konvencionalno kmetijstvo, v primerjavi z integriranim in ekološkim kmetijstvom povzroča večje onesnaženje okolja in slabšo kakovost pridelkov. V okviru raziskave smo ugotavljali vpliv načina pridelave na vsebnost nitratov v vzorcih vrtne solate (*Lactuca sativa* L.). Vzorce smo prejeli neposredno iz pridelovalnih površin iz različnih delov Slovenije. V laboratoriju smo vzorce analizirali na vsebnost nitrata ($NO_3^-$) po akreditirani metodi. Kot pričakovano, so vzorci iz konvencionalne pridelave pokazali največjo zastopanost vzorcev (51 %), z vrednostmi v najvišjem koncentracijskem območju (1000 – 2500 mg $NO_3^-$ $kg^{-1}$ sveže mase, en vzorec je presegel vrednost 2500 mg $kg^{-1}$). V okviru integrirane pridelave je bilo takšnih vzorcev manj (34 %), izmed vzorcev iz ekološke pridelave pa ni bilo vzorca, ki bi presegel vrednost 1000 mg $NO_3^-$ $kg^{-1}$. Izmed 88 analiziranih vzorcev, je en vzorec presegel zakonsko predpisano mejno vrednost, ki velja za vrtno solato tipa ledenka ('Ljubljanska ledenka'). V povprečju so vzorci ledenk vsebovali več nitratov, kot druge vrste vrtne solate.

**Ključne besede:** ekološka; konvencionalna; integrirana pridelava; nitrat; vrtna solata

## 1 INTRODUCTION

Nitrogen is the most important element in plant nutrition. The plants need it as a nutrient for building amino acids, proteins, nucleic acids and other vital compounds. The plants receive the nitrogen in the form of nitrate ($NO_3^-$) and ammonium ion ($NH_4^+$). Due to the oxidation of the ammonium nitrogen form to nitrate, which takes place in the soil in the presence of bacteria, the nitrate is the predominant form received by the plant (Below, 1994).

The intensity of farming and the associated use of nitrogen fertilizers cause nitrates to accumulate in plants. Most of nitrate is accumulated in vegetables (especially leafy vegetables), less in field crops, and much less in fruits (Schuddeboom, 1993; Ysart et al., 1999; Zhong et al., 2002). Vegetables are the largest source of nitrate input in the human body; that is 70-80 % of the total $NO_3^-$ input (Gangolli et al., 1994). Nitrate itselves is harmless to health, however, after ingestion, it is influenced by microbiological processes

[1] M.Sc. Chem., Agricultural Institute of Slovenia, Hacquetova 17, SI-1000 Ljubljana, Slovenia, e-mail: veronika.kmecl@kis.si

[2] Ph.D., Agr., University of Ljubljana, Biotechnical Faculty, Department of agronomy, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia, e-mail: dragan.znidarcic@bf.uni-lj.si

which convert it into nitrite that can cause "methemoglobinemia" (inability of oxygen transfer in organism). Nitrite is in the stomach precursor for the nitrosation of the compound (nitrosamines, nitrosamides), which are, according to the criteria of the International Agency for Research on Cancer, grouped into individual groups of carcinogenicity (Boink and Speijers, 1999, Santamaria, 2006).

The accumulation of nitrate in plants is influenced by various factors, such as the harvest time, the length of the growth age, soil fertility characteristics (pH), weather conditions (rainfall, light), cultivar type and to a great extent the use of nitrogen fertilizers (McCall and Willumsen, 1999; Dapoigny et al., 2000; Chen et al., 2004; Guadagnin et al., 2005; Weightman et al., 2006). The latter can be influenced by different methods of cultivation. Lately, great attention is being paid to the organic and integrated cultivation methods, which are, unlike conventional, more environmentally friendly.

Conventional cultivation allows the use of mineral fertilizers, plant protection products (FFS) and genetically modified organisms. Mineral fertilizers additionally improve the supply of the soil with nutrients and increase the fertility of the soil, but on the other hand pollute the environment (Kocjan Ačko, 2000).

Integrated cultivation represents a more friendly form of farming for the environment and for the consumer. This mode of cultivation is today widespread in most of Western European countries and ensures the consumers that vegetables do not contain harmful substances above the permitted limit (Osvald and Kogoj Osvald, 2003). Unlike conventional farming, integrated farming demands careful monitoring of the use of minerals and organic fertilizers, whereby the fertilization with organic fertilizers is preferred (livestock manure, animal or plant residues, compost). Soil analysis is needed before fertilization, from which we find out how much nutrient intake is needed. Plant protection products in integrated farming are used only when biological, mechanical and other measures do not work (Džuban, 2015).

Organic cultivation is the only sustainably oriented agricultural food production system. It is a way of farming, which also pays attention to the balance between living organisms. Also, among all the ways of farming, it is the least burdensome on the environment (Poštrak, 2010). Organic farming system prohibits the use of soluble mineral fertilizers, as well as the use of plant protection products and genetically modified organisms.

Crop plants absorb nitrogen mainly in the form of nitrate ions. Mineral nitrogen fertilizers used in conventional agriculture directly provide the nitrate, while many organic fertilizers gradually release their nitrogen content. The amount of nitrate absorbed by plants depends on the nitrate dissolved in the soil solution and, therefore, the type of fertilizer is not the only cause of nitrate accumulation in plant. Incorrect practices, such as overfertilization with nitrogen also favour this accumulation (Matallana Gonzàlez et al., 2010; Barker, 1975; Muramoto, 1999; Raigon et al., 2002).

Many studies have demonstrated that organically grown crops have lower nitrate content that integrated and conventionally grown crops (Muramoto, 1999; Pussemier et al., 2006, Merino et al., 2006), although this conclusion is not uniformly supported (De Martin and Restani, 2003; Guadagnin et al., 2005).

Woese et al. (1997) reviewed 41 comparative studies of nitrate content in conventional and organically grown vegetable and concluded that, in general, higher nitrate levels were found in leaf, root and tuber vegetables with mineral fertilization. Worthington (2001) summarized the results of 18 studies comparing nitrate levels of organic and conventional vegetable, and found that in 72 % of the cases nitrate levels were higher in the conventional products, while in 24 % of the cases nitrate levels were higher in the organic products. In Slovenia, a comparison between conventionally cultivated lettuce and organic lettuce was presented and confirmed 30 % higher values of nitrates using conventional method of cultivation (Hmeljak Gorenjak et al., 2012).

In recent years, organic farming is becoming an increasingly way of producing food. Consumers are becoming more aware of the importance of a healthy diet, and organic farming is thus becoming a fast growing industry in the world. The data show that the share of organic land in the EU is around 4 %. Some countries are more "ecologically aware", such as Liechtenstein (26 %), Austria (11 %), Switzerland (10 %), Italy (8 %), Sweden (6 %) (Poštrak, 2010; Repič, 2010). In 2016, 3518 Slovenian agricultural holdings, which represent 4.8 % of all Slovenian farms, were included in “Eco control – organic method of cultivation”, however, the number of them is increasing every year (MKGP RS, 2016).

Within the framework of the research, we wanted to determine how the cultivation method (conventional, integrated or organic) affects the nitrate content in the cultivated vegetables of Slovenian producers. We focused on the samples of lettuce.

## 2 MATERIAL AND METHODS

### 2.1 Sampling

In the laboratory of the Agricultural Institute of Slovenia we received 88 samples of lettuce from different regions of Slovenia. The samples belongs to various lettuce cultivar groups, such as Iceberg lettuce ('Ljubljanska ledenka'), Crisphead lettuce ('Margord', 'Beldi' and 'Vegor'), Looseleaf lettuce ('Green Oakleaf'), Romaine lettuce ('Salakis'), Batavian lettuce ('Noisete' and 'Vanity'), Frilly open lettuce type with an iceberg bite ('Crystal') and Butterhead lettuce ('Zimska rjavka').

Plants were harvested at the stage of market acceptance (5 May and 23 October). The samples were grown outdoors, with the exception of one ('Marcord') that was from a greenhouse. They were cultivated using organic, integrated or conventional method. Among them, there were 31 samples which were produced conventionally, 53 were cultivated using integrated method and only 4 samples were organically produced. A similar ratio represents the actual state of organic, integrated and conventional farming in Slovenia (MKGP RS, 2016). This might be a reason for a small number of samples in organic farming.

At organic cultivation method farmers used fertilizers of organic origin, such as compost manure, green manure and places emphasis on techniques such as crop rotation and companion planting.

In the frame of integrated production system, farmers mostly used fertilizers of organic origin and mineral nitrogen fertilizers. Synthetic pesticides were not included in their way of production, as they can be in conventional method of cultivation.

### 2.2 Chemical analysis

One lettuce sample consisted of several heads of lettuce. The scrap leaves of each head were removed and all inner and outer leaves were homogenized and prepared for the analysis. Water was added to homogenized sample and the nitrate ions were extracted into the solution by shaking with the shaker. The content of nitrate in the extraction solution was determined photometrically with a segment flow analyser (San, Skalar). The nitrate in the sample was reduced to nitrite with hydrazine sulphate. The nitrite forms, in reaction with sulphanilamide, a diazo compound, the latter turning red after the addition of NEDD (N-naphthylethylenediamine dihydrochloride). The colour intensity was measured photometrically at a wavelength ($\lambda$ = 540 nm) (EN 12014-7, 1998). The method for the determination of nitrate in vegetables is accredited at the Agricultural Institute of Slovenia (Kmecl and Žnidarčič, 2015).

### 2.3 Limit values of nitrates in lettuce in accordance with the legislation

**Table 1**: The prescribed threshold values for nitrates in lettuce (Commission Regulation (EU) No. 1258/2011)

| Foodstuffs | Harvesting time/ Place of production | Maximum levels ($mg\ NO_3^-\ kg^{-1}$) |
|---|---|---|
| 1.1. Lettuce (*Lactuca sativa* L.) (protected and open-growth lettuce) excluding lettuce listed in point 1.2. | Harvested 1 October to 31 March: | |
| | Lettuce grown under cover | 5000 |
| | Lettuce grown in the open air | 4000 |
| | Harvested 1 April to 30 September: | |
| | Lettuce grown under cover | 4000 |
| | Lettuce grown in the open air | 3000 |
| 1.2. Iceberg type lettuce | Lettuce grown under cover | 2500 |
| | Lettuce grown in the open air | 2000 |

## 3 RESULTS AND DISCUSSION

The Commission Regulation (EU) No. 1258/2011 defines the maximum permissible levels of nitrate for the lettuce cultivar groups (*Lactuca sativa* L.) and among this groups separately defines the permissible value for Iceberg type lettuce, which naturally accumulates less nitrates. In the light of the above classification, we present the values of nitrates in analyzed samples of lettuce (Table 2 and 3).

**Table 2:** Concentration of nitrates in the samples of lettuce

| | Nitrates ($NO_3^-$) | | | | |
|---|---|---|---|---|---|
| Sample | No. of samples | Min. (mg kg$^{-1}$) | Max. (mg kg$^{-1}$) | Average (mg kg$^{-1}$) | Median (mg kg$^{-1}$) |
| Iceberg lettuce | 20 | 47 | 3211 | 968 | 912 |
| Other types of lettuce | 68 | 11 | 2451 | 841 | 780 |

**Table 3:** Number and percentage of samples in a particular concentration range

| | Number and percentage of samples in the concentration range of nitrates ($NO_3^-$) | | | | |
|---|---|---|---|---|---|
| Sample | <200 (mg kg$^{-1}$) | 200-500 (mg kg$^{-1}$) | 500-1000 (mg kg$^{-1}$) | 1000-2500 (mg kg$^{-1}$) | >2500 (mg kg$^{-1}$) |
| Iceberg lettuce | 1 (5 %) | 4 (20 %) | 6 (30 %) | 8 (40 %) | 1 (5 %) |
| Other types of lettuce | 10 (15 %) | 9 (13 %) | 19 (28 %) | 30 (44 %) | 0 (0 %) |

The amount of nitrates in vegetables depends on the type of cultivar, the weather conditions (light, quantity of rainfall), method of cultivation and harvesting time. Vegetables that are grown in winter or early spring and those grown in greenhouses contain more nitrates. Lettuce grown in a greenhouse in winter in reduced light can contain up to 3500 mg of $NO_3^-$ kg$^{-1}$. If it is grown outdoors, at a time when the illumination is greater, the content of nitrates can be ten times smaller (Brown et al., 1993; Amr and Hadidi, 2001). In general, the cultivars of lettuce with looseleaf heads accumulate more nitrates than lettuce with tightly formed heads, such as Iceberg type lettuce (Commission Regulation (EU) No 1258/2011).

The latter statement could not be confirmed by our research. Iceberg type samples on average contained more nitrates (968 mg kg$^{-1}$), compared to other types of lettuce (841 mg kg$^{-1}$). The reason probably lies in the fact that a significant proportion of these samples were produced in a conventional way (35 %) and the rest of the samples used integrated cultivation method (65 %). No sample was produced organically. The highest concentration of nitrates was contained in a sample of Iceberg lettuce ('Ljubljanska ledenka'), 3211 mg kg$^{-1}$ (grown outdoors in June, using conventional cultivation method). Among other varieties of lettuce we found the highest value in the sample of 'Marcord', which was cultivated in a greenhouse in spring (2451 mg $NO_3^-$ kg$^{-1}$). The reason of so high value of nitrates is due to the conventional farming system and the way of cultivation in greenhouse. Individual samples contained very little nitrates (<50 mg $NO_3^-$ kg$^{-1}$), which is likely the result of lower intensification of fertilization with nitrogen fertilizers or other aforementioned factors.

Most samples of all types of lettuce (43 %) contained nitrate in the concentration range from 1000 to 2500 mg kg$^{-1}$ (38 samples). Of these, 4 samples exceeded 2000 mg $NO_3$ kg$^{-1}$. 28 % of the samples (25 samples) contained nitrate in the range of 500 to 1000 mg kg$^{-1}$, 15 % of the samples (13 samples) were in the range of 200 to 500 mg of $NO_3^-$ kg$^{-1}$, and in 13 % of the samples (11 samples) we determined lower values (< 200 mg $NO_3^-$ kg$^{-1}$). In one sample, the presence of nitrate was greater than 2500 mg of $NO_3^-$ kg$^{-1}$ (1 % of the samples).

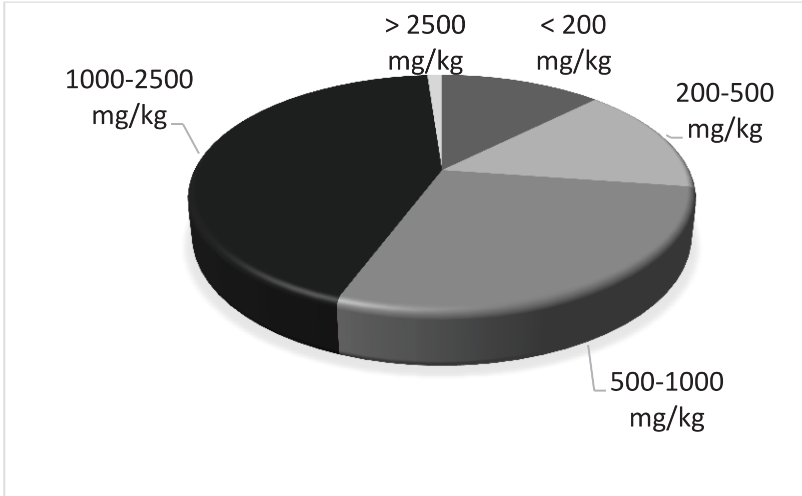



**Figure 1:** The proportion of samples in a particular concentration range of $NO_3^-$

Since 2011, the Slovenian legislation has prescribed nitrate limits for some leafy vegetables such as spinach, rocket and lettuce, and prepared baby food. For lettuce with looseleaf heads and tightly formed heads (Iceberg type), the limit values from 2000 to 5000 mg of $NO_3^-$ $kg^{-1}$ are prescribed. The values vary according to the harvest time (cultivation from October 1 to March 31, and from April 1 until September 30) and according to the method of cultivation (outdoor cultivation or in sheltered spaces). The standards are in accordance with the norms of the European Union (Commiss. Reg. (EU), 1258/2011).

All the samples that were analysed were growing the period from May to October, when the illumination of the plants is greater. During this time, less nitrogen accumulated in plants and $NO_3^-$ values, which are prescribed by the legislation, were not exceeded. In winter, the light is less intense, which causes slower rates of photosynthesis, and at the same time greater accumulation of nitrate in certain parts of plants (Schuddeboom, 1993). Of the 88 analysed samples, 1 sample of Iceberg type with the value of 3211 mg of $NO_3^-$ $kg^{-1}$, exceeded the legislative limit value (2000 mg $NO_3^-$ $kg^{-1}$; for Iceberg type lettuce grown in an open air). Other samples did not exceed the limit values.

With this investigation we wanted to determine how the cultivation method (organic, integrated or conventional) affects the nitrate content in the crops. According to the producers' statements, most of the lettuce was grown using the integrated cultivation method (60 %), 35 % of the samples used the conventional method and only 5% of farmers cultivated lettuce organically.

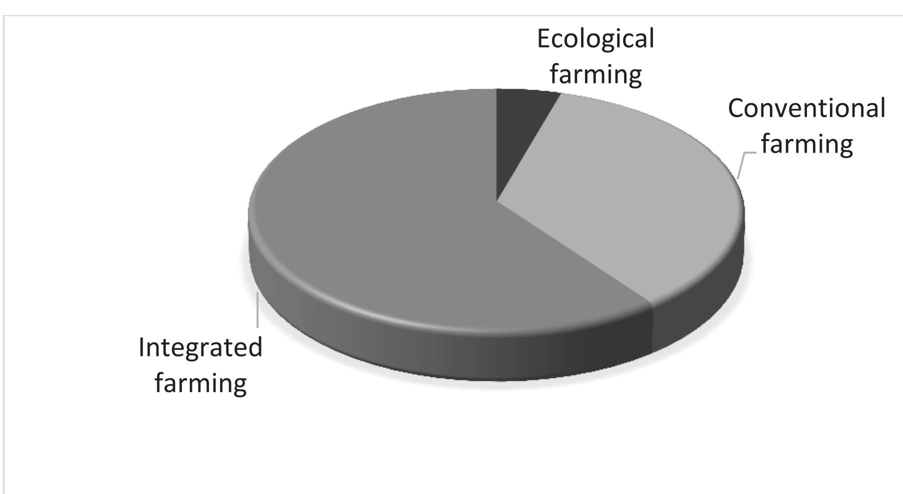



**Figure 2:** The proportion of organic, integrated or conventionally cultivated lettuce

A similar ratio represents the actual state of organic, integrated and conventional agriculture in Slovenia. Therefore, it is not surprising that we received only four samples of lettuce produced with organic method of cultivation (5 %), for which the producers used primarily organic fertilizers (compost, matured livestock manure, etc.). Most of the samples were received within the framework of the integrated cultivation method (53 samples), for which the use of mineral fertilizers (N, $P_2O_5$, $K_2O$ fertilizers) is otherwise allowed, however, carefully monitored. A relatively large proportion of the samples (31) was received from the conventionally oriented farms, where the intensive farming method has been maintained, with the emphasis on the quantity of produce and less on the quality (greater soil stocking with mineral fertilizers).

### 3.1 Organic method of cultivation

Two of the 4 lettuce samples of organic production ('Green Oakleaf' and 'Zimska rjavka') contained below 500 mg of $NO_3^-$ $kg^{-1}$ (138 and 429 mg $kg^{-1}$). Two samples ranged from 500 to 1000 mg of $NO_3^-$ $kg^{-1}$ (783 and 969 mg $kg^{-1}$; 'Crystal' and 'Noisette'). Values above 1000 mg of $NO_3^-$ $kg^{-1}$ were not detected in any sample. It is interesting that organic cultivation did not result in very low levels of nitrates, as opposed to individual samples that came from integrated and also conventional cultivation.

### 3.2 Integrated method of cultivation

Within the framework of integrated production, 53 samples of lettuce were analysed ('Ljubljanska ledenka', 'Marcord', 'Beldi', 'Salakis', 'Noisette', 'Vegor', 'Vanity' and 'Green Oakleaf'). 34 % of samples contained nitrate in higher concentration range (from 1000 to 2500 mg of $NO_3^-$ $kg^{-1}$). The highest proportion of samples (38 %) had values between 500 and 1000 mg $kg^{-1}$, while 28 % of the samples were in the <500 mg $NO_3^-$ $kg^{-1}$ range. The lowest measured value was 29 mg of $NO_3^-$ $kg^{-1}$ ('Marcord') and the highest 2200 mg of $NO_3^-$ $kg^{-1}$ ('Beldi').

### 3.3 Conventional method of cultivation

In the context of conventional production, 31 samples were analysed ('Ljubljanska ledenka', 'Noisette', 'Marcord' and 'Green Oakleaf'). The values of almost half of the samples (48 % or 15 samples) ranged from 1000 to 2500 mg of $NO_3^-$ $kg^{-1}$. 23 % of the samples (7) ranged between 500 and 1000 mg $kg^{-1}$, 26 % of the samples (8) between 0 and 500 mg $kg^{-1}$. Two samples contained very little nitrate, which is unusual for conventionally grown lettuce (11 mg $NO_3^-$ $kg^{-1}$, 'Noisette' and 47 mg $NO_3^-$ $kg^{-1}$, 'Green Oakleaf'). One sample exceeded the value of 2500 mg $NO_3^-$ $kg^{-1}$ ('Ljubljanska ledenka').

Figure 3 shows the distribution of nitrate content in lettuce according to the method of cultivation (in %). The highest values of nitrates (1000 to 2500 mg $NO_3^-$ $kg^{-1}$ and more) contain samples from the conventional production. In the case of the samples from integrated production, the proportion of such samples is lower, while the organically cultivated samples had values below 1000 mg of $NO_3^-$ $kg^{-1}$.

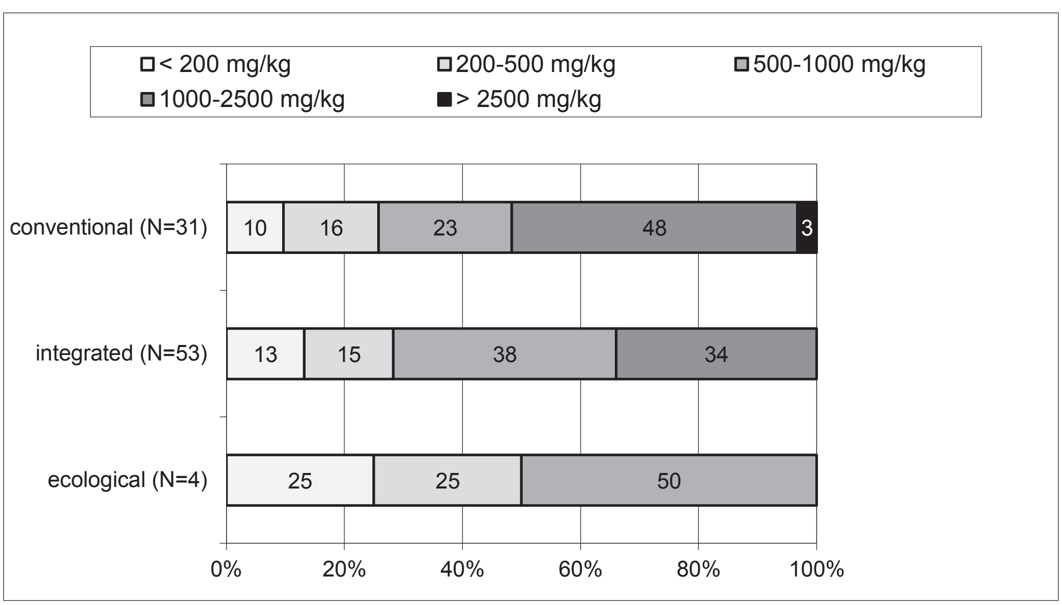



**Figure 3:** The distribution of nitrate content in lettuce according to the method of cultivation (in %)

## 4 CONCLUSION

Farmers advocated for a long time the conventional way of cultivation, where it was possible to produce more crops with less effort. Today, the opinion is different, especially with consumers who want healthy food and clean environment. In Slovenia, the share of producers who cultivate in the traditional way is still great. However, other methods of cultivation are becoming popular, such that use more environmentally friendly methods. The integrated cultivation of vegetables and fruits is becoming almost a demand on the Slovenian market, while more and more farmers are becoming interested in organic farming.

As part of our research we analysed the samples of lettuce of different types and cultivars. Of the 88 samples, there was 1 sample with a value of 3211 mg $NO_3^-$ $kg^{-1}$ which exceeded the statutory limit value of 2000 mg $kg^{-1}$, which applies to Iceberg type lettuce ('Ljubljanska ledenka'). The sample was cultivated outdoors and grown in the conventional way. Other samples did not exceed the statutory limit values, although for 43 % of the samples we determined relatively high values (from 1000 to 2500 mg of $NO_3^-$ $kg^{-1}$). On average, Iceberg type samples contained more $NO_3^-$ than other types of lettuce, which is probably because of different ways of cultivation and other factors that affect the accumulation of nitrates in plants (the use of nitrogen fertilizers, plant illumination, rainfall amount, soil fertility, length of growing, harvesting time and others).

Depending on the method of cultivation (conventional, integrated, organic), the laboratory received 35 % of the samples from conventional production, 60 % of samples from integrated and 4.5 % from organic production. Despite the fact that the number of analysed samples was relatively small, the results of the research confirm that the samples, cultivated in the conventional way, were the most contaminated with nitrates. On the other hand, it is surprising to know that the lowest values of nitrates (<50 $NO_3^-$ mg $kg^{-1}$) were detected in individual samples from integrated and conventional cultivation and not in organic lettuce.

## 5 REFERENCES


Amr, A., Hadidi, N. (2001). Effect of cultivar and harvest date on nitrate and nitrite content of selected vegetables grown under open field and greenhouse conditions in Jordan. *Journal of Agricultural and Food Chemistry, 14*, 59-67. doi:10.1006/jfca.2000.0950

Barker, A.V. (1975). Organic vs. inorganic nutritional and horticultural crop quality. *Hortscience, 10*(1), 50-53.

Below, F.E. (1994). Nitrogen metabolism and crop productivity. In: *Handbook of plant and crop physiology*. Mohammad Pessarakli (Ed.), New York, Marcel Dekker, 275-312.

Boink, A., & Speijers, G. (1999). Health effects of nitrates and nitrites: A review. Proceedings of the International Conference on Environmental Problems Associated with Nitrogen Fertilization of Field Grown Vegetable Crops. Postdam, Germany, ISHS. *Acta Horticulturae, 563*, 29-36. doi:10.17660/ActaHortic.2001.563.2

Brown, J.R., Christy, M., Smith, G.S. (1993). Nitrate in soils and plants. Retrieved from http://extension.missouri.edu/xplor/agguides/agchem/g09804.htm

Chen, BM., Wang, ZH., Li, SX., Wang, GX., Song, HX., Xi-Na, W. (2004). Effects of nitrate supply on plant growth, nitrate accumulation, metabolic nitrate concentration and nitrate reductase activity in three leafy vegetables. *Plant Science, 167*, 635-43. doi:10.1016/j.plantsci.2004.05.015

Commission regulation (EU) No. 1258/2011. (2011). *Amending regulation (EC) No 1881/2006 as regards maximum levels for nitrates in foodstuffs*, OJEU 15.

Dapoigny, L., de Tourdonnet, S., Roger-Estrade, J., Jeuffroy, M.H., Fleury, A. (2000). Effect of nitrogen nutrition on growth and nitrate accumulation in lettuce (*Lactuca sativa* L.), under various conditions of radiation and temperature. *Agronomie, 20*, 843-855. doi:10.1051/agro:2000162

De Martin, S., Restani, P. (2003). Determination of nitrates by a novel ion chromatographic method: occurance in leafy vegetables (organic and conventional) and exposure assessment for Italian consumers. *Food Additives & Contaminants, 20* (9), 787-792. doi:10.1080/0265203031000152415

European Norms EN 12014-7. (1998). *Foodstuffs – Determination of nitrate and/or nitrite content – Part 7: Continuous Flow method for the determination of nitrate content of vegetables and vegetable products after cadmium reduction* (EN standard No. 12014-7). Retrieved from https://standards.globalspec.com/std/415364/cen-en-12014-7

Gangolli, SD., van den Brandt, P., Feron, V., Janzowsky, C., Koeman, J., Speijers, G., Winshnok, J. (1994). Assessment of nitrate, nitrite, and N-nitroso compounds. *European Journal of Pharmaceutics and Biopharmaceutics, 29*, 1-38.

Guadagnin, SG., Rath, S., Reyes, FGR. (2005). Evaluation of the nitrate content in leaf vegetables produced through different agricultural systems. *Food Additives & Contaminants, 22* (12), 1203-08. doi:10.1080/02652030500239649

Hmeljak Gorenjak, A., Rizman Koležni,k U., Cenčič, A. (2012). Nitrate content in dandelion (Taraxacum officinale) and lettuce (Lactuca sativa) from organic and conventional origin: intake assessment. *Food Additives & Contaminants, Part B, 5*(2), 93-99. doi:10.1080/19393210.2012.658873

Kmecl, V., Žnidarčič, D. (2015). Accreditation of analytical method used for determination of nitrate in vegetable. *Archives of Biological Sciences, 67*, 295-302. doi:10.2298/ABS140428046K

Kocjan Ačko, D. (2000). Alternativne oblike kmetovanja. *Novi izzivi v poljedelstvu 2000*, *Moravske Toplice, 14. in 15. december 2000*. Zbornik simpozija. Slovensko agronomsko društvo, Ljubljana, (pp. 244-251).

Matallana Gonzáles, M.C., Martinez-Tomé, M.J., Torija Isasa, M.E. (2010). Nitrate and nitrite content in organically cultivated vegetables. *Archives of Biological Sciences, Part B, 3*(1), 19-29.

McCall, D., & Willumsen, J. (1999). Effect of nitrogen availability and supplementary light on the nitrate content of soil-grown lettuce. *Journal of Horticultural Science and Biotechnology, 74*(4), 458-463. doi:10.1080/14620316.1999.11511137

Merino, L., Darnerund, P.O., Edberg, U., Åman, P. Castillo, MdP. (2006). Levels of nitrate in Swedish lettuce and spinach over the past ten years. *Food Additives & Contaminants, 23*(12), 1283-1289. doi:10.1080/02652030600930543

MKGP RS, Analiza stanja ekološkega kmetovanja. (2016). Retrieved from http://www.mkgp.gov.si/si/delovna_podrocja/kmetijstvo/ekolosko_kmetovanje/analiza_stanja ekoloskega_kmetovanja/

Muramoto, J. (1999). Comparison of nitrate content in leafy vegetables from organic and conventional farms in California. University of California, Santa Cruz: Center for Agroecology and Sustainable Food systems. Retrieved from http://envs.ucsc.edu/shennan/Joji/leafnitrate-1.pdf

Osvald, J., Kogoj Osvald, M. (2003). Integrirano pridelovanje zelenjave, *Kmečki glas* (pp. 1-295). Ljubljana.

Poštrak, N. (2010). Krompir, vaša hrana in zdravilo, Ekološko kmetijstvo v Sloveniji in svetu. *Sončni hribček*, 27-31.

Pussemier, L., Larondelle, Y., Van Peteghem, C., Huyghebaert, A. (2006). Chemical safety of conventionally and organically produced foodstuffs: a tentative comparison under Belgian conditions. *Food Control, 17*, 14-21. doi:10.1016/j.foodcont.2004.08.003

Raigon, M.D., Dominguez Gento, A., Carrot Sierra, J.M., Vidal, E. (2002). Comparación de parámetros de calidad en hortalizas de hoja ancha bajo sistemas de producción ecológica y convencional. *Agricola Vergel*, *241*, 26-32.

Repič, P. (2010). Krompir, vaša hrana in zdravilo, Ekološko kmetijstvo, razvoj in prepoznavanje ekoloških živil. *Sončni hribček*, 33-43.

Sanatamaria, P. (2006). Nitrate in vegetables: toxicity, content, intake and EC regulation. *Food Additives & Contaminants, 86*, 10-17.

Schuddeboom, L. J. (1993). *Nitrates and nitrites in foodstuffs*. Council of Europe Press, Strasbourg.

Džuban, T. (2015). *Tehnološka navodila za integrirano pridelavo zelenjave za leto 2015*. Ministrstvo za kmetijstvo, gozdarstvo in prehrano, Ljubljana.

Uredba komisije (EU) št. 1258/2011. (2011). *Sprememba Uredbe Komisije (ES) št. 1881/2006 glede mejnih vrednosti nitratov v živilih*.

Weightman, RM., Dyer, C., Buxton, J., Farrington, DS. (2006). Effects of light level, time of harvest and position within field on the variability of tissue nitrate concentration in commercial crops of lettuce (*Lactuca sativa*) and endive (*Cichorium endiva*). *Food Additives & Contaminants, 23*, 462-69. doi:10.1080/02652030500522606

Woese, K., Lange, D., Boess, C., Werner Bögl, K. (1997). A comparison of organically and conventionally grown foods. Results of a review of the relevant literature. *Journal of the Science of Food and Agriculture, 74*, 281-293. doi:10.1002/(SICI)1097-0010(199707)74:3<281::AID-JSFA794>3.0.CO;2-Z

Worthington, MS. (2001). Nutritional Quality of Organic Versus Conventional Fruits, vegetables and Grains. *Journal of Alternative and Complementary Medicine*, *7*(2), 161-173. doi:10.1089/107555301750164244

Ysart, G., Clifford, R., Harrison, N. (1999). Monitoring for nitrate in UK-grown lettuce and spinach. *Food Additives & Contaminants, 16*, 301-306. doi:10.1080/026520399283966

Zhong, W., Hu, C., Wang, M. (2002). Nitrate and nitrite in vegetables from north China: Content and intake. *Food Additives & Contaminants, 19*, 1125-1129. doi:10.1080/0265203021000014806

doi:10.14720/aas.2018.111.3.17 Original research article / izvirni znanstveni članek

# Assessing government grants: evidence from greenhouse tomato and pepper farmers in Kosovo

Blend FRANGU[1], Jennie SHEERIN POPP[1], Michael THOMSEN[1] and Arben MUSLIU[2*]

Received September 10, 2018; accepted October 27, 2018.
Delo je prispelo 10. septembra 2018, sprejeto 27. oktobra 2018.

**ABSTRACT**

Genetic matching with an evolutionary algorithm was applied to evaluate the impact of the Ministry of Agriculture, Forestry and Rural Development (MAFRD) grant programs to support greenhouse vegetable production in Kosovo. The primary contribution of the paper is to assess whether grants have an impact on the farmers' gross seasonal revenue after matching similar grantees to non-grantees. The findings showed that greenhouse tomato grantees make 2,151.80 euros more per growing season in comparison to the non-grantees (95 % confidence interval -324.71 to 4,628.31 euros). Similarly, greenhouse pepper grantees make 2,866.69 euros more per growing season compared to non-grantees (95 % confidence interval 446.42 to 5,286.96 euros). The study identified farmers' education and region as important matching variables which may be of interest to policy researchers in Kosovo.

**Key words:** greenhouse economics; genetic matching; government farm grants; Kosovo agriculture

**IZVLEČEK**

**UGOTAVLJANJE UČINKOVITOSTI VLADNIH POMOČI: PRIMERI PRIDELOVALCEV PARADIŽNIKA IN PAPRIKE V RASTLINJAKIH NA KOSOVU**

Genetsko ujemanje in razvojni alagoritem sta bila uporabljena pri vrednotenju vpliva programov pomoči Ministrstva za kmetijstvo, gozdarstvo in razvoj podeželja pri vzpodbujanju pridelave zelenjave v rastlinjakih na Kosovu. Glavni pripevek te raziskave je ocena pomoči na sezonski bruto prihodek kmetov, ki so vladno pomoč dobili v primerjavi s tistimi, ki je niso prejeli. Izsledki so pokazali, da je pomoč pri pridelavi paradižnika v rastlinjakih prispevala 2.151,80 EUR več na sezono v primerjavi s tistimi, ki pomoči niso dobili. (95 % interval zaupanja je znašal -324,71 do 4.628,31 EUR). Podobno je pomoč pri pridelavi paprike v rastlinjaku dala za 2.866,69 EUR več na sezono v primerjavi s tistimi, ki pomoči niso dobili (95 % interval zaupanja je bil 446,42 do 5.286,96 EUR). Raziskava je pokazala, da sta izobrazba kmetov in območje pridelave pomembni vplivni spremenljivki, ki bi lahko zanimali raziskovalce agrarne politike na Kosovu.

**Ključne besede:** ekonomika rastlinjakov; genetsko ujemanje; vladna pomoč kmetom; kmetijstvo Kosova

## 1 INTRODUCTION

The development of Kosovo's agriculture has long been adversely affected by the Kosovo war, a conflict that shaped the dynamics of the agricultural sector and left it vulnerable with large losses of productivity. After the war, the newly formed government of Kosovo invested in agriculture to reestablish a well-functioning economy. Over the last decade, some government investments in the form of competitive grants were targeted to support greenhouse farming. Across Kosovo, tomatoes and peppers are among the main crops grown in greenhouses. In Kosovar greenhouses, tomatoes are more common than peppers (Kaciu, 2008), however, the Ministry of Agriculture, Forestry and Rural Development or MAFRD (2016) suggests that the cultivation of both of these crops have increased since the end of the war.

1 Department of Agricultural Economics and Agribusiness, University of Arkansas, Fayetteville, AR 72701, USA

2 Department of Agricultural Economics, Faculty of Agriculture and Veterinary, University of Prishtina "Hasan Prishtina", Pristina, Kosovo. *Corresponding author: arben.musliu@uni-pr.edu

This paper is part of the MS thesis of B.F. under the supervision of J.S.P.

In Kosovo, agricultural production has intensified because of increasing support through grant programs (Miftari et al., 2016). These grants have helped support desperately needed upgrades in farm facilities. These grants may also affect farmers' gross revenue levels, however, details regarding gross revenue changes are lacking. Similarly, reliable evidence regarding the relationship between government financial support and higher gross farm revenue per growing season could be valuable to policy makers. This study considers whether awardees of the MAFRD grants for the purchase of new greenhouses (grantees) have gross seasonal revenues that differ from non-grantees. We chose to examine the tomato and pepper crops because of their increasing levels of production in a greenhouse setting and because tomatoes and peppers remain among the main vegetables produced in Kosovo (Kaciu et al., 2016). In fact, vegetable farms are among the highest income generating farms (Martinovska Stojceska et al., 2008). Understanding the gross revenue impacts of the grant programs are also important given the increasing inequality in productivity between small and large greenhouse tomato and pepper farms. This may be one factor that is leading to different gross seasonal revenue levels.

Governmental reports address the effectiveness of the MAFRD grant programs (MAFRD, 2017), but empirical studies on the effects of these grants to advance the greenhouse vegetable subsector are lacking. Without the backing of empirical evidence, conclusions reached about the effectiveness of MAFRD's provision of grants for the greenhouse farmers can be misleading. It is important to know what factors impact greenhouse tomato and pepper farmers' ability to win grants. One approach to understand the gross revenue differences between farmer grantees and non-grantees is the use of matching to compare grantees to similar non-grantees. There are many methods available to perform matching and no consensus has emerged in the literature as to the best matching method (Stuart, 2010; Ruiz et al., 2017). There are several studies which have reviewed propensity score matching methods (see e.g. D'Agostino, 1998; Terza et al., 2008; Caliendo & Kopeinig, 2008).

A review of the literature revealed no studies since the early 2000s that have looked at the financial determinants of the greenhouse tomato and pepper production in Kosovo. A matching method known as genetic matching was selected to estimate casual treatment effects of the farmers who received an MAFRD grant. The analysis using this method allows us to quantify the treatment effects of grants on the farmers' gross revenues.

# 2 MATERIALS AND METHODS

## 2.1 Data

The data for the study were obtained from surveys completed by greenhouse tomato and pepper farmers in Kosovo from June to August 2017. Two research surveys (one for each crop) were developed to interview the farmers. There were three steps involved in gathering data from the field: (a) prioritize municipalities and villages with a greater number of farmers growing greenhouse tomatoes and peppers; (b) interview farmers over the age of 18; and (c) evaluate the data for quality and outlying values. The initial sample covered 136 greenhouse farms which, after accounting for data outliers, decreased to 127 greenhouse farms. The farmers producing tomatoes were from the four regions of Ferizaj, Gjakova, Peja and Prizren and those producing peppers were from the four regions of Ferizaj, Gjakova, Peja and Mitrovica.

## 2.2 Descriptive Statistics

The summary statistics of the covariates used for analysis are separated by grant status (grantee or non-grantee) for both greenhouse tomato and pepper farmers (Table 1). These covariates included distance to market in km, education in years, experience in years, and region indicators that take values of 0 or 1. The grantees producing tomatoes are located on average 12.9 km further away from the market than non-grantees and they have roughly two more years of education than non-grantees. However, there is no large difference in years of experience (0.11 years) between grantees and non-grantees producing tomatoes. The majority of these grantees (50 %) are from the region of Prizren. The largest percentage of non-grantees (32 %) are also from the region of Prizren. While grantees producing peppers are located on average only 3.2 km further away from the market than non-grantees and they have roughly three more years of education than non-grantees. The mean level of experience is 5.75 years among grantees showing that these farmers do not have extensive experience in growing greenhouse peppers. Similarly, non-grantees have only one more year of experience than grantees. Grantees producing peppers come mainly from the region of Peja (38 %), however, non-grantees are mostly from the region of Ferizaj (31 %).

**Table 1:** Descriptive statistics of the covariates by grant status

| Grant Status | Grantees | | | | Non-Grantees | | | |
|---|---|---|---|---|---|---|---|---|
| Covariates (x) | Mean | St. Dev. | Min | Max | Mean | St. Dev. | Min | Max |
| *Tomato Farmers* | N = 10 | | | | N = 77 | | | |
| Distance to market in km | 28.23 | 27.52 | 0.30 | 66 | 15.36 | 14.11 | 0.50 | 62 |
| Education in years | 12.20 | 5.01 | 8 | 20 | 10.48 | 2.95 | 8 | 20 |
| Experience in years | 9.60 | 3.41 | 5 | 17 | 9.71 | 7.72 | 2 | 30 |
| Region of Ferizaj | 0.30 | 0.48 | 0 | 1 | 0.16 | 0.37 | 0 | 1 |
| Region of Gjakova | 0.10 | 0.32 | 0 | 1 | 0.04 | 0.20 | 0 | 1 |
| Region of Peja | 0.10 | 0.32 | 0 | 1 | 0.06 | 0.25 | 0 | 1 |
| Region of Prizren | 0.50 | 0.53 | 0 | 1 | 0.32 | 0.47 | 0 | 1 |
| *Pepper Farmers* | N = 8 | | | | N = 32 | | | |
| Distance to market in km | 28.38 | 20.58 | 10 | 60 | 25.22 | 21.45 | 3 | 84 |
| Education in years | 13.88 | 4.22 | 8 | 20 | 11.06 | 2.65 | 8 | 15 |
| Experience in years | 5.75 | 2.92 | 2 | 9 | 6.41 | 2.80 | 2 | 13 |
| Region of Ferizaj | 0.25 | 0.46 | 0 | 1 | 0.31 | 0.47 | 0 | 1 |
| Region of Gjakova | 0.25 | 0.46 | 0 | 1 | 0.13 | 0.34 | 0 | 1 |
| Region of Peja | 0.38 | 0.52 | 0 | 1 | 0.13 | 0.34 | 0 | 1 |
| Region of Mitrovica | 0.13 | 0.35 | 0 | 1 | 0.09 | 0.30 | 0 | 1 |

### 2.3 Methods

Early work to develop propensity score matching (PSM) was conducted by Rosenbaum and Rubin (1983), and has become a widely used approach to estimate causal treatment effects (Caliendo & Kopeinig, 2008). Propensity score matching can be performed using various methods to match subjects. One method includes genetic matching as a multivariate matching method. In this study, the genetic matching algorithm is used to find covariate balance after matching between MAFRD grantees and non-grantees. The implementation of this method enables us to estimate the average treatment effect on the treated (ATT), which we use to assess the average differences in the farmers' gross seasonal revenue between grantees and non-grantees. According to Diamond and Sekhon (2013), genetic matching is performed by reducing a generalized version of the Mahalanobis distance (GMD). In contrast to the Mahalanobis distance, genetic matching includes an extra weight parameter W.

$$GMD(X_i,X_j,W) = \sqrt{(X_i-X_j)^T (S^{-1/2})^T W S^{-1/2} (X_i-X_j)}$$

From equation (1), $X_i$ and $X_j$ are covariates from farmers i and j, respectively. The matrix from the model contains the covariates described in Table 1. W is a $k \times k$ positive definite weight matrix, S is the sample covariance of matrix S, and $S^{-1/2}$ is the Cholesky decomposition of S (Diamond & Sekhon, 2013). Replacement was used to ensure that a farmer who received a grant (treatment group) has a proper match with a non-grantee (control group). It is noted in the literature that matching with replacement can provide better matches (Stuart & Rubin, 2008), and is preferred to use in methods with a control group that has similar values relative to a treatment group (Dehejia & Wahba, 2002). Lastly, we do our analysis with the help of the R-CRAN package "Matching" pioneered by Sekhon (2011).

### 2.4 Considerations in covariate selection

Four factors that we measure may have influence on a MAFRD grantee's ability to match with a non-grantee. The first covariate is distance to market in km. Farmers' markets bring consumers closer to producers (Ling & Newman, 2011), and the farmer's distance to market may impact both produce quantity and ability to sell the produce in a timely manner. Distance from farm to market can also be an important factor determining the farmer's access to the product markets (Ahmed, et al., 2016).

Agricultural education may influence productivity gains (Fîntîneru & Madsen, 2012). An earlier study that used propensity score matching found that education was positive and significant for cherry production (Ali et al., 2013). In a later study, education was found to be a contributing factor affecting the farmer's income (Panda, 2015). Based on these previous studies, we concluded that education was an important matching variable. Farm experience is included as a matching variable for similar reasons. Farmers' years of experience vary by region in Kosovo when growing greenhouse tomatoes and peppers. For example, given the strong tradition of tomato production in the region of Prizren, it is expected that Prizren greenhouse tomato producers may have more years of farm experience than producers in other regions. For greenhouse pepper producers, however, years of farm experience may differ little from region to region.

Lastly, we consider using covariates to control for four greenhouse tomato and pepper producing regions. Potentially, region may be an important variable in matching MAFRD grantees to non-grantees. For example, Kosovo's regions can have differences in the production of greenhouse vegetable crops because of the climatic conditions, or because of regional differences in family farming traditions. However, reasons may vary as why farmers from one region or another are more or less likely to acquire government farm grants. Therefore, it is important to use the covariate region in the matching procedures.

## 3 RESULTS AND DISCUSSION

When analyzing gross revenue per growing season, it was suggested from both groups of farmers that grantees compared to non-grantees were associated with higher revenue levels (Figure 1). The box plot analysis from Figure 1 shows that farmers producing tomatoes who received MAFRD grants have a mean of 5,759.30 euros while non-grantees have a lower mean of 3,178.84 euros of gross revenue per growing season. Grantees producing peppers suggest a mean of 5,080.43 euros while non-grantees suggest a lower mean of 3,739.02 euros of gross seasonal revenue. Figure 1 indicates also that when observing the densities of both groups of farmers, grantees highlight higher seasonal revenue levels than non-grantees. There were only few grantees producing tomatoes and peppers with gross seasonal revenue greater than 10,000.00 and less than 3,000.00 euros. However, there were more non-grantees producing tomatoes and peppers with revenue levels less than 3,000.00 euros. Considering that grants could have a positive impact on farmers' gross seasonal revenues, we estimated possible differences using gross seasonal revenue as the outcome variable in the model. As explained above, covariates including distance to market in km, education in years, and farm experience in years were used in the matching of greenhouse tomato and pepper grantees to non-grantees. Depending on the region, however, farmers were sometimes from different locations. For the matching of greenhouse tomato grantees to non-grantees, we included the regions of Ferizaj, Gjakova, Peja and Prizren. While, the regions of Ferizaj, Gjakova, Peja and Mitrovica were used in the matching of greenhouse pepper grantees to non-grantees.

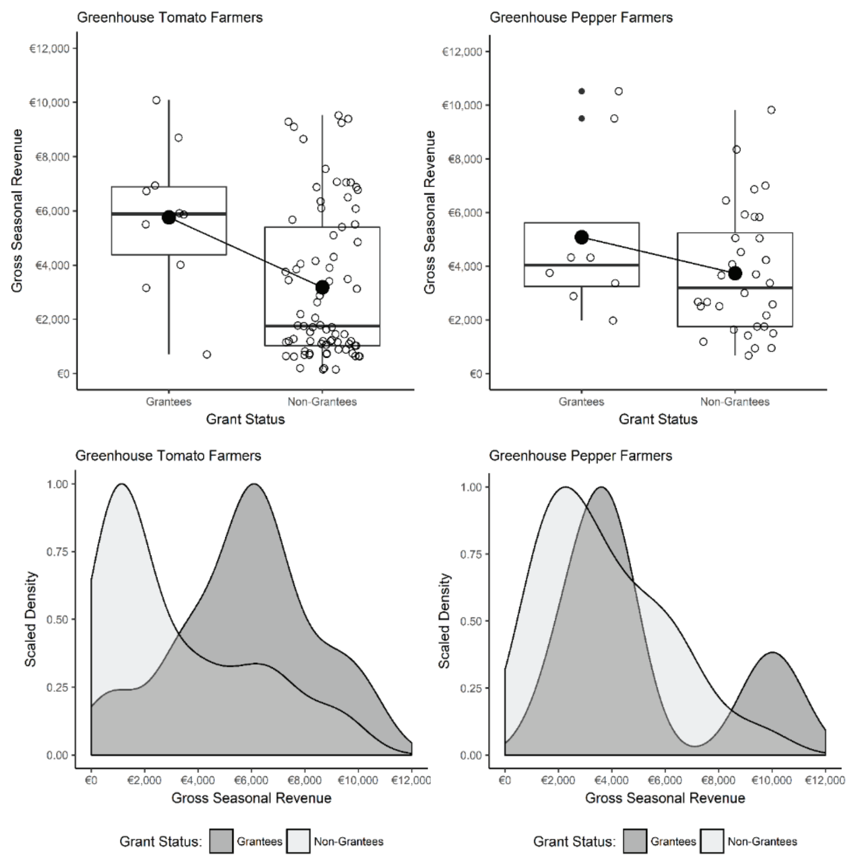



**Figure 1:** Greenhouse Tomato and Pepper Farmers' Gross Seasonal Revenue Levels

The average treatment effect on the treated (ATT) estimates revealed significant differences in gross seasonal revenue among greenhouse tomato and pepper farmer grantees and non-grantees. There was a statistically significant impact of grants for both greenhouse tomato and pepper farmers (Tables 2 and 3). Table 2 shows a positive and marginally significant ($p < 0.1$) ATT among greenhouse tomato farmers. The estimate of a difference of 2,151.80 euros in gross revenue per growing season was estimated for grantees relative to non-grantees. The 95 % confidence interval is -324.71 to 4,628.31 euros per growing season. However, it should be noted that the study contains a smaller sample of farmers than was desired. Therefore, ATT results could vary with a larger sample. The findings here suggest that it is possible MAFRD grant programs positively affect gross seasonal revenue levels of greenhouse tomato farmers that were awarded grants.

**Table 2**: Greenhouse Tomato Grantees' Average Treatment Effect on the Treated

| | Greenhouse Tomato Grantees | | | | | |
|---|---|---|---|---|---|---|
| | | | | | 95% CI | |
| Outcome Variable | Unit | Mean | T-stat | p-value | Lower | Upper |
| *Gross Seasonal Revenue* | | | | | | |
| Estimate | euro | 2,151.80 | 1.703 | 0.088* | -324.71 | 4,628.31 |

Note: T-stat, t statistic; CI, confidence interval. The statistical significance of the estimate is denoted by *p < 0.1; **p < 0.05; ***p < 0.01.

Similarly, Table 3 shows a positive and significant ($p < 0.05$) ATT for greenhouse pepper farmers. The estimate of a difference of 2,866.69 euros in gross seasonal revenue was suggested for grantees relative to non-grantees. The 95 % confidence interval is 446.42 to 5,286.96 euros per growing season. The MAFRD grant programs seem to influence positively the gross seasonal revenue levels of greenhouse pepper farmers.

**Table 3:** Greenhouse Pepper Grantees' Average Treatment Effect on the Treated

| | Greenhouse Pepper Grantees | | | | | |
|---|---|---|---|---|---|---|
| | | | | | 95% CI | |
| Outcome Variable | Unit | Mean | T-stat | p-value | Lower | Upper |
| *Gross Seasonal Revenue* | | | | | | |
| Estimate | euro | 2,866.69 | 2.322 | 0.020** | 446.42 | 5,286.96 |

Note: T-stat, t statistic; CI, confidence interval. The statistical significance of the estimate is denoted by *p < 0.1; **p < 0.05; ***p < 0.01.

The evolutionary algorithm of genetic matching determines the weight each covariate receives (Diamond & Sekhon, 2013). These weights are used in the matching estimate of the ATT. Improvements in the standardized mean difference (SMD) between pre and post matched samples are influenced by these covariate weights. Covariate balance results for tomato producers are presented in Table 4. There was a slight improvement in the mean value of distance to market. This covariate's SMD went from 46.77 to -36.95 showing that it was not considered important by the algorithm. In fact, distance to market had a low weight of only 3.86. Education in years showed a mean improvement of roughly a year and a half of education. Its SMD reduced from 34.34 to 5.99, and with a much larger weight of 601. Although experience in years had a relatively high weight of 440, yet its SMD did not improve. Regions of Ferizaj, Gjakova, and Peja marked SMD improvements from 29.84 to 0, 19.30 to 0, and 11.10 to 0, respectively. The three regions showed balance in mean values in the post-match phase (Table 4). Accordingly, the three regions were assigned high weights (594, 655, and 619) which could support the improvement in balance for these region indicators. However, region of Prizren registered a smaller improvement in its SMD and mean value. This covariate highlighted only a weight of 229. Overall balance was favored for the measure of education and indicators for the regions of Ferizaj, Gjakova, and Peja.

**Table 4:** Greenhouse Tomato Covariate Balance Results

| Covariate | Pre-Match (N=87) | | | Post-Match (N=10) | | |
|---|---|---|---|---|---|---|
| Tomato Farmers | Grantees | Non-Grantees | d | Grantees | Non-Grantees | d |
| Distance to market | 28.23 | 15.36 | 46.77 | 28.23 | 38.40 | -36.95 |
| Education in years | 12.20 | 10.48 | 34.34 | 12.20 | 11.90 | 5.99 |
| Experience in years | 9.60 | 9.71 | -3.36 | 9.60 | 9.90 | -8.81 |
| Region of Ferizaj | 0.30 | 0.16 | 29.84 | 0.30 | 0.30 | 0 |
| Region of Gjakova | 0.10 | 0.04 | 19.30 | 0.10 | 0.10 | 0 |
| Region of Peja | 0.10 | 0.06 | 11.10 | 0.10 | 0.10 | 0 |
| Region of Prizren | 0.50 | 0.32 | 33.27 | 0.50 | 0.40 | 18.97 |

Note: N, number of observations; d, standardized mean difference.

As in the case of tomato farms, distance to market similarly continued to have an imbalance in in the post-matched sample of pepper farms. This measure received a low weight of 126. Likewise, education in years did not show a large reduction in its mean or SMD. Its SMD decreased from 66.59 to 38.47 and it had a weight of 225. Experience in years had the highest weight (909) and the balance improved from -22.51 to -8.57. The SMDs of regions of Ferizaj and Gjakova decreased from -13.50 to 0 and 27 to 0, respectively. The two regions showed balance in the post-match phase (Table 5). In addition, the former region had a weight of 814 and the latter a weight of 899. Region of Peja had a small balance in its mean and SMD and a low weight of 71. While the mean of region of Mitrovica improved, its SMD degraded from 8.84 to -35.36. However, this indicator had a very small weight (4.94) among the covariates. In comparison to greenhouse tomato farmers, balance was favored partly for the covariate experience in years and indicators for the regions of Ferizaj and Gjakova.

**Table 5:** Greenhouse Pepper Covariate Balance Results

| Covariate | Pre-Match (N=40) | | | Post-Match (N=8) | | |
|---|---|---|---|---|---|---|
| Pepper Farmers | Grantees | Non-Grantees | d | Grantees | Non-Grantees | d |
| Distance to market | 28.38 | 25.22 | 15.33 | 28.38 | 27.50 | 4.25 |
| Education in years | 13.88 | 11.06 | 66.59 | 13.88 | 12.25 | 38.47 |
| Experience in years | 5.75 | 6.41 | -22.51 | 5.75 | 6.00 | -8.57 |
| Region of Ferizaj | 0.25 | 0.31 | -13.50 | 0.25 | 0.25 | 0 |
| Region of Gjakova | 0.25 | 0.13 | 27.00 | 0.25 | 0.25 | 0 |
| Region of Peja | 0.38 | 0.13 | 48.31 | 0.38 | 0.25 | 24.15 |
| Region of Mitrovica | 0.13 | 0.09 | 8.84 | 0.13 | 0.25 | -35.36 |

Note: N, number of observations; d, standardized mean difference.

## 4 CONCLUSIONS

The presence of the government grant programs as an agricultural policy may provide the opportunity to promote Kosovo's greenhouse production given that each year more and more farmers apply to the MAFRD grant programs. In this study, important matching variables for greenhouse tomato farmers were education and indicators for the regions of Ferizaj. Gjakova, and Peja. While indicators for the regions of Ferizaj and Gjakova and partly experience in years were most important for greenhouse pepper farmers.

Policy researchers in Kosovo may take interest in the evidence of the positive gross seasonal revenue difference of 2,151.80 euros for the greenhouse tomato grantees relative to the non-grantees, and 2,866.69 euros for the greenhouse pepper grantees relative to the non-grantees. This evidence may help to identify which group of greenhouse farmers are likely to be influenced from the MAFRD grant programs. The study results should also be of interest to nonprofit organizations and agencies for development that invest to help MAFRD's efforts in Kosovo for the provision of new and upgraded farm facilities and greenhouses. Regarding the impact estimates, this study found the genetic matching method with a good convergence of the results with our sample of surveyed farmers. Despite the large or small mean differences of the covariates pertaining to the greenhouse tomato grantees and non-grantees prior to matching, the genetic matching method provided a significant improvement in the covariate balance. Nevertheless, it should be noted that the study contained a sample of farmers that was not sufficiently large, and ATT results could vary with a larger sample.

In conclusion, these overall results suggest that grants awarded to the greenhouse farmers improved their gross revenue levels per growing season. Greenhouse tomato and pepper MAFRD grantees attained higher gross seasonal revenue levels relative to the non-grantees. Considering balance on the covariates, it was found that based on the farmers' education and depending on the region, balance was possible for MAFRD grantees and non-grantees.

## 5 REFERENCES

Ahmed, U. I., Ying, L., Bashir, M. K., Abid, M., Elahi, E. and Iqbal, M. A. (2016). Access to output market by small farmers: The case of Punjab, Pakistan. *Journal of Animal and Plant Sciences*, *26*(3), 787–793.

Ali, A., Sharif, M., Mahmood, K. and Akmal, N. (2013). Determinants of cherry production and marketing in Pakistan: A propensity score matching approach. *Agricultural Economics Review*, *14*(1).

Caliendo, M. and Kopeinig, S. (2008). Some practical guidance for the implementation of propensity score matching. *Journal of Economic Surveys*, *22*, 31–72, doi:10.1111/j.1467-6419.2007.00527.x

D'Agostino, R. B. (1998). Propensity score methods for bias reduction in the comparison of a treatment to a non-randomized control group. *Statistics in Medicine*, 17, 2265-2281, doi:10.1002/(SICI)1097-0258(19981015)17:19<2265::AID-SIM918>3.0.CO;2-B

Dehejia, R. H. and Wahba, S. (2002). Propensity score matching methods for non-experimental causal studies. *The Review of Economics and Statistics*, *84*, 151–161, doi:10.1162/003465302317331982

Diamond, A. and Sekhon, J. S. (2013). Genetic matching for estimating causal effects: A general multivariate matching method for achieving balance in observational studies. *The Review of Economics and Statistics, 95*, 932–945, doi:10.1162/REST_a_00318

Fîntîneru, G. and Madsen, O. (2012). Farmers' education and farm productivity. Evidence from Denmark and from Romania. *AgroLife Scientific Journal*, *2*(1), 175–180.

Kaciu, S. (2008). *Study of the current situation of pepper production in Kosovo*. Retrieved from http://helvetas-ks.org/documentation/technical_reports/Study_of_the_current_situation_of_pepper_production_in_Kosovo_HPK_Prishtina_35.pdf.

Kaciu, S., Babaj, I., Aliu, S. and Demaj, I. (2016). Potential of protected vegetable production in Kosovo and future perspectives. *Acta Hortic, 1142,* 461–466, doi:10.17660/ActaHortic.2016.1142.70

Ling, C. and Newman, L. L. (2011). Untangling the Food Web: Farm-to-market distances in British Columbia, Canada. *Local Environment*, *16*, 807–822, doi:10.1080/13549839.2010.539602

Martinovska Stojcheska A., Georgiev, N. and Erjavec, E. (2008). Farm income analysis of agricultural holdings in Macedonia using FADN methodology. *Acta agriculturae Slovenica*, *92*(1), 41-51.

Miftari, I., Hoxha, B. and Gjokaj, E. (2016). *Kosovo*: Agricultural policy brief (CAPB).* Joint Research Centre: Institute for Prospective Technological Studies (IPTS).

Ministry of Agriculture, Forestry and Rural Development. (2016). *Green report*. Retrieved from http://www.mbpzhr-ks.net/repository/docs/Green_Report_Kosovo_2016_Final_050417.pdf.

Ministry of Agriculture, Forestry and Rural Development. (2017). *Green report*. Retrieved from http://www.mbpzhr-ks.net/repository/docs/Raporti_i_Gjelber_2017_Eng_Final.pdf.

Panda, S. (2015). Farmer education and household agricultural income in rural India. *International Journal of Social Economics*, *42*, 514–529, doi:10.1108/IJSE-12-2013-0278

Ruiz, D., Stout, D. and Herlihy, C. (2017). Use of genetic matching in program evaluation: The case of RAD. *Cityscape*, *19*(2), 337–349.

Sekhon, J. S. (2011). Multivariate and propensity score matching software with automated balance optimization: The Matching package for R. *Journal of Statistical Software*, *42*, 1–52. doi:10.18637/jss.v042.i07

Stuart, E. (2010). Matching methods for causal inference: A review and a look forward. *Statistical Science: A Review Journal of the Institute of Mathematical Statistics*, *25*(1), 1–21, doi:10.1214/09-STS313

Stuart, E. and Rubin, D. B. (2008). *Best Practices in Quasi–Experimental Designs: Matching Methods for Causal Inference*. Thousand Oaks, CA: SAGE Publications Ltd.

Terza, J. V., Basu, A. and Rathouz, P. J. (2008). Two-stage residual inclusion estimation: Addressing endogeneity in health econometric modeling, *Journal of Health Economics*, *27*(3), 531–543, doi:10.1016/j.jhealeco.2007.09.009

doi:10.14720/aas.2018.111.3.18 Review article / pregledni znanstveni članek

# Deficitni princip namakanja vinske trte (*Vitis vinifera* L.) – pregled dosedanjih izkušenj in izhodišča za Slovenijo

Tina SMRKE[1], Vesna ZUPANC[2*]

Received November 10, 2017; accepted November 25, 2018.
Delo je prispelo 10. novembra 2017, sprejeto 25. novembra 2018.

**IZVLEČEK**

Deficitno namakanje je eden izmed najbolj raziskanih načinov upravljanja namakanja vinske trte, katerim so raziskovalci potrdili veliko pozitivnih učinkov na pridelavo grozdja. Za doseganje optimalne rasti in mase grozdja pri posamezni sorti moramo vinski trti med rastno dobo zagotoviti ustrezno količino vode. Vinsko trto bo potrebno v bližnji prihodnosti zaradi vse pogostejše suše ponekod namakati tudi v Sloveniji. Pri deficitnem namakanju z manjšo količino dodane vode, kot bi bilo optimalno, vplivamo na rast vinske trte in s tem na kakovost in količino pridelka. Pričakovani rezultat deficitnega namakanja so krajše mladike (15,5 % pri sorti 'Monastrell', manjša listna površina, manjša masa lesa po rezi), manjše jagode in s tem manjši pridelek za 38 % do 57 % pri sorti 'Monastrell' in 24 % do 27 % pri sorti 'Tempranillo', manjše število grozdov na trto, primernejšo sestavo jagod (več sladkorjev in antocianov, manj kislin) in učinkovitejšo rabo vode, pomeni več pridelka na enoto dodane vode. Zelo primeren način za nadzor deficitnega namakanja pri vinski trti je merjenje vodnega potenciala rastline. Za uspešen prenos principa deficitnega namakanja v prakso je potrebno poznati tudi kritične fenofaze različnih sort vinske trte, in odziv sorte v dotičnem okolju. Uspešen prenos deficitnega namakanja v prakso bo mogoč le ob finančni in strokovni podpori pridelovalcem.

**Ključne besede:** vinska trta; namakanje; deficitno namakanje; rast; rodnost; sestava grozdnih jagod

**ABSTRACT**

**DEFICIT IRRIGATION OF VINES (*Vitis vinifera* L.) – REVIEW OF EXPERIENCES AND POTENTIAL FOR SLOVENIA**

Deficit irrigation is one of the most researched irrigation water management techniques for vines with many potential benefits for successful grape production. For optimal growth and grape quantity of individual variety, suitable water quantity over growing season should be provided. Due to more frequent droughts vine irrigation will be needed also in Slovenia. The principle of deficit irrigation is affecting vine growth and quality and quantity of the yield by adding smaller amount of water than optimal. Decreased vine growth (reduced growth of shoots, 15.5 % for 'Monastrell, reduced leaf area, reduced pruning mass), smaller berries, and thus yield quantity from 38 % to 57 % for 'Monastrell' and 24 % to 27 % for 'Tempranillo', respectively, improved berry composition (higher sugar and antocianin content, lower acid content), better water use efficiency, meaning higher yield per unit of added water, are expected. Most suitable method for deficit irrigation management of vines is by measuring plant water potential. For successful transfer of deficit irrigation in practice, good knowledge of critical growth stages of irrigated vine variety and its behavior in a certain environment is needed. Successful application of this irrigation method in practice will be possible only with financial and expert support.

**Key words:** vines; irrigation; deficit irrigation; growth; fertility; grape berry composition

[1] Mag. inž. hort., Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za agronomijo, Jamnikarjeva 101, Ljubljana, tina.smrke@bf.uni-lj.si

[2] Doc. dr., Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za agronomijo, Jamnikarjeva 101, Ljubljana, *corresponding author: vesna.zupanc@bf.uni-lj.si

**SEZNAM OKRAJŠAV**

$\Psi$ – vodni potencial
$\Psi_{PD}$ – vodni potencial pred zoro
$\Psi_s$ – opoldanski vodni potencial
$ET$ – evapotranspiracija
$ET_a$ – dejanska evapotranspiracija
$ET_C$ – potencialna evapotranspiracija rastline oziroma v primeru tega članka trte
$ET_0$ – referenčna evapotranspiracija
INK – izmenično namakanje korenin
IPNK – izmenično polovično namakanje korenin
$k_c$ – koeficient rastline
NDN – nadzorovano deficitno namakanje
OIV – Mednarodna organizacija za trto in vino (International Organisation of Vine and Wine)
PN – podzemno namakanje
TDN – trajno deficitno namakanje
WP –produktivnost rastlin
$Y_a$ – masa tržnega pridelka

## 1 UVOD

Glede na zadnje podatke Mednarodne organizacije za trto in vino (International Organisation of Vine and Wine; OIV, 2018) je površina svetovnih vinogradov leta 2017 pokrivala 7,6 milijonov ha. Največji doprinos k svetovni pridelavi grozdja imajo ravno evropske države, predvsem Španija, Francija ter Italija, saj pridelajo okoli 70 % celotnega svetovnega pridelka grozdja in zavzemajo okoli 60 % skupnih pridelovalnih površin grozdja v svetu (Malheiro in sod., 2010). V Sloveniji je bilo leta 2016 v Register pridelovalcev grozdja in vina (RPGV) vpisano okoli 16 000 ha vinogradov (Simončič in sod., 2017).

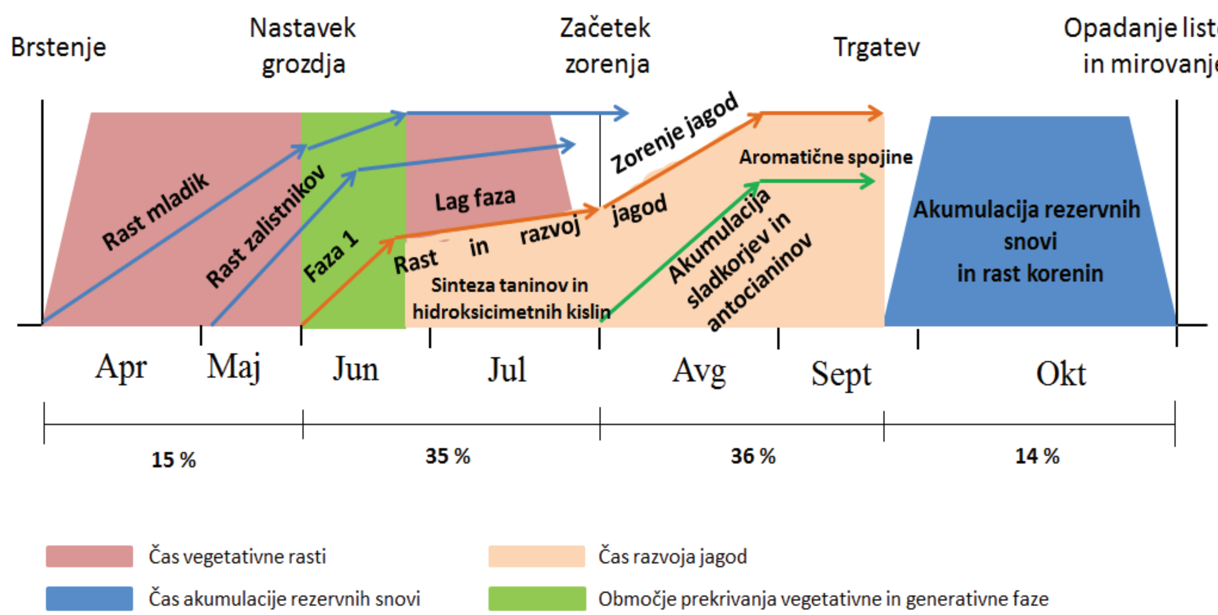
Območje pridelave grozdja v Evropi se zaradi segrevanja ozračja pomika proti severu, medtem ko se bodo pridelovalci grozdja v državah južne Evrope in drugod po svetu (Kajfež-Bogataj, 2009; Hannah in sod., 2013) vse pogosteje spopadali s sušo (Malheiro in sod., 2010). Zaradi tovrstnih razmer je namakanje neizbežen tehnološki ukrep, v mnogih državah pridelovalkah grozdja, kot so Alžirija, Avstralija, Čile, Italija, Črna Gora, Nova Zelandija, Srbija, Slovenija, Španija, Švica in Urugvaj (Matthews in sod., 1987; FAO database, 2016). Po podatkih Statističnega urada Republike Slovenije (Stat, www.stat.si) smo vinograde in trsnice v Sloveniji prvič namakali leta 2007, in sicer 4 ha, leta 2014 se je že namakalo 12 ha. Dandanes namakanje slovenskih vinogradov ni pogosta praksa, za katero nimamo uradnih podatkov.

Zaradi nenadzorovane porabe vode pri namakanju kmetijskih rastlin in hkrati globalnega segrevanja ozračja lahko pride v naravi do velike omejitve razpoložljivih vodnih virov (Döll, 2002; Kumar Kar, 2011), zato pridelovalci iščejo najbolj učinkovit način namakanja, s katerim bi dosegli optimalno razmerje med rastjo in rodnostjo, primerno sestavo jagod ter hkrati minimalno porabo vode (Cifre in sod., 2005; Edwards in Clingeleffer, 2013; Zhang in sod., 2014). Trti je potrebno dodati toliko vode, da se ji ustrezno omeji vegetativno rast, optimizira maso pridelka in izboljša sestavo jagod, kar je najbolj pogojeno z značilnostmi gojene sorte. Za doseganje opisanih ciljev je danes v vinogradništvu najbolj primerno deficitno namakanje, pri kateremu trtam dodamo manjšo količino vode, kot znaša njihova evapotranspiracija (Fereres in Soriano, 2007; Ruiz-Sanchez in sod., 2010; Lanari in sod., 2014). Deficitno namakanje vinske trte se v državah Sredozemlja ter v aridnih delih Severne in Južne Amerike uspešno uporablja s tehnologijo kapljičnega namakanja in namakanja z razpršilci (Moriana in sod., 2003, Ruiz-Sanchez in sod., 2010), vendar je zaradi večje učinkovitosti smotrnejša uporaba kapljičnega namakanja.

Raziskav o vplivu sušnega stresa oziroma uravnavanja vodnega režima vinske trte pri nas še ni, se pa podrobno raziskuje občutljivost vinske trte na bolezni in škodljivce (Hladnik in sod., 2014; Lamovšek in sod., 2014; Štrukelj in sod., 2016) ter preverja avtohtonost sort (Štajner, 2010; Pelengič in sod., 2012; Imazio in sod., 2014; Rusjan in sod., 2015), saj naj bi bile udomačene, lokalne sorte vinske trte najmanj prizadete v sušnih rastnih razmerah.

## 2 POTREBE VINSKE TRTE PO VODI

Na odziv vinske trte na sušni stres vpliva več dejavnikov. Z vidika pridelka je ključno vprašanje, za kateri namen gojimo vinsko trto, namizno grozdje ali pridelavo vina. V Sloveniji imamo 95 % vinske trte namenjene za pridelavo vina, zato v prispevku obravnavamo zgolj to. Vinska trta ima v posameznih fenofazah različne potrebe po vodi (Slika 1). Med brstenjem in razvojem mladik (BBCH 01-10) korenine črpajo vodo in v njej raztopljene rudninske snovi, ki so potrebne za uspešno rast vinske trte. Stalna preskrba z vodo med rastjo mladik in listov (BBCH 11-19) omogoči optimalen razvoj listne stene, ki je pomembna za absorpcijo svetlobe in tvorbo asimilatov. Od brstenja do začetka cvetenja (BBCH 07-60) znaša potreba vinske trte po vodi 9 % potreb celotne rastne dobe. Takrat je voda potrebna za optimalno rast korenin in razvoj listne stene ter nastavek grozdja za prihajajočo in naslednjo sezono. Med začetkom cvetenja in razvojem plodičev (BBCH 60-70) je zadostna količina vode, ki znaša 6 % nujna za tvorbo fertilnega peloda, pestiča in prašnikov ter za uspešno oprašitev in oploditev. Največ vode vinska trta potrebuje med debeljenjem jagod (BBCH 71-79), in sicer okrog 35 % celokupne potrebne količine v rastni dobi, to je od 1. 4. do 31. 10. (Maljevič, 2003). Rast jagod sprva poteka na račun hitre delitve celic in kasneje njihovega večanja. Če v tem času pride do pomanjkanja vode, bo manjše število celic in hkrati tudi njihova velikost, kar se bo odražalo v manjši končni velikosti jagod. Med zorenjem, vse do trgatve (BBCH 81-89) vinska trta potrebuje približno 36 % celokupne potrebne količine vode v rastni dobi, z namenom, da trte ne izpostavljamo sušnemu stresu po nepotrebnem in ji ohranjamo zdravo listno steno. Od trgatve in vse do odpadanja listov (BBCH 91-97) znaša poraba vode vinske trte 14 % porabe celotne rastne dobe. Pred mirovanjem je pomembno, da se v listih še vedno sintetizirajo sladkorji, ki se nato transportirajo in skladiščijo v lesu vinske trte za naslednjo rastno dobo (Moyer in sod., 2013; Goldammer, 2018).




**Slika 1**: Razpored fenofaz in delež potreb vinske trte po vodi v rastni dobi (prirejeno po Romero in sod., 2013)
**Figure 1**: Vine's growth stages and water demand in the growing season (adapted from Romero et al., 2013)

Vinski trti najbolj ustrezajo globoka in zračna tla, ki dobro prepuščajo vodo, da lahko razvije globok koreninski sistem. Slednji ji omogoča lažje preživetje sušnih razmer, da najde in izkoristi tudi najmanjše zaloge razpoložljive vode v tleh (Vršič in Lešnik, 2010). Koreninski sistem vinske trte se lahko razvije do globine več metrov, sesalna sila za vodo lahko znaša do −1,6 MPa (Deluc in sod., 2009; Vršič in Lešnik, 2010). Enako kot ostale rastline trta uravnava vodni status z regulacijo prevodnosti listnih rež. Glede na ohranjanje vodnega potenciala v listih ($\Psi$) ločimo izohidre in anizohidre sorte vinske trte (Schultz, 2003; Hochberg in sod., 2017). Izohidre sorte ob pomanjkanju vode v tleh natančno regulirajo transpiracijo listov in hitro zapirajo listne reže, da ohranjajo vodni status blizu optimalnega. Pri teh sortah, je $\Psi$ redko manjši od −1,5 MPa (Lovisolo in sod., 2010). Dosedanje raziskave kažejo, da so izohidre sorte vinske trte 'Montepulciano', 'Grenache', 'Modra frankinja', in 'Portugalka' (Zsófi in sod., 2008). Anizohidre sorte so tolerantnejše na pomanjkanje vode in listnih rež, ob manjši količini razpoložljive vode ne zapirajo tako hitro, kar pomeni, da njihov $\Psi$ bolj variira. Primer takšnega načina ohranjanja vodnega režima so sorte 'Shyrah', 'Sangiovese', 'Semillon' in 'Chardonnay' (Rogiers in sod., 2009). Pri omenjeni razdelitvi sort vinske trte lahko zaradi različnih okoljskih razmer pride do odstopanj, saj ima lahko ista sorta izohidrni in tudi anizohidrni odziv na pomanjkanje vode, ki je po navedbah mnogih avtorjev odvisen od fenofaze rastline in pojava suše v dnevnem ciklu in rastni dobi (Medrano in sod., 2003; Chaves in sod., 2007; Rogiers in sod., 2009; Lovisolo in sod., 2010; Conesa in sod., 2016). Sorte s tovrstnim odzivom so 'Syrah', 'Grenache', 'Merlot' in 'Modri Pinot' (Lovisolo in sod., 2010; Zhang in sod., 2012). Kljub dobrim fiziološkim, morfološkim in anatomskim prilagoditvam vinske trte na pomanjkanje vode v tleh, lahko pride do negativnih posledic suše, ki se najpogosteje kažejo kot omejena rast mladik, manjši pridelek in slabša sestava jagod (Intrigliolo in Castel, 2009).

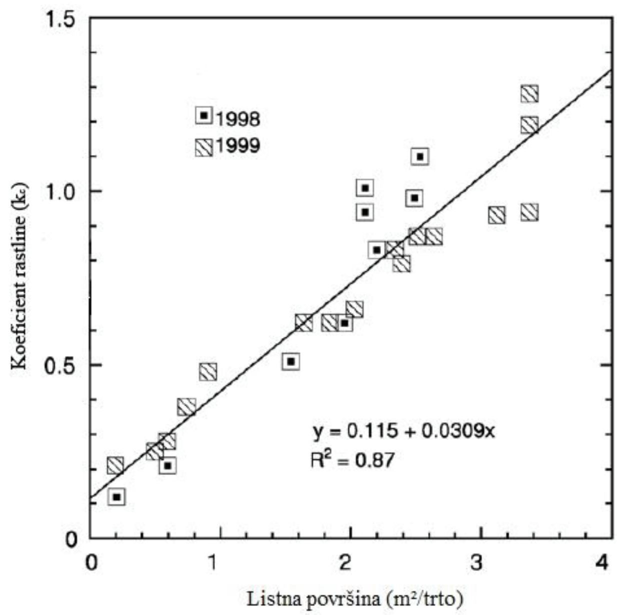
Pri vinskih sortah so zaželene manjše jagode, z manjšo vsebnostjo kislin in večjo vsebnostjo sladkorjev ter fenolnih spojin, ki so po McCarthy (1997) tudi edini parametri, ki vplivajo na končno kakovost vina. To se lahko doseže, če ima vinska trta na voljo manj vode, kot je potencialna evapotranspiracija ($ET_c$), kar pomeni, da se trte načrtno izpostavi blagemu sušnemu stresu. V številnih raziskavah so v jagodah trt pod sušnim stresom izmerili večjo vsebnost antocianov in drugih fenolnih snovi kot v jagodah trt, ki so bile zadostno preskrbljene z vodo (Matthews in Anderson, 1988; Matthews in sod., 1990; Roby in sod., 2004; Salon in sod., 2005). Prekomerno dodajanje vode lahko vodi do manj izrazite barve vina zaradi manjše vsebnosti antocianov v kožici jagod, manjše vsebnosti sladkorjev ter fenolnih spojin in kislinskega neravnovesja (Hepner in sod., 1985; Esteban in sod., 2001; Lanari in sod., 2014).

## 3 DOLOČANJE POTREB PO VODI

Na volumen trti razpoložljive vode v vinogradnih se lahko vpliva z različnimi tehnološkimi ukrepi (Cancela in sod., 2017; Tomaz in sod., 2017), katerih izvajanje je pomembno pred ureditvijo in v obdobju rodnosti vinograda. Pomembna je izbira lege (Stajnko in sod., 2010), obdelava tal pred sajenjem, predvsem je pomembno globoko rigolanje, izbira podlage (Lavrenčič in sod., 2007) in sorte vinske trte, redno gnojenje, predvsem z dušikom, od brstenja do začetka cvetenja, sadilne razdalje ter gojitvene oblike, urejen odtok vode oziroma osuševanje tal ob pojavu prevelike količine vode. Med rastno dobo je pomembno odstranjevanje zalistnikov, redčenje listov in grozdov (Reščič in sod., 2015, 2016), košnja ali mulčenje travne ruše večkrat v sezoni (od brstenja do trgatve), ki preprečuje izsušitev tal in namakanje ob pomanjkanju vode v tleh (Novello in sod., 1992; Kosta, 1998; Pintar, 2006).

Rastlini razpoložljiva voda v tleh je v območju matričnega potenciala tal, ki je med poljsko kapaciteto in točko venenja (Romano in Santini, 2002). Poljska kapaciteta predstavlja največjo količino vode, ki jo tla lahko zadržijo; le-ta se vzpostavi, ko odteče gravitacijsko odcedna voda. V literaturi je največkrat privzeta poljska kapaciteta −0,033 MPa. Točka venenja predstavlja tisto količino vode, pri kateri rastline ireverzibilno uvenijo. Splošno privzeta sila vezave vode pri točki venenja je −1,5 MPa. Voda, ki je vezana šibkeje kot poljska kapaciteta, gravitacijsko odteče, voda, ki je vezana močneje od točke venenja predstavlja rastlinam nedostopno vodo.

Evapotranspiracija (*ET*) je prehod vode v obliki vodne pare z vodne površine ali iz tal ter iz rastline v atmosfero (Pintar, 2006). Referenčna evapotranspiracija ($ET_0$) predstavlja *ET* iz površine aktivno rastoče travne ruše, ki popolnoma prekriva površino povprečne višine 12 cm, ki je zadostno preskrbljena z vodo, s povprečno upornostjo rastlinskega pokrova 70 m $s^{-1}$ in albedom 0,23. $ET_c$ predstavlja *ET* v primeru zadostne količine vode v tleh za izbrano rastlino. Izračunamo jo po Penman-Monteith metodi, ki upošteva temperaturo zraka, sončno sevanje, relativno zračno vlago in hitrost vetra. Pri izračunu referenčno evapotranspiracijo ($ET_0$) pomnožimo s koeficientom rastline ($k_c$), ki pri vinski trti običajno ni večji od 0,8 (pogosto med 0,35 in 0,76) (Slika 2). Slednji nam pove, koliko vode potrebuje vinska trta v primerjavi s travno rušo. Večji kot je $k_c$, več vode trta potrebuje, kar kaže na veliko površino listne stene ter senčenje pod trto in medvrstnega prostora (Allen in sod., 1998; Evapotranspiration…, 2015).




**Slika 2**: Razmerje med izračunanim koeficientom rastline ($k_c$) in listno površino (m²/trto) sorte 'Thompson Seedless' (prirejeno po Williams in Ayars., 2005)
**Figure 2**: Ratio between calculated crop coefficient ($k_c$) and leaf area ($m^2$/vine) for 'Thompson Seedless' (adapted from Williams and Ayars, 2005)

Pri namakanju rastlinam dodajamo toliko vode, da je rastlina optimalno preskrbljena, t.j. do poljske kapacitete (Pintar, 2006). Hkrati pri načrtovanju namakalnega sistema upoštevamo načelo, da pokrivamo potrebe najbolj zahtevne rastline, pri čemer pokrivamo 80−90 % verjetne potencialne evapotranspiracije (Cvejič in Pintar, 2013), izračunane po Penman Monteithovi metodi (Allen in sod., 1998). Pri deficitnem namakanju je količina dodane vode pod točko optimalne preskrbe, zato je dovoljen manjši vodni stres, ki ima minimalen učinek na pridelek (English in Raja, 1996). Primanjkljaj vode pri deficitnem namakanju torej ni prepuščen naključju, temveč količino vode nadzorovano zmanjšamo glede na razvoj rastline in lastnosti tal (Lu in sod., 2003).

Dodajanje manjše količine vode, kot je trta izgubi z evapotranspiracijo, a še vedno dovolj, da le-ta nima negativnega vpliva na pridelek, imenujemo deficitno namakanje (Intrigliolo in Castel, 2009). Z dodajanjem manjše, nezadostne količine vode, se izboljša sestava jagod in hkrati manj obremenjuje naravne zaloge vode v tleh (Fereres in Soriano, 2007; Podgornik in Bandelj, 2015).

Voda v tleh, in sicer del, ki je dostopen, prehaja iz tal v korenine na osnovi razlike v $\Psi$. Sprejem je možen, kadar je $\Psi$ tal večji kot $\Psi$ korenin. Razliko v vodnem potencialu v razmerah, ko rastline transpirirajo, generira matrični potencial (tenzija) v listih. Ta je tudi osnova za snovni tok vode iz korenin v nadzemne dele. V razmerah, ko je transpiracija omejena, se lahko razlika v $\Psi$ med koreninami in tlemi ustvarja na račun sprejema ionov mineralnih hranil v centralni cilinder korenine. S tem se ustvarja osmotski učinek, ki omogoča sprejem vode, tlačna sila, ki nastaja v koreninah (koreninski tlak), prispeva k transportu po ksilemu. V sušnih razmerah lahko rastline z aktivnim kopičenjem osmotikov vzdržujejo sprejem vode iz tal in ohranjajo turgor (Steudle, 2001; Vodnik, 2012).

Razpon intervala rastlinam dostopne vode je odvisen od lastnosti tal, predvsem od teksture in strukture (Tuller in Or, 2005). Praksa pri konvencionalnem namakanju za doseganje optimalnega pridelka je dodajanje vode do poljske kapacitete, kjer se, odvisno od rastline dopusti 30 ali 50 % primanjkljaj glede na rastlinam dostopno vodo v tleh. Pri vinski trti se pri deficitnem pristopu namaka, ko je dosežen 70 % primanjkljaj (Du in sod., 2008). Za uspešno deficitno namakanje je nujno poznavanje vodnozadrževalnih lastnosti tal (Montalebifard in sod., 2013). V peščenih tleh lahko rastline hitreje občutijo vodni stres, medtem ko imajo v tleh finejših teksturnih frakcij možnost postopne prilagoditve na večji matrični potencial (Katerji in sod., 2008).

Za uspeh deficitnega namakanja sta ključna dobro poznavanje rastline in nadzor nad primanjkljajem vode, ki mu je rastlina izpostavljena (Lu in sod., 2003; Pintar in Zupanc, 2017). Nadomeščanje primanjkljaja vode lahko uravnavamo na podlagi potreb rastlin po vodi, t.j. rastlinam je v namakalnem obroku dodan za nek količnik zmanjšan delež potencialne evapotranspiracije rastline (Moriana in sod., 2003); na osnovi meritev stanja rastline, t.j. merjenje vodnega potenciala rastlin (Moriana in sod., 2003; Fernandez in sod., 2008; Fernandez, 2014), merjenje vodnega toka v ksilemu (Yunusa in sod., 2004; Sousa in sod., 2006; Zhang in sod., 2011; Zhang in sod., 2012; Edwards in Clingeleffer, 2013); dodajanje vode v obsegu intervala rastlinam dostopne vode na podlagi meritev količine vode v tleh (Moriana in sod., 2003; Ruiz-Sanchez in sod., 2010). Slednji pristopi zahtevajo opremo, ki omogoča meritve vsaj vodnega potenciala trt neposredno v vinogradu.

Vodni status rastline ugotavljamo z merjenjem vodnega potenciala v listih ali deblu rastlin (Patakas in sod., 2005). To sta vodni potencial pred zoro ($\Psi_{PD}$) ali opoldanski vodni potencial ($\Psi_S$), ki predstavljata maksimum in minimum v 24 urnem hodu vodnega potenciala. V splošnem velja, da imajo rastline, ki niso v sušnem stresu $\Psi_S$ večji od −1,0 MPa; rastline v blagem sušnem stresu imajo $\Psi_S$ med −1,0 in −1,2 MPa, medtem ko imajo rastline v močnem sušnem stresu $\Psi_S$ med −1,2 in −1,5 MPa (Acevedo-Opazo in sod., 2010; Vodnik, 2012).

Po Podgornik in Bandelj (2015) deficitno namakanje temelji na načelu, da vodo dodamo takrat, ko jo rastlina najbolj gospodarno uporabi. Namakanje lahko izvajamo v kritični razvojni fazi vinske trte (NDN – nadzorovano deficitno namakanje), med celo rastno dobo v enakih odmerkih (TDN – trajno deficitno namakanje) oziroma del korenin v enem odmerku optimalno oskrbimo z vodo in drugega izpostavimo sušnemu stresu (IPNK – izmenično polovično namakanje korenin) (Ruiz-Sanchez in sod., 2010; Podgornik in Bandelj, 2015). Pri NDN trte, se le-to v dotični fenofazi ohranja na točno določeni stopnji sušnega stresa. Običajno se ga prvič izvaja po nastavku grozdja (BBCH 71), z namenom zmanjšanja rasti mladik, listov ter jagod, in drugič po začetku zorenja z namenom izboljšanja sestave jagod (Romero in sod., 2013), vendar le v vinogradih, ki so že v obdobju polne rodnosti. Glavni cilj pri oskrbi mladega vinograda je pospešiti razvoj trsov, da jih čim hitreje uredimo v želeno gojitveno obliko in pri tem se je vsakršnemu pomanjkanju vode najbolje izogniti. NDN upošteva tudi sposobnost rastline za preživetje v sušnih razmerah in njeno fenofazo, od česa je odvisen tudi odziv vinske trte na omejeno razpoložljivost vode, čas njene aplikacije ter trajanje in intenziteto sušnega stresa (Ruiz-Sanchez in sod., 2010).

Manjša količina vode v namakalnem obroku zmanjša evapotranspiracijo (*ET*) in rast rastlin predvsem zaradi manjše transpiracije vode iz jagod (Greenspan in sod., 1996) in liste zaradi zapiranja listnih rež ter posledično manjše asimilacije ogljika (Medrano in sod., 2003). Ker je transpiracija na pripiranje rež bolj odzivna kot fotosinteza je mogoče, da ob zmernem pripiranju rež, slednja ni nujno zmanjšana. V takem primeru se zelo poveča trenutna učinkovitost izrabe vode (razmerje fotosinteza/transpiracija). Ko prihaja do omejitev fotosinteze, je rast lahko zmanjšana, spremeni se tudi premeščanje sladkorjev. Tako se lahko z deficitnim namakanjem omeji tudi rast jagod in izboljša njihova kemijska sestava (poveča se vsebnost sladkorjev, zmanjša vsebnost kislin ter izboljša razmerje med sladkorji in kislinami). Do tega pride zaradi koncentriranja omenjenih snovi v jagodah na račun manjše vsebnosti vode v jagodah ter večje izpostavljenosti grozdov svetlobi (Grimplet in sod., 1970; Barroso in sod., 2017). Če rastlinam dodamo toliko vode, da so v stanju močnega sušnega stresa oz. prevelikega pomanjkanja vode, lahko pride do manjšega pridelka in slabše kakovosti grozdja. Ključno vprašanje je, katera je še sprejemljiva spodnja meja zmanjšanja namakalnega obroka, da se ohrani ali celo poveča koristi tovrstnega načina dodajanja vode (English in Raja, 1996; Intrigliolo in Castel, 2009), kar se s poskusi ugotavlja za posamezno sorto v različnih okoljskih razmerah.

Cilj deficitnega namakanja je povečati produktivnost rastlin z uporabo vode (*WP* – crop water productivity). Po Geerts in Raes (2009) produktivnost rastlin (*WP*) opišemo kot razmerje med maso pridelka ($Y_a$) in količino vode, ki jo rastlina porabi ($ET_a$). Na območjih, kjer pogosto prihaja do pomanjkanja razpoložljive vode v tleh, so zaželene vrste in sorte z večjo *WP*, kar pomeni večji pridelek in manjšo porabo vode (Geerts in Raes, 2009).

$$WP\ (\mathrm{kg\ m^{-3}}) = Y_a/ET_a$$

Za dosego deficitnega namakanja je na voljo več tehnologij. Najbolj pogosto uporabljena tehnologija v intenzivni rastlinski pridelavi je kapljično namakanje, kjer so cevi s kapljači položene nad površino tal ali celo v tleh, t.i. podzemno namakanje (Camp, 1998). Če ob enem obroku namakanja omočimo samo eno stran vrste ter ob drugem obroku drugo, namakanje izvajamo z metodo izmeničnega polovičnega namakanja korenin (IPNK). To se lahko poleg kapljičnega namakanja izvaja z namakanjem v brazde, ki v Sloveniji ni uveljavljena praksa in je z vidika porabe vode tudi neracionalna (Pintar, 2006; Araujo in sod, 1995a,b). V prispevku bomo v nadaljevanju obravnavali kapljično namakanje za štiri sorte vinske trte.

**Kapljično namakanje**

Pri kapljičnem namakanju se vodo aplicira neposredno v območje korenin, kar poveča izkoristek dodane vode, in sicer na od 85 do 95 % (Pintar, 2006). V svetovno znanih vinorodnih območjih v Italiji, Španiji in Čilu so izpeljali raziskave, kjer so proučevali vpliv različnih obravnav NDN na rast, pridelek in sestavo jagod ob trgatvi, pri različnih sortah vinske trte. Izbrani poskusi na sortah 'Cabernet Sauvignon', 'Monastrell', 'Sangiovese' in 'Tempranillo' z rezultati so prikazani v preglednici 1.

Pri sorti 'Cabernet Sauvignon' deficitno namakanje ni vplivalo na vegetativno rast, za razliko od sorte 'Monastrell'. Izmerjene razlike med parametri rasti so bile pri 'Cabernet Sauvignon' majhne, kjer voda v globokih ilovnatih tleh z velikim volumskim deležem vode (420 mm) ni bila omejujoč dejavnik rasti trt, ki so imele koreninski sistem vse do 350 cm globine tal. Pri sorti 'Monastrell' je prišlo do značilnega zmanjšanja rasti pri trtah z NDN, kjer so zabeležili krajše mladike in manjšo maso lesa po rezi v primerjavi s TDN. Rezultati nakazujejo, da je vegetativna rast pri omenjeni sorti zelo občutljiva na pomanjkanje vode, predvsem rast mladik v dolžino (Romero in sod., 2013). To potrjujejo tudi rezultati raziskave na sorti 'Tempranillo', saj je bila rast mladik in debla ter masa lesa po rezi zmanjšana zaradi vpliva manj razpoložljive vode (Preglednica 1). Če primerjamo dva deficitna obroka vode se je masa lesa po rezi najbolj zmanjšala pri NDN2, kjer je bila najmanjša izmerjena 0,34 kg, največja pa 0,49 kg (Acevedo-Opazo in sod., 2010; Santesteban in sod., 2011). Pri sorti 'Sangiovese' je na maso lesa po rezi močno vplivala letna količina padavin. Povprečno najmanjša masa je znašala 0,53 kg na trto in je bila izmerjena pri $I_2$, medtem ko je bila povprečno največja masa izmerjena pri obravnavanju $I_1$ (0,65 kg na trto) (Lanari in sod., 2014).

Pri sortah 'Monastrell' in 'Tempranillo' je nadzorovano deficitno namakanje (NDN, Preglednica 1) značilno zmanjšalo pridelek ob trgatvi, predvsem na račun manjših jagod. Pri sorti 'Monastrell' je bil pridelek pri trtah z NDN-1 zmanjšan za od 38 do 47 % in pri NDN-2 za od 52 do 57 % (Preglednica 1). Zmanjšalo se je število grozdov na trto pri sorti 'Tempranillo' (Santesteban in sod., 2011; Romero in sod., 2013), medtem ko pri sorti 'Sangiovese' ni prišlo do značilnih razlik med obravnavanji, vendar so pri slednji, kljub temu, značilno največji pridelek izmerili pri namakanih trtah (Lanari in sod., 2014).

Pri sortah 'Cabernet Sauvignon' in 'Monastrell' se je v jagodah trt pod deficitnim namakanjem vsebnost sladkorjev povečala. Rezultati nakazujejo, da blag sušni stres pred začetkom zorenja pozitivno vpliva na vsebnost sladkorjev in hkrati na končno sestavo jagod sort v poskusu (Santesteban in sod., 2011; Romero in sod., 2013). Med zorenjem jagod se vsebnost sladkorjev veča in hkrati vsebnost kislin manjša, kar pomeni da večja vsebnost sladkorjev nakazuje manjšo vsebnost kislin. To potrjujejo rezultati poskusa na sortah 'Monastrell' in 'Sangiovese', kjer so v jagodah trt pod največjim sušnim stresom izmerili največjo vsebnost sladkorjev ter najmanjšo vsebnost titrabilnih kislin (Romero in sod., 2013; Lanari in sod., 2014). Pri vseh proučevanih sortah različna namakanja niso vplivala na pH jagod (Acevedo-Opazo in sod., 2010; Santesteban in sod., 2011; Romero in sod., 2013; Lanari in sod., 2014).

Kapljično namakanje je učinkovit način, s katerim lahko izvajamo nadzorovano deficitno namakanje na rastlinah vinske trte in jih s tem izpostavimo blagemu sušnemu stresu. Zmerno pomanjkanje vode vodi do manjše rasti mladik v dolžino, manjšega pridelka na trto in boljše sestave jagod, kar je pri vinskih sortah ključnega pomena za pridelavo kakovostnega vina (Cifre in sod., 2005). Kljub obetavnim rezultatom raziskav se še vedno opazi velik vpliv okolja na sam potek poskusov oziroma na nadzor dodajanja vode za dosego deficitnega namakanja. Moyer in sod. (2013) navajajo, da se globina največje gostote korenin razlikuje pri namakanih in nenamakanih trtah. Pri kapljičnem namakanju naj bi se glavnina korenin nahajala blizu površja, pod kapljači (Araujo in sod., 1995a, b). Na takšno namakanje tip tal nima značilnega vpliva, saj se vodo dodaja večkrat in počasi, v manjših količinah, ki jo tla lahko sproti absorbirajo (Poling in Spayd, 2015).

**Preglednica 1:** Opis raziskav nadzorovanega deficitnega namakanja (NDN) na sortah 'Cabernet Sauvignon', 'Monastrell', Sangiovese' in 'Tempranillo' ter vpliv posameznega obravnavanja na rast, pridelek in sestavo jagod (Prirejeno po Intrigliolo in Castel, 2009, Acevedo-Opazo in sod., 2010, Santesteban in sod., 2011, Romero in sod., 2013, Lanari in sod., 2014).
**Table 1:** Description of studies with regulated deficit irrigation on 'Cabernet Sauvignon', 'Monastrell', 'Sangiovese' and 'Tempranillo' and impact of different treatments on growth, yield and berry composition (adapted from Intrigliolo and Castel, 2009, Acevedo-Opazo et al., 2010, Santesteban et al., 2011, Romero et al., 2013, Lanari et al., 2014).

| Način namakanja | Sorta | Fenofaza | Mejni parameter za razpoložljivost vode v obravnavanju | Lastnosti tal | Rast | | Pridelek | | | Sestava jagod | | | Referenca |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $\Psi_S$ | | Masa lesa po rezi (kg trto$^{-1}$) | Dolžina mladik (cm) | Kg trto$^{-1}$ | Št. grozdov trto$^{-1}$ | Masa grozda (g) | Vsebnost sladkorjev (°Brix) | Vsebnost titrabilnih kislin (g l$^{-1}$) | pH | |
| Nadzorovano deficitno namakanje (NDN) | Cabernet Sauvignon | Nastavek grozdja - trgatev | −0,80 do −0,95 MPa (T1) | I, globoka | 1,29 | 148,1 | 3,15 | 26 | 122,6 | 23,6 | - | 3,6 | Acevedo-Opazo in sod., 2010 |
| | | | −1,00 do −1,20 MPa (T2) | | 1,29 | 147,1 | 2,90 | 24,8 | 117,7 | 24,5 | - | 3,6 | |
| | | | −1,25 do −1,40 MPa (T3) | | 1,19 | 134,3 | 2,76 | 25,1 | 109,2 | 24,8 | - | 3,6 | Čile |
| | | | $ET_c$ | | | | | | | | | | |
| | Monastrell | TDN | 40% | G (48% gline, 30% melja, 22% peska) | 0,75 | 129 | 3,71 | 18,2 | 207 | 22,2 | 3,04 | 3,89 | Romero in sod., 2013 |
| | | Brstenje – nastavek grozdja | 30% | | | | | | | | | | |
| | | Nastavek grozdja – začetek zorenja | 20% | | 0,51 | 109 | 2,08 | 15 | 141,8 | 22,9 | 2,84 | 4,01 | Španija |
| | | Začetek zorenja - trgatev | 12,3% | | | | | | | | | | |
| | | Brstenje – nastavek grozdja | 0 | | | | | | | | | | |
| | | Nastavek grozdja – začetek zorenja | 20,7% | | 0,46 | 111 | 1,72 | 14,1 | 121,4 | 22,4 | 2,98 | 4,02 | |
| | | Začetek zorenja - trgatev | 22,3% | | | | | | | | | | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $ET_C$ | | | | | | | | | | |
| NDN (nadaljevanje) | 'Sangiovese' | Nastavek grozdja – začetek zorenja | 0 % ($I_0$) | MGI (55% melja, 37,5% gline, 7,5% peska ) | 0,59 | - | 4,80 | 13,7 | 358,7 | 23,1 | 6,3 | 3,37 | Lanari in sod., 2014 Italija |
| | | | 33 % ($I_1$) | | 0,65 | - | 5,43 | 13,2 | 411,7 | 21,7 | 6,6 | 3,34 | |
| | | | 70 % ($I_2$) | | 0,53 | - | 5,16 | 13,5 | 380,3 | 22,1 | 6,4 | 3,35 | |
| | | | $\Psi_{PD}$ | | | | | | | | | | |
| | Tempranillo | Nastavek grozdja – začetek zorenja | −0,2 do −0,9 MPa | - | 0,64 | - | 4,18 | 18,35 | 227,5 | - | 5,26 | - | Santesteban in sod., 2011 |
| | | | < −0,8 MPa | - | 0,45 | - | 3,05 | 14,63 | 208,5 | - | 4,65 | - | |
| | | Začetek zorenja - trgatev | < −0,8 MPa | - | 0,40 | - | 3,18 | 14,65 | 217,1 | - | 4,52 | - | Španija |
| | | | −0,2 do −0,9 MPa<br>< −0,8 MPa<br>< −0,6 MPa | | | | | | | | | | |
| | | | $ET$c | | | Listna površina ($m^2$ $trto^{-1}$) | | | | | | | |
| Izmenično polovično namakanje korenin (IPKN) | Tempranillo | Začetek cvetenja - trgatev | 0% | | 1,20 | 6,6 | 5,92 | 16 | 370 | 20,9 | 5,3 | 3,23 | Intrigliolo in Castel, 2009 |
| | | | 50% | GI-G, globina tal več kot 2 m | 1,46 | 7,5 | 6,54 | 16 | 409 | 22 | 5,4 | 3,33 | Španija |
| | | | 50% (+ IKN) | | 1,37 | 7,6 | 6,59 | 16 | 412 | 22,1 | 5,2 | 3,35 | |
| | | | 100% | | 1,59 | 8,3 | 6,8 | 16 | 425 | 22,8 | 5,5 | 3,37 | |
| | | | 100% (+ IKN) | | 1,59 | 8,8 | 7,43 | 17 | 437 | 22,1 | 5,3 | 3,32 | |

Pri sorti 'Cabernet Sauvignon' so obravnavanja brez sušnega stresa ($\Psi_{PD}$ −0,80 do −0,95 MPa), blag sušni stres (−1,00 do −1,20 MPa) in močan sušni stres (−1,25 do −1,4 MPa).

Pri sorti 'Monatrell' je TDN trajno deficitno namakanje (40 % celotne $ET_c$), NDN nadzorovano deficitno namakanje (NDN-1: 30 % celotne $ET_c$ med brstenjem in nastavkom grozdja, 12,3 % $ET_c$ med nastavkom grozdja in začetkom zorenja, 20,7 % $ET_c$ med začetkom zorenja in trgatvijo; NDN-2: 20 % celotne $ET_c$ med brstenjem in nastavkom grozdja, med nastavkom grozdja in začetkom zorenja niso namakali, med začetkom zorenja in trgatvijo 22,3 % celotne $ET_c$).

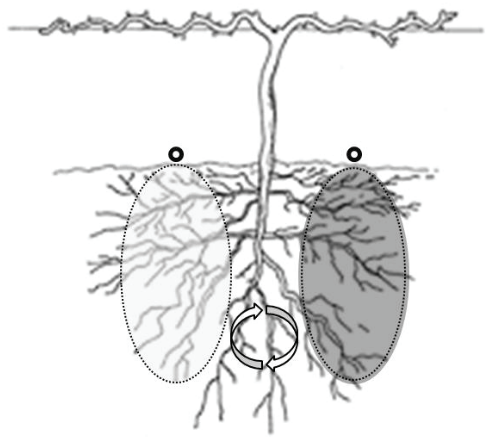
Pri sorti 'Sangiovese' pomeni $I_0$ kontrola (nenamakane trte), pri $I_1$ so trte namakane s 33 % $ET_c$ in pri $I_2$ s 70 % $ET_c$ zadnjih 7 dni, med nastavkom grozdja in začetkom zorenja. Trte so bile namakane, ko je $\Psi_S$ dosegel okvirno −0,9 MPa, neto fotosinteza okvirno 11 µmol $CO_2$ $m^{-2}s^{-1}$ in ko je razpoložljiva voda v tleh na 0,6 m globine pod listno steno dosegla točko venenja.

Pri sorti 'Tempranillo' in nadzorovanem deficitnem namakanju – NDN je KN konvencionalno namakanje ($\Psi_{PD}$ od −0,2 MPa do −0,9 MPa), NDN je nadzorovano deficitno namakanje (NDN-1 namakali ko je $\Psi_{PD}$ padel pod −0,8 MPa, pri NDN-2 med nastavkom grozdja in začetkom zorenja, ko je $\Psi_{PD}$ padel pod −0,8 MPa, med začetkom zorenja do trgatve, ko je $\Psi_{PD}$ padel pod −0,6 MPa).

Pri sorti 'Tempranillo' in izmeničnem polovičnem kapljičnemu namakanju - IPK je kontrola nenamakane trte, pri 50 (50 % $ET_c$) in 100 (100 % $ET_c$) so vodo dodali na obe strani trt, pri 50-IKN (50 % $ET_c$) in 100-IKN (100 % $ET_c$) so vodo dodali le na eno stran trt.

**Izmenično polovično namakanje korenin (IPNK)**

Izmenično polovično namakanje korenin (IPNK) temelji na izmenjujočem dodajanju vode dvema deloma korenin vinske trte (Slika 2). Del korenin v mokrih tleh naj bi trto preskrboval z vodo, del v suhih tleh naj bi listom in mladikam poslal signal o pomanjkanju vode, ki bi posledično zmanjšale prevodnost listnih rež in s tem transpiracijo (dos Santos in sod., 2003; Intrigliolo in Castel, 2009). Dosedanji rezultati o vplivu izmeničnega namakanja na rast, rodnost in sestavo jagod pri vinski trti kažejo na velik vpliv sorte, okoljskih razmer, lastnosti tal in načina aplikacije vode na fiziološke odzive posamezne trte. Posledično še ni moč zagotovo trditi ali se IPNK dobro obnese v vseh pridelovalnih razmerah (Gu in sod., 2004; Du in sod., 2013).



**Slika 3**: Izmenično kapljično namakanje (prirejeno po Du in sod., 2008)
**Figure 3**: Alternate partial drip irrigation (adapted from Du et al., 2008

Poskus, kjer so ugotavljali vpliv IPNK na rast, pridelek in sestavo jagod so izvedli v Španiji na sorti 'Tempranillo'. Pri obravnavanjih so upoštevali $ET_0$ in $k_c$, stran namakanja so zamenjali na vsaka dva tedna. Pri obravnavanjih z izmeničnim namakanjem so najprej namakali severno in nato južno stran trt (preglednica 1).

Izmenično kapljično namakanje (IKN) ni značilno vplivalo na vegetativno rast vinske trte. Masa lesa po rezi ter površina listne stene sta se opazno povečala pri bolj namakanih trtah (100 % in 100 % IKN), vendar razlik med običajnim in izmeničnim namakanjem niso opazili. Pri 50 % je bila površina listne stene na trto 7,5 $m^2$, pri 50 % IKN pa 7,6 $m^2$ (Intrigliolo in Castel, 2009).

Pridelek in parametri rodnosti (št. grozdov na trto in masa posameznega grozda) se pod vplivom različnega načina namakanja niso značilno razlikovali. Razlike so bile opazne le med trtami, ki so jim dodali različen volumen vode (Preglednica 1). Pri kontroli brez namakanja so izmerili najmanjši povprečni pridelek na trto (5,92 kg/trto) in maso grozdov (370 g), medtem ko so polno namakane trte pod običajnim namakanjem dale največ grozdja (7,43 kg/trto, masa enega grozda v povprečju 437 g). Število grozdov na trto se med obravnavanji ni spremenilo (Intrigliolo in Castel, 2009; preglednica 1).

Način dodajanja vode ni vplival na končno sestavo jagod. Pri vseh obravnavanjih je vsebnost sladkorjev variirala med 20,9 °Brix pri kontroli in 22,8 °Brix pri trtah pod običajnim 100 % kapljičnim namakanjem. Med obravnavanji se tudi pri vsebnosti titrabilnih kislin in pH vrednosti niso pokazale značilne razlike (Intrigliolo in Castel, 2009).

Dosedanje raziskave vpliva izmeničnega kapljičnega namakanja na rast in rodnost vinske kažejo nasprotujoče rezultate, predvsem zaradi velikega števila dejavnikov, ki vplivajo na procese v sistemu tla – vinska trta – listna stena. Omeniti velja predvsem vremenske razmere, značaj sorte, lastnosti tal in načine namakanja, zato bi morali tovrstno namakanje preizkusiti v različnih okoljskih razmerah z različnimi tipi tal, na več sortah in pod različnimi načini dodajanja vode (Gu in sod., 2004). Še bolj kot pri kapljičnem namakanju, kjer namakamo celotno območje korenin, tip tal močno vpliva na izmenično namakanje, pri katerem je pomembno, da dosežemo očitno mejo med mokrim in suhim delom tal. Težja tla se počasneje segrevajo ter ohlajajo in hkrati osušujejo, kar pomeni, da mora cikel namakanja ene in osuševanja druge strani vinske trte trajati dlje časa, sicer izmenično namakanje ne pride do izraza (Du in sod., 2008).

## 4 NAMAKANJE VINSKE TRTE V SLOVENIJI

Namakanje vinske trte v Sloveniji nima takšne tradicije izvajanja kot v drugih državah pridelovalkah grozdja s toplejših območij, vendar se je tudi že pri nas nekaj vinarjev odločilo za ureditev namakalnega sistema. Prvi poskusi vpeljave deficitnega namakanja so v teku pri oljkah (Podgornik in Bandelj, 2015), medtem ko vinsko trto pri nas namakajo predvsem na Primorskem, kjer izrazito plitva tla ne omogočajo zadrževanja vode. Po podatkih javnih baz namakanih vinogradov v Sloveniji ni. V zadnjih letih se je na nekaterih omejenih območjih Goriških brd in Krasa uredilo posamezne kapljične namakalne sisteme. Najverjetneje je vodni vir vodovodna voda ali deževnica. V drugih vinorodnih okoliših se namakanje v pridelavi grozdja ne izvaja (Rusjan, 2018). Za strokovno pravilno namakanje ter prenos principa deficitnega namakanja v prakso bo potrebno preveriti odziv sort v danem okolju. Na uspešnost izbranega pristopa k namakanju oziroma deficitnemu principu namakanja imajo velik vpliv številni dejavniki, kot so sorta in podlaga vinske trte, poznavanje kritičnih faz namakane sorte, lastnosti tal. Zato je smiselno tovrstni princip namakanja dobro preučiti v različnih okoljskih in eksperimentalnih razmerah ter ločeno za vsak vinorodni okoliš v Sloveniji. Dobro poznavanje nadzora deficita je ključno, za kar je potrebno znanje ter dostop do opreme, ki bo omogočala nadzor deficita. V Sloveniji se namaka pogosto le po občutku (Cvejić s sod., 2015), zato sta strokovna in finančna podpora pridelovalcem pri vpeljavi te tehnologije namakanja v vinogradništvu nujni.

## 5 ZAKLJUČKI

V pregledu vsebin dosedanjih objav obravnavamo vpliv različnih pristopov deficitnega namakanja na rast in rodnost žlahtne vinske trte (*Vitis vinifera* L.).

Deficitno namakanje je eno izmed najbolj raziskanih načinov upravljanja namakanja vinske trte, pri katerem raziskovalci navajajo veliko pozitivnih učinkov za uspešno pridelavo grozdja. Takšen način upravljanja namakanja vinske trte se je v svetu začel uporabljati zaradi zmanjševanja zalog rastlinam razpoložljive vode v tleh. Pri deficitnem namakanju se nadzorovano dodaja manjši volumen vode, kot jo trta potrebuje. Rezultati tovrstnega namakanja so manjša rast trte (krajše mladike, manjša listna površina, manjša masa lesa po rezi), manjše jagode in s tem sprejemljivo manjši pridelek, boljša sestava jagod (več sladkorjev in antocianov, manj kislin), večja učinkovitost rabe vode (več pridelka na enoto dodane vode) in manjša poraba vode. Najboljši način za nadzor deficitnega namakanja pri vinski trti je sprotno merjenje vodnega potenciala oziroma ksilemskega toka trte.

Izkušnje z dosedanjimi poskusi nakazujejo, da sušni stres pred in po začetku zorenja vodi do najboljšega razmerja med rastjo in količino pridelka ter sestavo grozdja sort 'Cabernet Sauvignon', 'Monastrell' in 'Sangiovese'. Na uspešnost izbranega pristopa k deficitnemu namakanju imajo velik vpliv sorta in podlaga vinske trte ter tekstura tal, zato je smiselno to tehnologijo namakanja dobro preučiti v različnih okoljskih in eksperimentalnih razmerah ter za vsak vinorodni okoliš v Sloveniji. Za uspešen prenos principa deficitnega namakanja v prakso je potrebno poznati tudi kritične fenofaze namakanih sort vinske trte, kakšen je odziv sorte v nekem okolju, potreben je dostop do opreme, ki bo omogočala nadzor deficita. Uspešen prenos v prakso bo mogoč le ob finančni in strokovni podpori pridelovalcem pri vpeljavi te tehnologije namakanja.

## 6 VIRI

Acevedo-Opazo C., Ortega-Farias S., Fuentes S. (2010). Effects of grapevine (*Vitis vinifera* L.) water status on water consumption, vegetative growth and grape quality: An irrigation scheduling application to achieve regulated deficit irrigation. *Agricultural Water Managament*, *97*, 956-96. doi:10.1016/j.agwat.2010.01.025

Allen R.G., Pereira L.S., Raes D., Smith, M. (1998). Crop evapotranspiration: guidelines for computing crop water requirements. Irrigation and drainage paper, 56. Rome, Food and Agriculture Organization of the United Nations: 300 str.

Araujo F., Williams L. E., Grimes D. W., Mathews M. A. (1995a). A comparative study of young ‘Thompson Seedless’ grapevines under drip and furrow irrigation. I. Root and soil water distributions. *Scientia Horticulturae*, 60, 235-249. doi:10.1016/0304-4238(94)00710-W

Araujo F., Williams L. E., Mathews M. A. (1995[b)]. A comparative study of young ‘Thompson Seedless’ grapevines (*Vitis vinifera* L. ) under drip and furrow irrigation. II. Growth, water use efficiency and nitrogen partitioning. *Scientia Horticulturae*, 60, 251-265. doi:10.1016/0304-4238(94)00711-N

Barroso J.M., Pombeiro L., Rato A.E. (2017). Impacts of crop level, soil and irrigation management in grape berries of cv 'Trincadeira (*Vitis vinifera* L.). Journal of Wine Research, 28 (1), 1-12. doi:10.1080/09571264.2016.1238350

Camp C. R. (1998). Subsurface drip irrigation: a review. *Transactions of the ASAE*, 41, 5, 1353-1367. doi:10.13031/2013.17309

Cancela, J.J., Rey, B.J., Fandiño, M., Martínez, E.M., Lopes, C.M., Egipto, R., Silvestre, J.M. (2017) Tools for management of irrigation in vineyards: An approach to farmers. *Acta Horticulturae*, 1150, 471-476, doi:10.17660/ActaHortic.2017.1150.65

Chaves M.M., Zarrouk, O., Francisco, R., Costa, J.M., Santos, T., Regalado, A.P., Rodrigues M.L. Lopes, C.M. (2007). Grapevine under deficit irrigation: hints from physiological and molecular data. *Annals of Botany*, 105, 661–676. 2010 doi:10.1093/aob/mcq030

Cifre J., Bota J., Escalona J.M., Medrano H., Flexas J. (2005). Physiological tools for irrigation scheduling in grapevine (*Vitis vinifera* L.). An open gate to improve water-use efficiency? *Agriculture, Ecosystems and Environment*, 106, 159-170. doi:10.1016/j.agee.2004.10.005

Conesa M.R., de la Rosa J.M., Domingo R., Baȷ̃on S., Pérez-Pastor A. (2016). Changes induced by water stress on water relations, stomatal behaviour and morphologyof table grapes (cv. Crimson Seedless) grown in pots. *Scientia Horticulturae*, 202, 9-16. doi:10.1016/j.scienta.2016.02.002

Cvejič R., Pintar M. (2013). Namakanje. Šentjur, 27 november 2013. Oddelek za agronomijo, Biotehniška fakulteta, Univerza v Ljubljani

Cvejić R., Zupanc V., Pintar M. (2015). Primerjava razvoja namakanja v Sloveniji z globalnim trendom = Development of irrigation in Slovenia from a global perspective. *Hmeljarski bilten*, 22, 74-85.

Deluc L.G. Quilici D.R., Decendit A., Grimplet J., Wheatley M.D., Schlauch K.A., Merillon J. Cushman J.C., Cramer G.R. (2009). Water deficit alters differentialy metabolic pathways affecting important flavor and quality traits in grape berries of Cabernet Sauvignon and Chardonnay. BMC Genomics, 10.

Döll P. (2002). Impact ofclimate change an variability on irrigation requirements: A global perspective. Climatic change, 54, 269-293. doi:10.1023/A:1016124032231

dos Santos T.P., Lopes C.M., Rodrigues M.L., de Souza C.R., Maroco J.P., Pereira J.S., Silva J.R., Chaves M.M. (2003). Partial rootzone drying: effects on growth and fruit quality of field-grown grapevines (*Vitis vinifera*). *Functional plant biology*, 30, 663-671. doi:10.1071/FP02180

Du T., Kang S., Zhang J., Li F., Yan B. (2008). Water use efficiency and fruit quality of table grape under alternate partial root-zone drip irrigation. *Agricultural water management*, 95, 659-668. doi:10.1016/j.agwat.2008.01.017

Du T., Kang S., Yan B., Zhang J. (2013). Alternate furrow irrigation: A practical way to improve grape quality and water use efficiency in arid northwest China. *Journal of Integrative Agriculture*, 12(3), 509-519. doi:10.1016/S2095-3119(13)60252-X

Edwards E.J., Clingeleffer P.R. (2013). Interseasonal effects of regulated deficit irrigation on growth, yield, water use, berry composition, and wine attributes of Cabernet Sauvignon grapevines. Australian Journal of Grape and Wine Research, 19, 261-276. doi:10.1111/ajgw.12027

English M., Raja S.N. (1996). Perspectives on deficit irrigation. *Agricultural Water Management*, 32, 1-14. doi:10.1016/S0378-3774(96)01255-3

Esteban M.A, Villanueva M.J., Lissarrague J. (2001). Effect of irrigation on changes in the anthocyanin composition of the skin of cv. Tempranillo (*Vitis vinifera* L.) grape berries during ripening. *Journal of the Science of Food and Agriculture*, 81, 409-420. doi:10.1002/1097-0010(200103)81:4<409::AID-JSFA830>3.0.CO;2-H

Evapotranspiration and Grapevine Water Use. (2015). WSU viticulture and enology, Washington State University. http://wine.wsu.edu/research-extension/weather/evapotranspiration/ (1. sept. 2015)

FAO database. Food and Agriculuture Organisation of the United Nations. Aquastat. http://www.fao.org/nr/water/aquastat/data/query/results.html (18.feb. 2016)

Fereres E., Soriano M.A. (2007). Deficit irrigation for reducing agricultural water use. *Journal of Experimental Botany*, 58(2), 147-159. doi:10.1093/jxb/erl165

Fernandez J.E., Green S.R., Caspari H.W., Diaz-Espejo A., Cuevas M.V. (2008). The use of sap flow measurements for scheduling irrigation in olive, apple and Asian pear trees and in grapevines. *Plant* *and Soil*, 305(1-2), 91-104. doi:10.1007/s11104-007-9348-8

Fernandez J.E. (2014). Understanding olive adaptation to abiotic stresses as a tool to increase crop performance. *Environmental and Experimental Botany*, 103, 158–179. doi:10.1016/j.envexpbot.2013.12.003

Geerts S., Raes D. (2009). Deficit irrigation as an on-farm strategy to maximize crop water productivity in dry areas. *Agricultural Water Management*, 96, 1275-1284. doi:10.1016/j.agwat.2009.04.009

Goldammer T. (2018). Grape Grower's Handbook: A Guide to Viticulture for Wine Production. Third edition. U.S.A., Apex Publishers: 482 str.

Greenspam M.D., Schultz H.R., Matthews M.A. (1996). Field evaluation of water transport in grape berries during water deficits.*Physiologia Plantarum*, 97(1), 55-62. doi:10.1111/j.1399-3054.1996.tb00478.x

Grimplet J., Deluc L.G., Cramer G., Cushman J.C. (1970). Integrating functional genomics with salinity and water deficit stress responses in wine grape – *Vitis vinifera*. Advances in molecular breeding toward drought and salt tolerant crops, 643-668.

Gu S., Du G., Zoldoske D., Hakim A., Cochran R., Fugelsang K., Jorgensen G. (2004). Effects of irrigation amount on water relations, vegetative growth, yield and fruit composition of Sauvignon blanc grapevines under partial rootzone drying and conventional irrigation in the San Joaquin Valley of California, USA. *Journal of Horticultural Sciense and Biotechnology*, 79(1), 26-33. doi:10.1080/14620316.2004.11511732

Hannah L., Roehrdanz P.R., Ikegami M., Shepard A.V., Shaw M.R., Tabor G., Zhi L., Marquet P.A., Hijmans R.J. (2013). Climate change, wine and conservation. Proceedings of the National Academy of Sciences of the Unated States of America, 110(17), 6907-6912. doi:10.1073/pnas.1210127110

Hepner Y., Bravdo B., Loinger C., Cohen S., Tabacman H. (1985). Effect of drip irrigation schedules on growth, yield, must composition and wine quality of Cabernet Sauvignon. *American Journal of Enology and Viticulture*, 36, 77-85.

Hladnik M., Jakše J., Bandelj D., Vuk I. (2014). The characterisation of *Vitis vinifera* 'Refošk' with AFLP and SSR molecular markers and ampelographic traits. *Acta Agriculturae Slovenica*, 103(1), 55-64. doi:10.14720/aas.2014.103.1.06

Hochberg U., Degu A., Fait A., Rachmilevitch S. (2017). Grapevines hydraulic diversity - A critical consideration for irrigation management. *Acta Horticulturae*, 1150, 443-448. doi:10.17660/ActaHortic.2017.1150.61

Imazio S., De Lorenzis G., Scienza A., Failla O., Vouillamoz J., Korošec-Koruza Z., Rusjan D., Nikolao N. (2014). 'Ribolla Gialla' from North Eastern Italy, 'Rebula' from Northern Balkans and 'Robola' from Ionian Islands; Do they belong to the same population variety or are they genetically different? *Acta Horticulturae*, 1046, 645-652. doi:10.17660/ActaHortic.2014.1046.88

Intrigliolo D.S., Castel J.R. (2009). Response of *Vitis vinifera* cv. 'Tempranillo' to partial rootzone drying in the field: Water relations, growth, yield and fruit and wine quality. *Agricultural Water Managament*, 96, 282-292. doi:10.1016/j.agwat.2008.08.001

Kajfež Bogataj L. (2009). Climate change and future adaptation. Economic and business review, 11(1), 9-27.

Katerji N., Mastrorilli M., Rana G. (2008). Water use efficiency of crops cultivated in the Mediterranean region: Review and analysis. *European Journal of Agronomy*, 28(4). doi:10.1016/j.eja.2007.12.003

Kosta H. (1998). Vinogradniški nasveti. Ljubljana, Kmečki glas: 149 str.

Kumar Kar R. (2011). Plant responses to water stress. Role of reactive oxygen species. Plant Signaling and Behavior, 6(11), 1741-1745. doi:10.4161/psb.6.11.17729

Lamovšek, J., Zidarič, I., Mavrič Pleško, I., Urek, G., Trdan, S. (2014). Comparative study of diagnostic methods used for monitoring of common grape vine (Vitis vinifera L.) crown gall (Agrobacterium vitis Ophel & Kerr) in Slovenia. *Acta Agriculturae Slovenica*, 103(2), 313-321. doi:10.14720/aas.2014.103.2.16

Lanari V., Pallioti A., Sabbatini P., Howell G.S. (2014). Optimizing deficit irrigation strategies to manage vine performance and fruit composition of field-grown 'Sangiovese' (*Vitis vinifera* L.) grapevines. *Scientia Horticulturae*, 179, 239-247. doi:10.1016/j.scienta.2014.09.032

Lavrenčič P., Peterlunger P., Sivilotti E. (2007). Water stress and root hydraulic conductivity in grapevine grafted on different rootstocks. *Acta Horticulturae*, 754, 283-288. doi:10.17660/ActaHortic.2007.754.36

Lovisolo, C., Perrone, I., Carra, A., Ferrandino, A., Flexas, J., Medrano, H., Schubert, A. (2010). Drought-induced changes in development and function of grapevine (Vitis spp.) organs and in their hydraulic and non-hydraulic interactions at the whole-plant level: a physiological and molecular update. *Functional Plant Biology*, 2010(37), 98–116. doi:10.1071/FP09191

Lu P., Yunusa I.A.M., Walker R.R., Müller W.J. (2003). Regulation of canopy conductance an transpiration and their modeling in irrigated grapevines. *Functional Plant Biology*, 30, 689-698. doi:10.1071/FP02181

Malheiro A.C., Santos J.A., Fraga H., Pinto J.G. (2010). Climate change scenarios applied to viticultural zoning in Europe. *Climate Research*, 43, 163-177. doi:10.3354/cr00918

Maljevič J. (2003). Naravi in ljudem prijazno vinogradništvo. Novo mesto, KGZS – Zavod: 93 str.

Matthews M.A., Anderson M.M., Schultz H.R. (1987). Phenologic and growth responses to early and late season water deficits in Cabernet franc. *Vitis – Journal of Grapevine Research*, 26, 147-160.

Matthews M.A., Anderson M.M. (1988). Fruit ripening in *Vitis vinifera* L.: Responses to seasonal water deficits. *American Journal of Enology and Viticulture*, 39(4), 313-320.

Matthews M.A., Ishii R., Anderson M.M., O'Mahony M. (1990). Dependence of wine sensory attributes on wine water status. *Journal of the Science of Food and Agriculture*, 51(3), 321-335. doi:10.1002/jsfa.2740510305

McCarthy M.G. (1997). The effect of transient water deficit on berry development of cv. Shiraz (*Vitis vinifera* L.). *Australian Journal of Grape and Wine Research*, 3, 102-108. doi:10.1111/j.1755-0238.1997.tb00128.x

Medrano H., Escalona J.M., Cifre J., Bota J., Flexas J. (2003). A ten-year study on the physiology of two Spanish grapevine cultivars under field conditions: effects of water availability from leaf photosynthesis to grape yield and quality. *Functional Plant Biology*, 30, 607-619. doi:10.1071/FP02110

Montalebifard R., Najafi N., Oustan S. Nyshabouri M. R., Valizadeh M. (2013). The combined effects of phosporous and zinc on evapotranspiration, leaf water potential, water use efficiency and tuber attributes of potato under water deficit conditions. *Scientia Horticulturae*, 162, 31 – 38. doi:10.1016/j.scienta.2013.07.043

Moriana A., Orgaz F., Pastor M., Fereres E. (2003). Yield responses of a mature olive orchard to water deficits. *Journal of the American Society of Horticultural Science*, 128(3), 425-431.

Moyer M., Peters R. T., Hamman R. (2013). Irrigation Basics for Eastern Washington Vineyards. Washington State University Extension. http://cru.cahe.wsu.edu/CEPublications/EM061E/EM061E.pdf (12.dec. 2015)

Novello V., Schubert A., Antonietto M., Boschi A. (1992). Water relations of grapevine cv. Cortese with different training systems. Vitis – Journal of Grapevine research, 31(2), 65-75.

OIV (International Organisation of Vine and Wine). (2011). http://www.oiv.int/en/ (9.11.2018)

Patakas A., Noitsakis B., Chouzouri A. (2005). Optimization of irrigation water use in grapevines using the relationship between transpiration and plant water status. *Agriculture, Ecosystem and Environment*, 106, 253-259. doi:10.1016/j.agee.2004.10.013

Pelengič R., Pipan B., Meglič V., Rusjan D. (2012). Ovrednotenje genskih virov belih sort žlahtne vinske trte (*Vitis vinifera* L.). *Acta agriculturae Slovenica*, 99, 433-438.

Pintar M. (2006). Osnove namakanja s poudarkom na vrtninah in sadnih vrstah v zahodni, osrednji in južni Sloveniji. Ljubljana, Ministrstvo za kmetijstvo, gozdarstvo in prehrano: 55 str.

Pintar M., Zupanc V. (2017). Deficitno namakanje v poljedelstvu in zelenjadarstvu - izzivi in perspektive. V: Čeh B. (ur.), et al. Novi izzivi v agronomiji 2017 : zbornik simpozija, Laško, 2017 Ljubljana: Slovensko agronomsko društvo. 2017, str. 272-276

Podgornik M., Bandelj D. (2015). Deficitni princip namakanja oljčnih nasadov v Slovenski Istri. *Acta agriculturae Slovenica*, 105, 337-344.

Poling B., Spayd S. (2015). Grapevine water relations and vineyard irrigation. The North Carolina Winegrape Grower's Guide. http://content.ces.ncsu.edu/chapter-10-grapevine-water-relations-and-vineyard-irrigation.pdf (12.dec. 2015)

Reščič J., Mikulič-Petkovšek M., Štampar F., Zupan A., Rusjan D. (2015). The impact of cluster thinning on fertility and berry and wine composition of 'Blauer Portugieser' (*Vitis vinifera* L.) grapevine variety. *Journal International des Sciences de la Vigne et du Vin*, 49(4), 275-291. doi:10.20870/oeno-one.2015.49.4.16

Reščič J., Mikulič-Petkovšek M., Rusjan D. (2016). The impact of canopy managements on grape and wine composition of cv. 'Istrian Malvasia' (*Vitis vinifera* L.). *Journal of the Science of Food and Agriculture*, 96(14), 4724-4735. doi:10.1002/jsfa.7778

Roby G., Harbertson J.F., Adams D.A., Matthews M.A. (2004). Berry size and vine water deficits as factors in winegrape composition: Anthocyanins and tannins. *Australian Journal of Grape and Wine Research*, 10, 100-107. doi:10.1111/j.1755-0238.2004.tb00012.x

Rogiers S. Y., Greer D. H., Hutton R. J., Landsberg J. J. (2009). Does night-time transpiration contribute to anisohydric behaviour in a *Vitis vinifera* cultivar? *Journal of Experimental Botany*, 60(13), 1-13. doi:10.1093/jxb/erp217

Romano N, Santini, A.. (2002). Water retention and storage: Field. V: Methods of Soil Analysis, Part 4, Physical Methods, Edition: SSSA Book Series N.5, Chapter: Water retention and storage: Field., Publisher: Soil Science Society of America, Editors: J.H. Dane, G.C. Topp, pp.721-738

Romero P., Gil-Muñoz R., M. del Amor F., Valdés E., Fernandez J.I., Martinez-Cutillas A. (2013). Regulated deficit irrigation based upon optimum water status improves phenolic composition in Monastrell grapes and vines. *Agricultural Water Managament*, 121, 85-101. doi:10.1016/j.agwat.2013.01.007

Ruiz-Sanchez M.C., Domingo R., Castel J.R. (2010). Review. Deficit irrigation in fruit trees and vines in Spain. *Spanish Journal of Agricultural Research*, 8, S5-S20. doi:10.5424/sjar/201008S2-1343

Rusjan D. (2018). Osebna komunikacija.

Rusjan D., Bubola M., Janjanin D., Užila Z., Radeka S., Poljuha D., Pelengić R., Javornik B., Štajner N. (2015). Ampelographic characterisation of grapevine accesions denominated 'Refošk', 'Refosco', Teran' and 'Terrano' (*Vitis vinifera* L.) from Slovenia, Croatia and Italy. *Vitis – Journal of Grapevine Research*, 54, 77-80.

Salon J.L., Chirivella C., Castel J.R. (2005). Response of cv. Bobal to timing on deficit irrigation in Requena, Spain: Water relations, yield and wine quality. *American Journal of Enology and Viticulture*, 56, 1, 1-8.

Santesteban L.G., Miranda C., Royo J.B. (2011). Regulated deficit irrigation effects on growth, yield, grape quality and individual anthocyanin composition in *Vitis vinifera* L. cv. 'Tempranillo'. *Agricultural Water Managament*, 98, 1171-1179. doi:10.1016/j.agwat.2011.02.011

Schultz H.R. (2003). Differences in hydraulic architecture account for near-isohydric and anisohydric behaviour of two field-grown *Vitis vinifera* L. cultivars during drought. *Plant, Cell and Environment*, 26, 1393-1405. doi:10.1046/j.1365-3040.2003.01064.x

Simončič J., Mavrič Štrukelj M., Brdnik M., Štabuc R., Novak E. (2017). Slovenski vinogradi. V: Zbornik prispevkov. 5. Slovenski vinogradniško-vinarski kongres, Šentjernej, 12. maj 2017. Čuš F., Košmerl T., Vanzo A. (ur.). Ljubljana, Kmetijski Inštitut Slovenije: 2-36

Sousa T.A., Oliveira M.T., Pereira J.M. (2006). Physiological indicators of plant water status of irrigated an non-irrigated grapevines grown in a low rainfall area of Portugal. *Plant and Soil*, 282, 127-134. doi:10.1007/s11104-005-5374-6

Stajnko D., Pulko B., Rakun J. (2010). Possible application of didifferental global positioning siystem (DGPS) to harvesting date and precision viticulture. *African Journal of Biotechnology*, 9(48), 8182-8191. doi:10.5897/AJB10.1245

Statistični urad Republike Slovenije. Irrigation by type of area, Slovenia, annually by IRRIGATED AREA, MEASURES and YEAR. http://pxweb.stat.si/pxweb/Dialog/Saveshow.asp (18.feb. 2016)

Steudle E., (2001). The cohesion-tension mechanism and the acquisition of water by plant roots. *Annual Review of Plant Physiological and Molecular Biology*, 52**,** 847-875. doi:10.1146/annurev.arplant.52.1.847

Štajner N., (2010). Mikrosatelitski markerji uporabni za identifikacijo kultivarjev vinske trte (*Vitis vinifera* L.). Acta agriculturae Slovenica, 95, 183-192.

Štrukelj, M., Razinger, J., Grubar, B., Žibrat, U., Mavrič Pleško, I., Vodnik, D., Urek, G. (2016) Physiological response of grapevine Vitis vinifera L. to grapevine leafroll associated viruses (GLRaV-1 and GLRaV-1 + GLRaV-3) | [Fiziološki odziv žlahtne vinske trte Vitis vinifera L. na okužbo z zvijanjem listov vinske trte povezanih virusov (GLRaV-1 in GLRaV-1 + GLRaV-3)] *Acta Agriculturae Slovenica*. 107(2), 519-529. doi:10.14720/aas.2016.107.2.22

Tomaz A., Pacheco C.A., Coleto Martinez J.M. (2017). Influence of cover cropping on water uptake dynamics in an irrigated Mediterranean vineyard. *Irrigation and Drainage*, 66(3), 387-395. doi:10.1002/ird.2115

Tuller M., Or D. (2005). Water films and scaling of soil characteristic curves at low water contents. *Water Resources research*, 41(9), W09403. doi:10.1029/2005WR004142

Vodnik D. (2012). Osnove fiziologije rastlin. Ljubljana, Oddelek za agronomijo, Biotehniška fakulteta: 141 str.

Vršič S., Lešnik M. (2010). Vinogradništvo. 2. dopolnjena izdaja, Ljubljana, Kmečki glas: 403 str.

Williams L.E., Ayars J.E. (2005). Grapevine water use and the crop coefficient are linear functions of the shaded area measured beneath the canopy. *Agricultural and Forest Meteorology*, 132, 201-211. doi:10.1016/j.agrformet.2005.07.010

Yunusa I.A.M., Walker R.R., Lu P. (2004). Evapotranspiration components from energy balance, sapflow and microlysimetri techniques for an irrigated vineyard in inland Australia. *Agricultural and Forest Meteorology*, 127, 93-107. doi:10.1016/j.agrformet.2004.07.001

Zhang Y., Kang S., Ward E.J., Ding R., Zhang X., Zheng R. (2011). Evapotranspiration components determined by sap flow and microlysimetry techniques of a vineyard in northwest China: Dyanmics and influental factors. *Agricultural Water Management*, 98, 1207-1214. doi:10.1016/j.agwat.2011.03.006

Zhang Y.,Oren R., Kang S. (2012). Spatiotemporal variation of crown-scale stomatal conductance in an arid *Vitis vinifera* L. cv. Merlot vineyard: direct effects on hydraulic properties and indirect effects of canopy leaf area. *Tree Physiology*, 32, 262-279. doi:10.1093/treephys/tpr120

Zhang Q., Wang S., Li L., Inoue M., Xiang J., Qui G., Jin W. (2014). Effects of mulching and sub-surface irrigation on vine growth, berry sugar content and water use of grapevines. *Agricultural Water Management*, 14, 1-8. doi:10.1016/j.agwat.2014.05.015

Zsófi Z., Tóth E., Váradi G., Rusjan D., Bálo B. (2008). The effect of progressive drought on water relations and photosynthetic performance of two grapevine cultivars (*Vitis vinifera* L.). *Acta Biologica Szegediensis*, 52(2), 321-322.

doi:10.14720/aas.2018.111.3.19 Review article / pregledni znanstveni članek

# On-farm seed priming interventions in agronomic crops

Neha CHATTERJEE[1], Deepranjan SARKAR[2], Ardith SANKAR[1], Sumita PAL[3], H. B. SINGH[3], Rajesh Kumar SINGH[1], J. S. BOHRA[1], and Amitava RAKSHIT[2]*

Received May 07, 2018; accepted November 12, 2018.
Delo je prispelo 07. maja 2018, sprejeto 12. novembra 2018.

**ABSTRACT**

Priming techniques are gaining importance in agriculture with the increase in environmental stresses. Resource-poor farmers are in urgent need of such techniques as they are simple, economical, and value-added intervention associated with low-risk bearing factors. Seed enhancement methods are key to improve seed performance and achieve a good stand establishment. Worldwide beneficial effects of priming are recorded. But these technologies have still not reached most farmers. This review highlights the importance of on-farm priming strategies in modern crop production system to yield better productivity and obtain higher economic returns. Stimulation of the pre-germination metabolic changes by priming is necessary to overcome the environmental challenges that a plant can encounter. Thus, the study also focuses on mechanisms associated with priming-induced stress tolerance of crops. Various safe practical methods of seed priming can be easily adopted by the farming community to alleviate the levels of different stresses which can hamper productivity. Simultaneously they can produce good quality seeds and use them further for the next crop cycle cutting the costs of seed purchase.

**Key words:** priming methods; priming agents; stress; stress tolerance; plant growth

**IZVLEČEK**

**UVAJANJE PREDSETVENE OBDELAVE SEMEN POLJŠČIN NA KMETIJAH**

Tehnike predsetvene obdelave semen pridobivajo na pomenu v kmetijstvu s povečevanjem okoljskih stresov. Zaradi preprostosti uporabe, ekonomičnosti in dodane vrednosti zaradi zmanjšanja tveganja so te metode nujno potrebne za revne kmete. Metode pospeševanja kalitve semen so ključne za izboljšanje setve in za vzpostavitev dobrih posevkov. Blagodejni učinki predsetvene obdelave semen so zabeleženi širom po svetu, vendar te tehnologije še vedno niso dosegle večine kmetov. Ta pregled osvetljuje pomen teh postopkov na kmetijah v modernih sistemih pridelovanja poljščin za boljšo produktivnost in doseganje večjih iztržkov. Vzpodbujanje predkalitvene presnovne aktivnosti semen z njihovo predsetveno obdelavo je potrebno za preseganje okoljskih izzivov s katerimi se soočajo rastline. Raziskava se osredotoča tudi na mehanizme, povezane s predsetveno obdelavo semen vzpodbujene tolerance na stres pri kmetijskih rastlinah. Različne varne in praktične metode predsetvene obdelave semen bi lahko bile z lahkoto uporabljene pri kmetovalcih za zmanjševanje okoljskih stresov, ki ovirajo produktivnost rastlin. Hkrati bi tako pridelali kvalitetna semena za naslednjo setev in s tem zmanjšali stroške njihovega nakupa.

**Ključne besede:** metode predsetvene obdelave semen; sredstva za predsetveno obdelavo; stres; toleranca na stres; rast rastlin

[1] Department of Agronomy, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi 221005, Uttar Pradesh, India

[2] Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi 221005, Uttar Pradesh, India; *corresponding author: amitavar@bhu.ac.in

[3] Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi 221005, Uttar Pradesh, India

## 1 INTRODUCTION

Farmers in developing countries often encounter poor crop establishment in crop production practices. The reasons for this failure might be due to some basic factors like availability of good quality seeds, knowledge of seed technology, proper sowing techniques, or climate change and various environmental stresses (Figure 1). The extra costs in labour, draft power, and materials hinder the small and marginal farmers to alleviate these constraints efficiently. Therefore, we need to popularize the seed enhancement technologies which could be easily adopted by all farmers, irrespective of their socio-economic conditions. Because successful crop production depends on good quality seeds.

Abiotic and biotic stresses are the norm for any plant while completing its phenology (Suzuki et al., 2014). Among others, the predominant abiotic stresses include extremes of temperature, drought, high salinity, nutrient, and oxidative stress. While pathogens (fungi, bacteria, viruses, and nematodes) and pests (insects, arachnids, herbivores, and weeds) are threats to plants as biotic stress. These stresses are known to cause physiological, biochemical, and metabolic changes in crop plants affecting the metabolism, performance, and ultimately adversely reducing the yield of plants (Anjum et al., 2011; Rejeb et al., 2014).

High salt stress is the cause for disruption of water relations and ionic distribution in plants (Munns & Tester, 2008). Basically, salinity has a negative impact on seed germination and seedling establishment either by inhibiting water uptake with development of high external osmotic potential or through accumulation of toxic ions ($Na^+$ and $Cl^-$) in the system (Nasri et al., 2015; Negrão et al., 2017). Water is a primary factor of production, and hence drought stress, particularly at the critical growth stages is one of the major environmental limiting factor affecting crop growth and productivity (Araus et al., 2002). The negative consequences of drought stress can be seen in every aspect of plant life (Rahman et al., 2004). Drought stress is known to bring about a series of adverse effect in biochemical and physiological processes of plants, viz., disturbance in ion homeostasis and enzymatic activities, increased levels of reactive oxygen species (ROS), decreased cell division, leaf parameters (area, size, and chlorophyll contents), $CO_2$ assimilation, photosynthesis, root proliferation, stem elongation, and water use efficiency, and consequently reduction in grain yield (Farooq et al., 2009; Anjum et al., 2011; Farooq et al., 2012; Nikju et al., 2015). Moreover, seeds sown in dry soil often delays in germination as they absorb too little water from the soil, following delay in several imbibition processes. If a method of germination could be devised to overcome with this time lag in germination by pre-soaking of the seeds in water, germination would occur swiftly ensuing in a better crop stand.

Another major global constraint in productivity of agricultural crops is the inaccessibility of nutrients by plants due to their deficiency in soil, commonly known as nutrient stress (Baligar et al., 2001; Sun et al., 2011). Both macro- and micronutrients have great roles in meeting crops demands and improving the yield levels. Micronutrient deficiency has already been found in the food chain; proper management strategies focusing on nutritional quality is a serious concern in sustainable agriculture (Khoshgoftarmanesh et al., 2010). Several other abiotic stresses like cold, heat, light, or irradiation can adversely alter the plant physiology and govern the occurrence of biotic stresses in the environment (Suzuki et al., 2014; Pandey et al., 2017).

Plants in response to multiple environmental stresses activate various defense mechanisms and signaling pathways regulated by ROS, hormones (salicylic acid, abscisic acid, jasmonic acid, ethylene, gibberellic acid, auxins, and cytokinins), proteins, and transcription factors (Verma et al., 2016; Gimenez et al., 2018). Understanding these mechanisms will not only help in saving important crops but also preventing economic losses. Searching suitable priming agents can significantly contribute to acclimation pathways of plants developed against the threats of stress-induced infections.

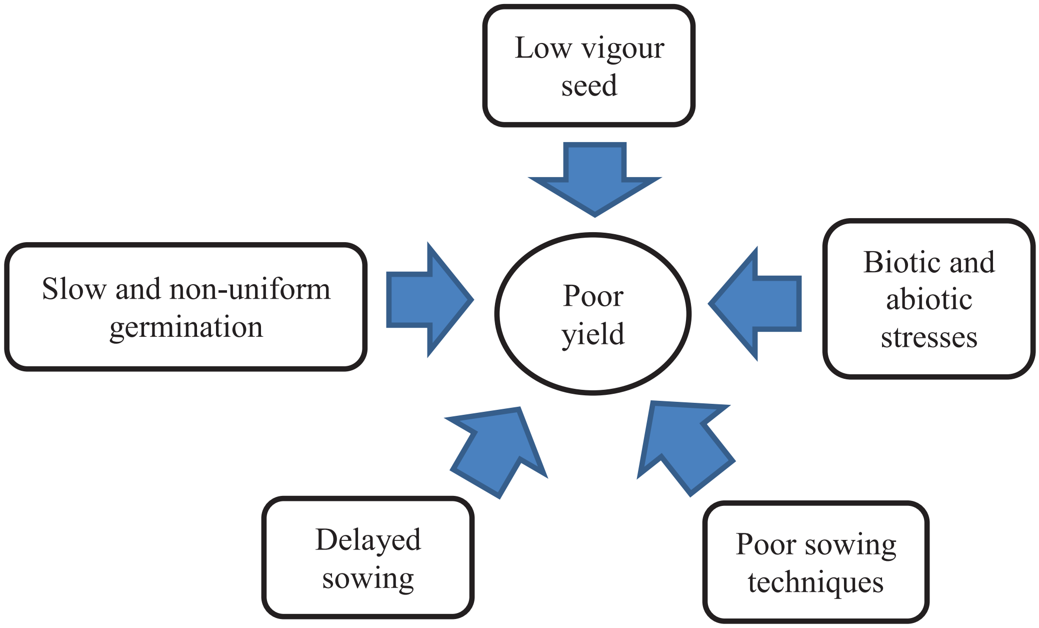



**Figure 1:** Basic issues in low productivity of crops

While attempting to maintain an optimum external environment for crops, the overdependence on natural resources and fossil fuel-based technology, over the years, has led to the gradual depletion of global water resources, escalating greenhouse gas emissions, and a gradual decline in productivity particularly in the high input-intensive agriculture systems. This scenario is likely to worsen and the brunt of the menace will supposedly be faced by rainfed agriculture systems globally on account of their inherent resource limitation. While 40 % of the world's and 68 % of Indian agriculture is rainfed, 42 % of the Indian food requirement is met through dryland agriculture (Singh et al., 2004). Climate change is a real intimidation to the developing world which unchecked, will become a major hindrance to poverty eradication. Besides, the contamination of the natural water bodies and soil strata through excessive agrochemical use coupled with improper irrigation techniques in intensive-irrigated agriculture systems call for alternative options under low-input sustainable agriculture systems with the ultimate aim of acquiring global food security.

## 2 POSSIBLE ALTERNATIVES

Throughout the history of agriculture, several methods have been adopted to achieve better crop tolerance to stresses and production in unfavorable environmental conditions. These entail breeding strategies (selection and hybridization, molecular breeding, genetic engineering) (Athar & Ashraf, 2009; Waqar et al., 2014), agronomic strategies (variety selection, date of sowing, soil management, irrigation) (Mariani & Ferrante, 2017; Lamaoui et al., 2018), physiological approaches (seed priming, foliar spray) (Bakhtavar et al., 2015), etc. The success of breeding techniques is often limited due to huge requirement of skilled manpower and energy, complexity associated with stress tolerance traits, tedious and costly methods, and ethical regulations. These drawbacks have forced the researchers to go for the alternatives which are simple and low-cost solution so that they are easily introduced at the field scale by resource-poor farmers.

Pre-sowing seed treatment (seed priming, seed coating, and seed pelleting), in this regard, is an effective strategy to overcome different stresses. This pragmatic and short-term approach is basically used as seed enhancement aimed at value adding or upgrading the quality of seed (Taylor et al., 1998). Such intervention involves the application of physical, chemical, and/or biological agents to stimulate seed germination, seedling vigour, and crop yield in a sustainable manner (Sharma et al., 2015). Seed priming is an effective pre-germination physiological method that mends seed performance and delivers quicker and synchronized seed germination (Matsushima & Sakagami, 2013; Nawaz et al., 2015) by prior exposure of seed to a stress situation, which endows plant to better withstand the future stress imposition (Yadav et al., 2011; Ibrahim, 2016). It involves soaking of seeds in water (hydropriming) or solutions of lower water potential (osmotic solutions) composed of polyethylene glycol (PEG) (osmopriming) or salts ($CaCl_2$, $CaSO_4$, $KH_2PO_4$, KCl, NaCl, etc.) (halopriming) prior to germination (Jisha et al., 2013; Paparella et al., 2015; Wojtyla et al., 2016). In priming, controlled imbibition is provided so as to induce the metabolic process of germination without actual germination and seminal root emergence (Binang et al., 2012; Nejad & Farahmand, 2012). Seed coating is a process of application of adhesive polymers with active ingredients (nutritional elements, plant growth regulators, insecticides, fungicides, and other chemicals) to the seed surface without altering its original shape or size (Avelar et al., 2012; Mandal et al., 2015; Pedrini et al., 2017). Seed pelleting came out as an advanced form of seed coating technology. The method includes enclosing of seed in a layer of inert material that may change the shape and size of raw seed, but produce a standard product (uniform round seeds) to facilitate improved planting (Mandal et al., 2015; Mei et al., 2017 ). Seed coating technologies are sophisticated and expensive (Sharma et al., 2015). Seed priming emerged as the most common method of pre-sowing treatments (Parera & Cantliffe, 1994; Jisha et al., 2013; Soleimanzadeh, 2013; Paparella et al., 2015; Lutts et al., 2016; Wojtyla et al., 2016).

## 3 ON-FARM PRIMING OPTIONS

In ‘‘on-farm’’ seed priming, seeds are soaked in water, surface dried, and sown in the field (Rashid et al., 2002). The term on-farm is used to differentiate it from the intensive agricultural systems using high input and advance technology for seed priming (Harris et al., 1999). This practice is very common in tropical environments or semi-arid agricultural lands as a low-cost and low-risk intervention. A plethora of priming techniques has been developed to enhance and stabilize field emergence, and those are categorized according to the priming agents used. The efficiency of these approaches is dependent upon certain factors like aeration and water potential of priming solution, light, temperature, priming duration, post-hydration drying, seed and storage condition, and plant species (Parera & Cantliffe, 1994; Wojtyla et al., 2016). Thus, it is essential to evaluate the efficacy of various priming options in different crops and agro-climatic conditions, and optimize our chosen priming technique.

### 3.1 Hydropriming

Slow and non-uniform germination of seeds induced the requirement of water-based seed priming. Hydropriming is a very simple, cost-effective, and eco-friendly technique which basically involves soaking seeds in water for a pre-determined time followed by re-drying to their initial moisture content (Farooq et al., 2006; Lutts et al., 2016). Submergence can also be performed in distilled water with or without aeration. Earlier, this practice was known as hardening, which was done by alternate soaking of seeds in water and drying before sowing. The process of seed germination occurs in three phases, viz., rapid water uptake or imbibition (phase I), lag or plateau phase (phase II), and protrusion of seminal root and resumption of growth (phase III) (Bewley, 1997). Hydropriming reduces the lag period, and ensures rapid and uniform germination for good stand establishment (Ahammad et al., 2014). Controlled seed hydration as a pre-sowing strategy triggers pre-germination metabolic activities in the form of cellular physiological, biochemical, and molecular changes (Figure 2) (Ibrahim, 2016; Wojtyla et al., 2016). Improved germination of hydroprimed seeds is a result of activation of enzymes (amylase, protease, phosphatase, lipase, etc.), ATP production, RNA and protein synthesis, DNA replication, detoxification of ROS and lowering of lipid peroxidation by antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and glutathione reductase (GPx)], accumulation of germination enhancing metabolites (proline, soluble sugars, etc.), higher utilization of seed reserves (proteins, carbohydrates, lipids, and phosphorus compounds), and other metabolic repairing mechanisms (McDonald, 2000; Ghassemi-Golezani & Hosseinzadeh-Mahootchi, 2013; Vaz Mondo et al., 2016).

Information on duration (maximum length of time) (Table 3) of hydration treatment can bring success at the farmer’s level. Mahmoodi et al. (2011) evaluated four hydropriming durations (6, 12, 18 and 24 h) to improve seedling vigour and field establishment of maize, and found 18 h to be the most effective priming period. In case of pinto bean, Ghassemi-Golezani et al. (2010) reported 7 and 14 h priming is sufficient to augment seed and seedling vigour, stand establishment, and grain yield instead of soaking the seeds for 21 h. For optimizing duration of hydropriming in mung bean, Shukla et al. (2018) chose five intervals of time viz., 2, 4, 6, 8, 24 h, and concluded 6 h of priming to be optimum as the germination responses were almost similar if the duration was increased after this priming treatment.

The major limitations associated with this technique include uncontrolled water uptake, which cannot be regulated because it is a property of seeds; and non-uniform hydration of seeds may result in unsynchronized germination (Taylor et al., 1998; McDonald, 2000; Di Girolamo & Barbanti, 2012). To overcome these challenges, drum priming is often used, where major focus is given on duration, temperature, and volume of water.

### 3.2 Osmopriming

Osmopriming is alternatively known as osmotic priming, osmotic conditioning or osmoconditioning. In this technique, seeds are soaked in osmotic solutions of organic compounds like PEG, mannitol, glycerol, sorbitol, etc. having low water potential so as to restrict the water uptake by seeds and allow the pre-germinative metabolic events to continue, but prevent the seminal root protrusion (Ashraf & Foolad, 2005). PEG with a molecular weight of 6000–8000 daltons having some osmotic potential ($\Psi s$) is dissolved in water for seed treatment. Out of the different concentrations (5, 10, 15, and 20 %) of PEG solutions, Faijunnahar et al. (2017) reported 10 % is sufficient to improve the germination, seedling growth, and water relation behaviour of wheat genotypes. Sadeghi et al. (2011) tested the efficacy of PEG-6000 with different levels of osmotic potentials (-0.4, -0.8, -1.2, -1.6, and -2 MPa) and priming durations (6, 12, 24, and 48) on germination behaviour (percentage, mean time, index) and vigour of soybean seeds. Better results were observed in the seeds primed with -1.2 MPa for 12 h.

This technique is further classified as halopriming when the seeds are soaked in low water potential solutions composed of inorganic salts. Osmopriming/halopriming are also used for developing stress tolerances in plants (Table 2 and 3). Eivazi (2012) found wheat seeds primed with 2.5 % KCl for 16 h developed drought tolerance in plants besides increasing the grain yield. Rice seeds primed with NaCl @ 50 and 75 mM performed higher seedling vigor, osmotic stress tolerance potential, and overall crop growth than hydropriming (Jisha & Puthur, 2014). However, both of the priming treatments were able to modulate antioxidant enzyme activities, reduce lipid peroxidation of biomembranes, and increase the protein, carbohydrate, photosynthetic pigment, photochemistry, and mitochondrial activities of rice seedlings under salt stress conditions. A similar study of Goswami et al. (2013) revealed that rice seedlings conferred drought resistance by seed priming with 5 % of PEG-6000 and NaCl, which strengthened GPx activity and reduced peroxidative damage of the crop. Interestingly, priming effects of NaCl and PEG was not visible in chickpea under water deficit stress conditions (Kaur et al., 2002); priming with mannitol (4 %) and water gave better results in growth by modulating enzymes (amylase, invertase, sucrose synthase, and sucrose phosphate synthase) of sucrose metabolism (Kaur et al., 2002, 2005). Seed pretreatment with NaCl (50 mM) moderated the adverse effect of salt stress by modifying the antioxidant enzymes like SOD, CAT, and catechol peroxidase (CPX), enhancing accumulation of osmolytes (proline), lowering malondialdehyde (MDA) and $H_2O_2$ contents of plants, and improving growth and photosynthetic pigments (total chlorophyll, chlorophyll-a, chlorophyll-b, and carotenoids) (Saha et al., 2010). Application of PEG may cause disruption in aeration of solution due to its high viscosity (Paparella et al., 2015).

### 3.3 Solid Matrix Priming

In solid matrix priming (SMP) or matric conditioning, solid or semi-solid medium is used instead of liquid medium (Copeland & McDonald, 1995). This technique is accomplished by mixing seeds with a solid or semi-solid medium and specified amounts of water. By the virtue of the physical and chemical characteristics of the matrix, the water uptake by the seeds is restricted. In SMP, a small amount of liquid per unit of seed and solid particles is used. During SMP, water is slowly provided to the seeds and thus, slow or controlled imbibition occurs, allowing cell repair mechanisms to function (Jisha et al., 2013). Predominant solid matrices are exfoliated vermiculite, expanded calcined clay, bituminous coal, sodium polypropionate gel or synthetic calcium silicate (Kubik et al., 1988). Some locally available materials that are generally utilized as solid matrices are sawdust, charcoal and volcanic cinder and they offer the scope for reducing priming costs (Lorenzo, 1991). In the study of Lorenzo (1991) on SMP using sawdust, ground charcoal and volcanic cinder, soybean seeds responded favorably to shorter incubation periods, i.e., 1, 2, and 5 days. The longer incubation periods and higher water levels were harmful to the seeds because they encouraged fungal growth. SMP was also found to be effective in improving soybean germination by Mercado & Fernandez (2002).

### 3.4 Biopriming

Biopriming or biological seed treatment is the application of beneficial microbes in seed–plant–soil system to enhance plant productivity and simultaneously maintain the ecological balance. The process is accomplished by controlled seed hydration followed by coating of seeds with biological agents (Sarkar et al., 2017). The priming agents help in plant growth promotion by supplying nutrients to crops, enhancing resistance of plants to biotic and abiotic stresses, improving soil diversity (Singh, 2016), ameliorating soil structure, bioremeding the polluted soils (Mahmood et al., 2016). The technology basically aims at reduction of chemical inputs in our production system.

Farmers can treat the seeds with microbial formulations at the rate of 10 g $kg^{-1}$ seed after soaking the seeds for 12 h and incubate at room temperature for 48 h to obtain microbial coating of seeds (Reddy, 2013). Carboxymethyl cellulose (CMC), gum arabic, rice water, etc. are used for adhesion of inoculums. The seeds are air-dried after incubation, and then used for sowing. Liquid formulations can also be used by the farmers as they mix well over the seed surface without any sticking agent. Root dipping of the seedlings for few hours before transplanting is also a common practice now-a-days. Biopriming agents are potential in promoting germination, controlling pathogens, and favouring growth and development in plants (Table 1). Microbial consortium of compatible microbes can also be used for better effects. Selection of microbes is an important step in biopriming as the growth-promoting abilities of microbes are highly specific to certain plant species, cultivars, and genotypes (Rakshit et al., 2015). Further, it can also promote synergistic interaction between microbes (e.g., *Trichoderma* and arbuscular mycorrhiza) (Meena et al., 2017). Arbuscular mycorrhizal fungi (AMF) (e.g., *Glomus* sp.) are getting special attention in biopriming techniques because of their multi-functional nature stimulating the growth and development of plants (Dhawal et al., 2016). Biopriming is a suitable tool to enhance the nutrient use efficiency in soil–plant–environment system (Meena et al., 2016).

**Table 1:** Some field experiments carried on biopriming

| Crop | Biological agent | Method | Major effect | References |
|---|---|---|---|---|
| Boro rice | *Trichoderma* sp. | Seed inoculation @ 4 % of seed weight before 4 h of sowing | Higher grain yield | Rahman et al. (2015) |
| Maize | *Trichoderma harzianum*Rifai, (1969) | Seed inoculation @ 10 g $kg^{-1}$ seed | Reduction of *Fusarium verticillioides* (Sacc.) Nirenberg (1976) and fumonisin infection, increased seed germination, vigour index, field emergence, yield, 1000 seed mass | Chandra Nayaka et al. (2010) |
| Barley | *Azospirillum* sp. | Seed inoculation @ 7 g $kg^{-1}$ seed | Increased plant height, spike length, number of spike per area, grains per spike, 1000 grain mass, grain yield | Shirinzadeh et al. (2013) |
| Soybean | *Rhizobium* sp. | Seed inoculation @ 7.5 x $10^6$ cells $seed^{-1}$ | Increased germination, nodulation, seed yield, harvest index, nitrogen harvest index | Amule et al. (2017) |
| Pearl millet | *Pseudomonas fluorescens* (Flügge 1886) Migula, 1856 | Seed inoculation | Enhanced germination, seedling vigour, plant height, leaf area, tillering capacity, 1000 seed weight, grain yield, induction of resistance against downy mildew | Niranjan Raj et al. (2004) |

### 3.5 Nutrient priming

Nutrient priming has been proposed as a novel technique that combines the dual benefits of seed priming with an improved nutrient supply (Al-Mudaris & Jutzi, 1999). In nutrient priming, seeds are primed in solutions containing the limiting nutrients instead of being soaked just in water (Arif et al., 2005). Of the mineral nutrients, potassium plays an important role in imparting stress tolerance to plants (Cakmak, 2005). Seed priming in $Zn^{2+}$ solutions improves grain yield of chickpea and wheat (Arif et al., 2007). Micronutrients are required in traces however, their deficiencies are quite common in crop plants (Abd El-Wahab, 2008). There are mainly 3 methods of micronutrient application in crops: application to soil, through foliar sprays and seed treatment (Johnson et al., 2005). Each method may affect plant growth distinctly. The use of micronutrient enriched seeds (seed priming) has been reported to be an easier and cost-effective strategy in overcoming micronutrient deficiencies (Harris et al., 1999; Rakshit et al., 2013). Seed priming has been shown to enhance the speed of germination (Deering & Young, 2006), reduce the emergence time, enhance seedling vigour (Harris, 1996) and obtain better stand establishment (Diniz et al., 2009), and increase yield (Yilmaz et al., 1998) in wheat, rice, maize, sorghum, chickpea, and soybean. There is evidence that sowing seeds enriched with micronutrients is also agronomically beneficial (Welch, 1986).

### 3.6 Redox priming

The cell processes are largely determined by "redox state". While redox state can categorically imply to the ratio of interconvertible oxidized and reduced species in a redox pair, lately this term is also correlated to define the cellular redox environment (Krebs, 1967; Schaefer & Buettner, 2001). It is opined that if the reduced redox state of a cell is possible to maintain, the extent of stress-induced damage can be significantly mitigated (Mittler, 2002). Srivastava et al. (2009) found that thiourea treatment to the seeds of Indian mustard (*Brassica juncea* (L.) Czem.) was helpful in maintaining the integrity and functioning of mitochondria in seeds as well as seedlings under salinity stress conditions. Srivastava et al. (2010b) treated *Brassica juncea* seeds with thiourea and observed different signaling and effector mechanisms to be regulated in a synchronized manner.

Seed priming with hydrogen peroxide was also reported in wheat (Wahid et al., 2007). Manjunatha et al. (2008) reported notable enhancement of seed germination and seedling vigour due to exogenous application of nitric oxide (NO) donors through seed treatment in pearl millet. Recently, Barba-Espín et al. (2012) reported that hydrogen peroxide could act as signaling molecule during the initiation of seed germination involving certain specific changes at proteomic, trancriptomic and hormonal levels. In the opinion of Draganić & Lekić (2012), priming with antioxidant substances like ascorbic acid, glutathione and tocopherol (vitamin E) was beneficial in increasing the vigour of sunflower seeds exposed to low temperatures. In sorghum, seed priming with cysteine reduced the injury caused by gamma radiations and the effects of cysteine were most prominent in primary root elongation (Reddy & Smith, 1978).

### 3.7 Hormonal priming

Plant growth regulators like auxins, cytokinins, and gibberellins can be utilised as a pre-sowing seed treatment to improve their germination and emergence in stress situations (Lee et al., 1998; Jisha et al., 2013). Particularly, abscisic acid (ABA) is extensively involved in plant responses to abiotic stresses such as drought, low temperature, and osmotic stress (Fujita et al., 2006). Besides inducing the expression of many salt-responsive genes (Chandler & Robertson, 1994), exogenous ABA application was found in some experiments to increase salt tolerance of the treated plants or plant tissues (Xiong & Zhu, 2002). At the molecular level, ABA is known to induce the expression of numerous plant genes (Rock, 2000). ABA priming showed increased rate of germination as compared to nonprimed seeds in Indian mustard (Srivastava et al. 2010a, b). The beneficial effects of gibberellic acid ($GA_3$) on germination are well known (Angrish et al., 2001; Radi et al., 2001). $GA_3$ (100 mg $l^{-1}$) applied as presowing treatment resulted in the highest $K^+$ and $Ca^{2+}$ content in the shoots of faba beans (*Vicia faba* L.) and cotton (*Gossypium barbadense* L.) (Harb, 1992). Auxin has also been used for priming. In wheat seed germination, auxin treatments increased the hypocotyl length, fresh and dry mass of seedlings and hypocotyl dry mass (Akbari et al., 2007).

Improved replication in root tips has been found by hormonal and vitamin priming (Shakirova et al., 2003) which could be attributed to rapidly dividing root apical meristem, consequently leading to better growth. Moreover, hormone applications maintain the auxin and cytokinin ratios in the tissues, which inadvertently are responsible for enhancing cell division (Sakhabutdinova et al., 2003). Hormonal priming, specifically ethylene (ET) and chloroethylphosphonic acid (CEPA) has also been found to impart tolerance to cadmium toxicity (50 μM cadmium chloride ($CdCl_2$) to pigeon pea (*Cajanus cajan* (L.) Mill.) (Sneideris et al., 2015).

Some seed priming options adopted in various crops over time are presented in Table 2. Detailed description of seed priming agents and their respective concentrations along with the treatment durations are mentioned in Table 3. The major events of seed priming responsible for improved plant performances are shown in Figure 2.

**Table 2:** Seed priming methods developed for various agro-ecologies and their effect on imparting stress tolerance in agronomic crops

| Crop | Agro-ecology | Treatment | Effect and protective mechanism | References |
|---|---|---|---|---|
| Cereals | | | | |
| Rice | Rainfed | Halopriming (NaCl, $KH_2PO_4$), osmopriming (PEG), hydropriming | Drought and salinity tolerance by increasing carbohydrate, protein, and photosynthetic pigment content, modulating antioxidant enzyme activities (SOD, POD, GPx), reducing lipid peroxidation of biomembranes, enhancing photochemistry and mitochondrial activities of rice seedlings | Goswami et al. (2013); Jisha & Puthur (2014) |
| | Subtropics | Se and SA priming | Chilling resistance by increasing membrane stability, antioxidant activity, starch metabolism (α-amylase activity, total soluble sugar contents) of primed seedlings | Wang et al. (2016a,b) |
| Wheat | Arid and semi-arid | Halopriming ($CaCl_2$), hydropriming, chemical priming ($H_2O_2$), hormonal priming (auxin), choline priming | Salt tolerance by increasing antioxidant activity (SOD, CAT), leaf water relations, nutritional status ($K^+$, $Ca^{2+}$, $NO_3^-$, $PO_4^{3-}$), improving $K^+$:$Na^+$ ratio, reducing toxic elements ($Na^+$, $Cl^-$), RMP leakage of ions, enhancing root | Afzal et al. (2006); Wahid et al. (2007); Akbari et al. (2007); Salama et al. (2011) |

| | | | | |
|---|---|---|---|---|
| | | | growth | |
| | | Hormonal priming (ascorbic acid) | Drought resistance due to accumulation of proline and phenolics leading to membrane stability, tissue water maintenance, reduced oxidative damages | Farooq et al. (2013) |
| Barley | Arid | Nutrient priming ($KH_2PO_4$, $ZnSO_4$) | Drought and nutrient stress tolerance due to increase in root biomass influencing nutrient uptake, water use efficiency | Ajouri et al. (2004) |
| Maize | Sub-tropical semi-arid | Halopriming ($CaCl_2$) | Drought resistance due to well-developed root system facilitating higher water and nutrient supplies | Khan et al. (2015) |
| Triticale | Rainfed | Hydropriming, halopriming ($KH_2PO_4$) | Drought and salt tolerance is related with higher water uptake ability of seeds enhancing the relative water content of shoot, increased root and shoot growth | Yağmur and Kaydan (2008) |
| Pulses | | | | |
| Mung bean | Subtropics | Halopriming (NaCl) | Salt stress tolerance by enhancing growth, photosynthetic pigments (chlorophyll, carotenoids), activities of antioxidant enzymes (SOD, CAT, CPX), accumulation of osmolytes (proline), lowering MDA and $H_2O_2$ contents of | Saha et al. (2010) |

| | | | | |
|---|---|---|---|---|
| | | | plants | |
| Chickpea | Semi-arid | Osmopriming (mannitol), hydropriming | Drought tolerance by rapid hydrolysis of transitory starch | Kaur et al. (2002, 2005) |
| Soybean | Dryland | Nutrient priming ($ZnSO_4$), halopriming ($CaCl_2$), vitamin priming (betaine hydrochloride), hormonal priming ($GA_3$) | Soda saline-alkali stress tolerance by better osmotic adjustment, antioxidant defense system, membrane integrity, higher photosynthetic pigment contents, starch accumulation | Dai et al. (2017) |
| Alfalfa | Arid and semi-arid | Hydropriming, osmopriming (mannitol) | Salinity tolerance with higher activity of antioxidant enzymes (POD, CAT, SOD), accumulation of proline, stabilizing membranes by reducing MDA accumulation and electrolyte leakage | Amooaghaie (2011) |
| Sugar crops | | | | |
| Sugarcane | Tropics and subtropics | Halopriming (NaCl), osmopriming (PEG) | Salt and drought tolerance by osmotic adjustment, antioxidant defense system | Patade et al. (2011) |

**Table 3:** Seed priming agents and treatment durations applied for developing tolerances in some crops under drought and salinity stress

| Crop species | Seed priming treatment | References |
|---|---|---|
| Rice | Halopriming (NaCl @ 50 and 75 mM) for 12 h; hydropriming with distilled water for 24 h; biopriming with *Trichoderma harzianum* @ 10 g $kg^{-1}$ of seed for 24 h; spermidine (0.5 mM) priming for 24 h | Jisha and Puthur (2014); Yuan-Yuan et al. (2010); Rawat et al. (2012); Zheng et al. (2016) |
| Wheat | Hydropriming (16 h); halopriming (2.5 % KCl) for 16 h; osmopriming (10 % PEG) for 12 h; hormonal priming (2 mM ascorbic acid solution) for 10 h; choline priming (5 mM choline chloride) for 24 h | Patra et al. (2016); Eivazi (2012); Faijunnahar et al. (2017); Farooq et al. (2013); Salama et al. (2011) |
| Barley | Hydropriming for overnight (12 to 16 h) | Rashid et al. (2006) |
| Maize | Osmopriming with aerated solution of $CaCl_2$ (ψs -1.25 MPa) for 24 h | Khan et al. (2015) |
| Mung bean | Hydropriming (6 h); chemical priming with β-amino butyric acid solution (1.0 mM) for 6 h | Shukla et al. (2018); Jisha and Puthur (2016) |
| Pea | Halopriming with KCl and KOH @ 250 and 500 ppm (1 h) | Naz et al. (2014) |
| Soybean | Hydropriming (12 h) and hormonal priming (gibberlic acid @ 50 ppm) for 14 h; biopriming with *Trichoderma harzianum* @ 10 g $kg^{-1}$ of seed | Langeroodi & Noora (2017); Khomari et al. (2018) |
| Indian mustard | Hydropriming with distilled water (18 h) | Srivastava et al. (2010a) |
| Sunflower | Hydropriming with distilled water (18 h) | Kaya et al. (2006); Moghanibashi et al. (2013) |
| Sugarcane | Halopriming (NaCl @ 100 mM) for 8 days | Patade et al. (2009) |

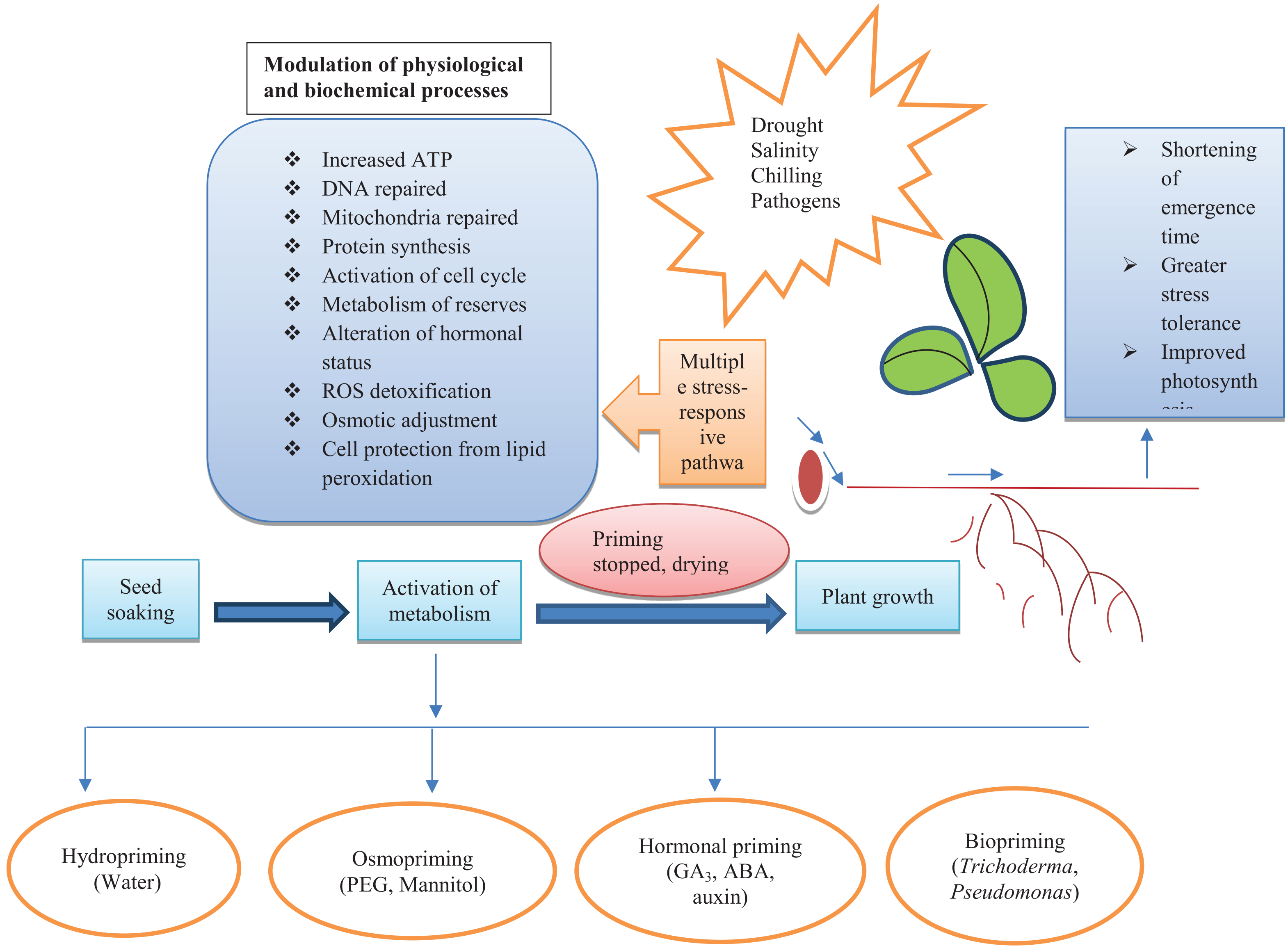



**Figure 2:** Schematic representation of the major processes induced by seed priming during pre- and post-germination stages

## 4 CHALLENGES IN ADOPTION OF SEED PRIMING

Many of the experiments which showed better results were practiced in greenhouse or controlled conditions, especially in the rainfed regions of Bangladesh, Pakistan, Nepal, Africa, and India. Their validation in field conditions is still unexplored. However, this customised method is gaining popularity, and emerged as a smart intervention across diversified agro-ecological regions. Harris et al. (1999, 2001) suggested on-farm seed priming can be revived through farmer-participatory approaches. Farm walks, group discussions, and other tools of extension should be adapted by the researchers/scientists. If the duration of priming is exceeded, then it may lead to seed or seedling damages. Special care is needed while transporting liquid inoculants and applying to the fields (Mahmood et al., 2016). Higher concentrations of priming agents may hamper or delay seed germination. The longevity of low vigour seeds are improved, but reduced in high vigour seeds (Varier et al., 2010). Reduced storability of primed seeds enhances the maintenance costs of farmers (Lutts et al., 2016). In hydropriming, the activation of the physiological processes are non-uniform because seeds are not equally hydrated (Girolamo & Barbanti, 2012). Heavy rainfall after sowing decays primed seeds in soils remaining saturated for a longer period of time (e.g., black soils) (Ramamurthy et al., 2005). Contamination of priming agents can heavily impair seed germination. The efficiency of the biological agents is often low or variable due to unfavourable environmental conditions (e.g., relative humidity, temperature, etc.), shorter shelf-life, low quality, and/or competition with local microbes (O'Callaghan, 2016). For better implementation of seed priming processes, crop species, location, duration of priming, priming agents, temperature, and storage conditions must be considered.

## 5 CONCLUSION

On-farm seed priming is an apt technology for the predominantly resource-poor farmers of the developing world. It is a simple, low-cost intervention which shows quick results in varied eco-systems ranging from arid and semi-arid tropics in India, Africa, the Middle East as well as in highly controlled temperate agriculture systems. Pre-sowing water hardening of seeds has been an age-old practice in several dryland agro-ecosystems. However, the recent advances in halopriming, chemical priming with $KNO_3$, $KH_2PO_4$, $ZnSO_4$, $MnSO_4$, etc., solid matrix priming, ascorbate priming, and redox priming have given this technique an added edge over the traditional system of seed hardening. Coupled with the success of modern science in agriculture, our understanding of the priming-induced responses of crops will open new vistas regarding their stress tolerance abilities, and devise further integrated and sustainable approach applicable in diverse agro-ecosystems. Biopriming with potent strains of *Trichoderma* spp. and *Pseudomonas* spp. among others is a stratagem of not only alleviating moisture stress but also imparting much needed biotic stress tolerance. Seed priming is known to activate certain signaling pathways during the early stages of plant phenology and result in quicker plant defence responses. Thereby, upon subsequent or future exposure to these biotic and abiotic stresses, a second signaling event would excite/stimulate the signaling proteins consequently amplifying the signal transduction, and therefore leading to more rapid and/or more intense activation of previously acquired defence responses (Conrath et al., 2006).

A better and refined transition from laboratory to field adaptability of the different seed priming methods is also needed, which essentially should be adjudged to extensive farmer participatory trials. While seed priming is indeed ascertained as a potential technology to mitigate the adverse effects of climate change and the ensuing alterations in water availability, extremes of temperatures, salinity stress, etc.; future work is needed on the usability of various priming options for varied agro-ecosystems and different crops.

## 6 ACKNOWLEDGEMENT

Sumita Pal is thankful to DST, New Delhi(SR/WOS-A/LS-1199/2015(G))for financial support.

## 7 REFERENCES


Abd El-Wahab, A.M., & Mohamed, A. (2008). Effect of some trace elements on growth, yield and chemical constituents of *Trachyspermum ammi* L. plants under Sinai conditions. *Research Journal of Agriculture and Biological Sciences*, *4*, 717–724.

Afzal, I., Basra, S. M. A., Hameed, A., & Farooq, M. (2006). Physiological enhancements for alleviation of salt stress in wheat. *Pakistan Journal of Botany*, *38*(5), 1649–1659.

Ahammad, K. U., Rahman, M. M., & Ali, M. R. (2014). Effect of hydropriming method on maize (*Zea mays*) seedling emergence. *Bangladesh Journal of Agricultural Research*, *39*(1), 143–150. doi:10.3329/bjar.v39i1.20164

Ajouri, A., Asgedom, H., & Becker, M. (2004). Seed priming enhances germination and seedling growth of barley under conditions of P and Zn deficiency. *Journal of Plant Nutrition and Soil Science*, *167*, 630–636. doi:10.1002/jpln.200420425

Akbari, G., Sanavy, S. A. M. M., & Yousefzadeh, S. (2007). Effect of auxin and salt stress (NaCl) on seed germination of wheat cultivars (*Triticum aestivum* L.). *Pakistan Journal of Biological Sciences*, *10*(15), 2557–2561. doi:10.3923/pjbs.2007.2557.2561

Al-Mudaris, M. A., & Jutzi, S. C. (1999). The influence of fertilizer based seed priming treatment on emergence and seedling growth of *Sorghum bicolor* and *Pennisetum glaucum* in pot trials under greenhouse conditions. *Journal of Agronomy & Crop Science*, *182*, 135–141. doi:10.1046/j.1439-037x.1999.00293.x

Amooaghaie, R. (2011). The effect of hydro and osmopriming on alfalfa seed germination and antioxidant defenses under salt stress. *African Journal of Biotechnology*, *10*(33), 6269–6275.

Amule, F. C., Rawat, A. K., & Rao, D. L. N. (2017). Mono and co-inoculation response of *Rhizobium* and PGPR on soybean in central India. *International Journal of Plant & Soil Science*, *20*(6), 1–13. doi:10.9734/IJPSS/2017/36997

Angrish, R., Kumar, B., & Datta, K. S. (2001). Effect of gibberellic acid and kinetin on nitrogen content and nitrate reductase activity in wheat under saline condition. *Indian Journal of Plant Physiology*, *6*, 172–177.

Anjum, S. A., Xie, X. -Y., Wang, L. -C., Saleem, M. F., Man, C., & Lei, W. (2011). Morphological, physiological and biochemical responses of plants to drought stress. *African Journal of Agricultural Research*, *6*(9), 2026–2032.

Araus, J. L., Slafer, G. A., Reynolds, M. P., & Royo, C. (2002). Plant breeding and drought in $C_3$ cereals: what should we breed for? *Annals of Botany*, *89*(7), 925–940. doi:10.1093/aob/mcf049

Arif, M., Ali, S., Shah, A., Javed, N., & Rashid, A. (2005). Seed priming maize for improving emergence and seedling growth. *Sarhad Journal of Agriculture*, *21*, 239–243.

Arif, M., Waqas, M., Nawab, K., & Shahid, M. (2007). Effect of seed priming in Zn solutions on chickpea and wheat. *African Crop Science Conference Proceedings*, *8*, 237–240.

Ashraf, M., & Foolad, M. R. (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

Athar, H. R., & Ashraf, M. (2009). Strategies for crop improvement against salinity and drought stress: an overview. In M. Ashraf, M. Ozturk, & H. R. Athar (Eds.), *Salinity and water stress* (pp. 1–16). Springer, Netherlands. doi:10.1007/978-1-4020-9065-3_1

Avelar, S. A. G., de Sousa, F. V., Fiss, G., Baudet, L., & Peske, S. T. (2012). The use of film coating on the performance of treated corn seed. *Revista Brasileira de Sementes*, *34*(2), 186–192. doi:10.1590/S0101-31222012000200001

Baligar, V. C., Fageria, N. K., & He, Z. L. (2001). Nutrient use efficiency in plants. *Communications in Soil Science and Plant Analysis*, *32*(7-8), 921–950. doi:10.1081/CSS-100104098

Barba-Espín, G., Hernández, J.A., Diaz-Vivancos, P. (2012). Role of $H_2O_2$ in pea seed germination. *Plant Signaling and Behavior*, 7, 193–195. doi:10.4161/psb.18881

Bakhtavar, M. A., Afzal, I., Basra, S. M. A., Ahmad, A-u-H, & Noor, M. A. (2015). Physiological strategies to improve the performance of spring maize (*Zea mays* L.) planted under early and optimum sowing conditions. *PLoS ONE*, *10*(4), e0124441. doi:10.1371/journal.pone.0124441

Bewley, J. D. (1997). Seed germination and dormancy. *The Plant Cell*, *9*, 1055–1066. doi:10.1105/tpc.9.7.1055

Binang, W. B., Shiyam, J. O., & Ntia, J. D. (2012). Effect of seed priming method on agronomic performance and cost effectiveness of rainfed, dry-seeded NERICA rice. *Research Journal of Seed Science*, *5*(4), 136–143. doi:10.3923/rjss.2012.136.143

Cakmak, I. (2005). The role of potassium in alleviating detrimental effects of abiotic stresses in plants. *Journal of Plant Nutrition and Soil Science*, *168*, 521–530. doi:10.1002/jpln.200420485

O'Callaghan, M. (2016). Microbial inoculation of seed for improved crop performance: issues and opportunities. *Applied Microbiology and Biotechnology*, *100*, 5729–5746. doi:10.1007/s00253-016-7590-9

Chandler, P. M., & Robertson, M. (1994). Gene expression regulation by abscisic acid and its relation to stress tolerance. *Annual Review of Plant Physiology and Plant Molecular Biology*, *45*, 113–141. doi:10.1146/annurev.pp.45.060194.000553

Chandra Nayaka, S., Niranjana, S. R., Uday Shankar, A. C., Niranjan Raj, S., Reddy, M. S., Prakash, H. S., & Mortensen, C. N. (2010). Seed biopriming with novel strain of *Trichoderma harzianum* for the control of toxigenic *Fusarium verticillioides* and fumonisins in maize. *Archives of Phytopathology and Plant Protection*, *43*(3), 264–282. doi:10.1080/03235400701803879

Copeland, L. O., & McDonald, M. B. (1995). *Principles of seed science and technology*. 3rd Edn. USA, Chapmann and Hall.

Dai, L. Y., Zhu, H. D., Yin, K. D., Du, J. D., & Zhang, Y. X. (2017). Seed priming mitigates the effects of saline-alkali stress in soybean seedlings. *Chilean Journal of Agricultural Research*, *77*(2), 118–125.

Deering, R. H., & Young, T. P. (2006). Germination speeds of exotic annual and native perennial grasses in California and the potential benefits of seed priming for grassland restoration. *Grasslands*, *16*, 14–15.

Dhawal, S., Sarkar, D. R., Yadav, R. S., Parihar, M., & Rakshit, A. (2016). Bio-priming with *Arbuscular mycorrhizae* for addressing soil fertility with special reference to phosphorus. *International Journal of Bioresource Science*, *3*(2), 35–40. doi:10.5958/2454-9541.2016.00013.X

Di Girolamo, G., & Barbanti, L. (2012). Treatment conditions and biochemical processes influencing seed priming effectiveness. *Italian Journal of Agronomy*, *7*, e25. doi:10.4081/ija.2012.e25

Diniz, K. A., Silva, P. A., Oliveira, J. A., & Evangelista, J. R. E. (2009). Sweet pepper seed responses to inoculation with microorganisms and coating with micronutrients, amino acids and plant growth regulators. *Scientia Agricola*, *66*, 293–297. doi:10.1590/S0103-90162009000300002

Draganić, I., & Lekić, S. (2012). Seed priming with antioxidants improves sunflower seed germination and seedling growth under unfavorable germination conditions. *Turkish Journal of Agriculture and Forestry*, *36*, 421–428.

Eivazi, A. (2012). Induction of drought tolerance with seed priming in wheat cultivars (*Triticum aestivum* L.). *Acta agriculturae Slovenica*, *99*(1), 21–29. doi:10.2478/v10014-012-0003-6

Faijunnahar, M., Baque, A., Habib, M. A., & Hossain, H. M. M. T. (2017). Polyethylene glycol (peg) induced changes in germination, seedling growth and water relation behavior of wheat (*Triticum aestivum* L.) Genotypes. *Universal Journal of Plant Science*, *5*(4), 49–57.

Farooq, M., Basra, S. M. A., Afzal, I., & Khaliq, A. (2006). Optimization of hydropriming techniques for rice seed invigoration. *Seed Science and Technology*, *34*, 507–512. doi:10.15258/sst.2006.34.2.25

Farooq, M., Irfan, M., Aziz, T., Ahmad, I., & Cheema, S. A. (2013). Seed priming with ascorbic acid improves drought resistance of wheat. *Journal of Agronomy and Crop Science*, *199*, 12–22. doi:10.1111/j.1439-037X.2012.00521.x

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., & Basra, S. M. A. (2009). Plant drought stress: effects, mechanisms and management. *Agronomy for Sustainable Development*, *29*(1), 185–212. doi:10.1051/agro:2008021

Farooq, M., Hussain, M., Wahid, A., & Siddique, K. H. M. (2012). Drought stress in plants: an overview. In R. Aroca (Ed.), *Plant responses to drought stress*: *from morphological to molecular features* (pp. 1–33). Springer-Verlag, Berlin, Heidelberg. doi:10.1007/978-3-642-32653-0_1

Fujita, M., Fujita, Y., Noutoshi, Y., Takahashi, F., Narusaka, Y., Yamaguchi-Shinozaki, K., & Shinozak, K. (2006) Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. *Current Opinion in Plant Biology*, *9*, 436–442. doi:10.1016/j.pbi.2006.05.014

Ghassemi-Golezani, K., Chadordooz-Jeddi, A., Nasrollahzadeh, S., & Moghaddam, M. (2010). Effects of hydro-priming duration on seedling vigour and grain yield of pinto bean (*Phaseolus vulgaris* L.) Cultivars. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *38*(1), 109–113.

Ghassemi-Golezani, K., & Hosseinzadeh-Mahootchi, A. (2013). Influence of hydro-priming on reserve utilization of differentially aged chickpea seeds. *Seed Technology*, *35*(1), 117–124.

Gimenez, E., Salinas, M., & Manzano-Agugliaro, F. (2018). Worldwide research on plant defense against biotic stresses as improvement for sustainable agriculture. *Sustainability*, *10*, 391. doi:10.3390/su10020391

Goswami, A., Banerjee, R., & Raha, S. (2013). Drought resistance in rice seedlings conferred by seed priming. *Protoplasma*, *250*, 1115–1129. doi:10.1007/s00709-013-0487-x

Harb, E. Z. (1992). Effect of soaking seeds in some growth regulators and micronutrients on growth, some chemical constituents and yield of faba bean and cotton plants. *Bulletin of Faculty of Agriculture University of Cairo*, *43*, 429–452.

Harris, D. (1996). The effects of manure, genotype, seed priming, depth and date of sowing on the emergence and early growth of *Sorghum bicolor* (L.) Moench in semi-arid Botswana. *Soil & Tillage Research*, *40*, 73–88.

Harris, D., Joshi, A., Khan, P. A., Khan, P. A., Gothkar, P., & Sodhi, P. S. (1999). On-farm seed priming in semi-arid agriculture: development and evaluation in maize, rice and chickpea in India using participatory methods. *Experimental Agriculture*, *35*, 15–29. doi:10.1017/S0014479799001027

Harris, D., Pathan, A. K., Gothkar, P., Joshi, A., Chivasa, W., & Nyamudeza, P. (2001). On-farm seed priming: using participatory methods to revive and refine a key technology. *Agricultural Systems*, *69*, 151–164. doi:10.1016/S0308-521X(01)00023-3

Ibrahim, E. A. (2016). Seed priming to alleviate salinity stress in germinating seeds. *Journal of Plant Physiology*, *192*, 38–46. doi:10.1016/j.jplph.2015.12.011

Jisha, K. C., & Puthur, J. T. (2014). Seed halopriming outdo hydropriming in enhancing seedling vigour and osmotic stress tolerance potential of rice varieties. *Journal of Crop Science and Biotechnology*, *17*(4), 209–219. doi:10.1007/s12892-014-0077-2

Jisha, K. C., & Puthur, J. T. (2016). Seed priming with BABA (β-amino butyric acid): a cost-effective method of abiotic stress tolerance in *Vigna radiata* (L.) Wilczek. *Protoplasma*, *253*, 277–289. doi:10.1007/s00709-015-0804-7

Jisha, K. C., Vijayakumari, K., & Puthur, J. T. (2013). Seed priming for abiotic stress tolerance: an overview. *Acta Physiologiae Plantarum*, *35*, 1381–1396. doi:10.1007/s11738-012-1186-5

Johnson, S. E., Lauren, J. G., Welch, R. M., & Duxbury, J. M. (2005). A comparison of the effects of micronutrient seed priming and soil fertilization on the mineral nutrition of chickpea (*Cicer arietinum*), lentil (*Lens culinaris*), rice (*Oryza sativa*) and wheat (*Triticum aestivum*) in Nepal. *Experimental Agriculture*, *41*, 427–448. doi:10.1017/S0014479705002851

Kaur, S., Gupta, A. K., & Kaur, N. (2002). Effect of osmo- and hydropriming of chickpea seeds on seedling growth and carbohydrate metabolism under water deficit stress. *Plant Growth Regulation*, *37*, 17–22. doi:10.1023/A:1020310008830

Kaur, S., Gupta, A. K., & Kaur, N. (2005). Seed priming increases crop yield possibly by modulating enzymes of sucrose metabolism in chickpea. *Journal of Agronomy & Crop Science*, *191*, 81–87. doi:10.1111/j.1439-037X.2004.00140.x

Kaya, M. D., Okcu, G., Atak, M., Cikili, Y., & Kolsaric, O. (2006). Seed treatments to overcome salt and drought stress during germination in sunfower (*Helianthus annuus* L.). *European Journal of Agronomy*, *24*, 291–295. doi:10.1016/j.eja.2005.08.001

Khan, M. B., Hussain, M., Raza, A., Farooq, S., & Jabran, K. (2015). Seed priming with $CaCl_2$ and ridge planting for improved drought resistance in maize. *Turkish Journal of Agriculture and Forestry*, *39*(2), 193–203. doi:10.3906/tar-1405-39

Khomari, S., Golshan-Doust, S., Seyed-Sharifi, R., & Davari, M. (2018). Improvement of soybean seedling growth under salinity stress by biopriming of high-vigour seeds with salt-tolerant isolate of *Trichoderma harzianum*. *New Zealand Journal of Crop and Horticultural Science*, *46*(2), 117–132. doi:10.1080/01140671.2017.1352520

Khoshgoftarmanesh, A. H., Schulin, R., Chaney, R. L., Daneshbakhsh, B., & Afyuni, M. (2010). Micronutrient-efficient genotypes for crop yield and nutritional quality in sustainable agriculture. A review. *Agronomy for Sustainable Development*, *30*(1), 83–107. doi:10.1051/agro/2009017

Krebs, H. A. (1967).The redox state of nicotinamide adenine dinucleotide in the cytoplasm and mitochondria of rat liver. *Advances in Enzyme Regulation*, *5*, 409–434. doi:10.1016/0065-2571(67)90029-5

Kubik, K. K., Eastin, J. A., & Eskridge, K. M. (1988). Solid matrix priming of tomato and pepper. In *Proceedings of the international conference on stand establishment for horticultural crops* (pp. 86–96). Lancaster, Pennsylvania, USA.

Lamaoui, M., Jemo, M., Datla, R., & Bekkaoui, F. (2018). Heat and drought stresses in crops and approaches for their mitigation. *Frontiers in Chemistry*, *6*, 26. doi:10.3389/fchem.2018.00026

Langeroodi, A. R. S., & Noora, R. (2017). Seed priming improves the germination and field performance of soybean under drought stress. *The Journal of Animal and Plant Sciences*, *27*(5), 1611–1621.

Lee, S. S., Kim, J. H., Hong, S. B., Yuu, S. H., & Park, E. H. (1998). Priming effect of rice seeds on seedling establishment under adverse soil conditions. *Korean Journal of Crop Science*, *43*, 194–198.

Lorenzo, M. C. B. (1991). *Seed invigoration of soybean and corn through solid matrix priming.* BS, Philippines.

Lutts, S., Benincasa, P., Wojtyla, L., Kubala, S., Pace, R., Lechowska, K., Quinet, M., & Garnczarska, M. (2016). Seed priming: new comprehensive approaches for an old empirical technique. In S. Araújo & A. Balestrazzi (Eds.), *New challenges in seed biology – basic and translational research driving seed technology* (pp. 1–46). InTechOpen. doi:10.5772/64420

Mahmood, A., Turgay, O. C., Farooq, M., & Hayat, R. (2016). Seed biopriming with plant growth promoting rhizobacteria: a review. *FEMS Microbiology Ecology*, *92*(8). doi:10.1093/femsec/fiw112

Mahmoodi, T. M., Ghassemi-Golezani, K., Habibi, D., Paknezhad, F., & Ardekani, M. R. (2011). Effect of hydro-priming duration on seedling vigour and field establishment of maize (*Zea mays* L.). *Research on Crops*, *12*(2), 341–345.

Mandal, A. B., Mondal, R., & Dutta, P. M. S. (2015). Seed enhancement through priming, coating and pelleting for uniform crop stand and increased productivity. *Journal of the Andaman Science Association*, *20*(1), 26–33.

Manjunatha, G., Raj, S. N., Shetty, N. P., & Shetty, H. S. (2008). Nitric oxide donor seed priming enhances defense responses and induces resistance against pearl millet downy mildew disease. *Pesticide Biochemistry and Physiology*, *91*, 1–11. doi:10.1016/j.pestbp.2007.11.012

Mariani, L., & Ferrante, A. (2017). Agronomic management for enhancing plant tolerance to abiotic stresses—drought, salinity, hypoxia, and lodging. *Horticulturae*, *3*, 52. doi:10.3390/horticulturae3040052

Matsushima, K. -I., & Sakagami, J. -I. (2013). Effects of seed hydropriming on germination and seedling vigor during emergence of rice under different soil moisture conditions. *American Journal of Plant Sciences*, *4*, 1584–1593. doi:10.4236/ajps.2013.48191

McDonald, M. B. (2000). Seed priming. In M. Black & J. D. Bewley (Eds.), *Seed technology and its biological basis* (pp. 287–325). Sheffield Academic Press, Sheffield.

Meena, S. K., Rakshit, A., & Meena, V. S. (2016). Effect of seed bio-priming and N doses under varied soil type on nitrogen use efficiency (NUE) of wheat (*Triticum aestivum* L.) under greenhouse conditions. *Biocatalysis and Agricultural Biotechnology*, *6*, 68–75. doi:10.1016/j.bcab.2016.02.010

Meena, S. K., Rakshit, A., Singh, H. B., & Meena, V. S. (2017). Effect of nitrogen levels and seed bio-priming on root infection, growth and yield attributes of wheat in varied soil type. *Biocatalysis and Agricultural Biotechnology*, *12*, 172–178. doi:10.1016/j.bcab.2017.10.006

Mei, J., Wang, W., Peng, S., & Nie, L. (2017). Seed pelleting with calcium peroxide improves crop establishment of direct-seeded rice under waterlogging conditions. *Scientific Reports*, *7*, 4878. doi:10.1038/s41598-017-04966-1

Mercado, M. F. O., & Fernandez, P. G. (2002). Solid matrix priming of soybean seeds. *Philippines Journal of Crop Science*, *27*, 27–35.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*, *9*, 405–410. doi:10.1016/S1360-1385(02)02312-9

Moghanibashi, M., Karimmojeni, H., & Nikneshan, P. (2013). Seed treatment to overcome drought and salt stress during germination of sunflower (*Helianthus annuus* L.). *Journal of Agrobiology*, *30*, 89–96.

Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology*, *59*, 651–681. doi:10.1146/annurev.arplant.59.032607.092911

Nasri, N., Saïdi, I., Kaddour, R., & Lachaâl, M. (2015). Effect of salinity on germination, seedling growth and acid phosphatase activity in lettuce. *American Journal of Plant Sciences*, *6*, 57–63. doi:10.4236/ajps.2015.61007

Nawaz, J., Hussain, M., Jabbar, A., Nadeem, G. A., Sajid, M., Subtain, M. U., & Shabbir, I. (2013). Seed priming a technique. *International Journal of Agriculture and Crop Sciences*, *6*(20), 1373–1381.

Naz, F., Gul, H., Hamayun, M., Sayyed, A., Khan, H., & Sherwani, S. (2014). Effect of NaCl stress on *Pisum sativum* germination and seedling growth with the influence of seed priming with potassium (KCL and KOH). *American-Eurasian Journal of Agricultural and Environmental Sciences*, *14*(11), 1304–1311.

Negrão, S., Schmöckel, S. M., & Tester, M. (2017). Evaluating physiological responses of plants to salinity stress. *Annals of Botany*, *119*(1), 1–11. doi:10.1093/aob/mcw191

Nejad, H. A., & Farahmand, S. (2012). Evaluating the potential of seed priming techniques in improving germination and early seedling growth of *Aeluropus macrostachys* under salinity stress condition. *Annals of Biological Research*, *3*(11), 5099–5105.

Nikju, M. B., Mobasser, H. R., & Ganjali, H. R. (2015). Influence of variety on biological yield, harvest index, percent of protein in *Zea mays*. *Biological Forum – An International Journal*, *7*(1), 662–667.

Pandey, P., Irulappan, V., Bagavathiannan, M. V., & Senthil-Kumar, M. (2017). Impact of combined abiotic and biotic stresses on plant growth and avenues for crop improvement by exploiting physio-morphological traits. *Frontiers in Plant Science*, *8*, 537. doi:10.3389/fpls.2017.00537

Paparella, S., Araújo, S. S., Rossi, G., Wijayasinghe, M., Carbonera, D., & Balestrazzi, A. (2015). Seed priming: state of the art and new perspectives. *Plant Cell Reports*, *34*, 1281–1293. doi:10.1007/s00299-015-1784-y

Parera, C. A., & Cantliffe, D. J. (1994). Presowing seed priming. *Horticultural Reviews*, *16*, 109–141. doi:10.1002/9780470650561.ch4

Patade, V. Y., Bhargava, S., & Suprasanna, P. (2009). Halopriming imparts tolerance to salt and PEG induced drought stress in sugarcane. *Agriculture, Ecosystems and Environment*, *134*, 24–28. doi:10.1016/j.agee.2009.07.003

Patade, V. Y., Bhargava, S., & Suprasanna, P. (2011). Salt and drought tolerance of sugarcane under iso-osmotic salt and water stress: growth, osmolytes accumulation, and antioxidant defense. *Journal of Plant Interactions*, *6*(4), 275–282. doi:10.1080/17429145.2011.557513

Patra, S. S., Mehera, B., Rout, S., Tomar, S. S., Singh, M., & Kumar, R. (2016). Effect of hydropriming and different sowing dates on growth and yield attributes of Wheat (*Triticum aestivum* L.). *Journal of Applied and Natural Science*, *8*(2), 971–980.

Pedrini, S., Merritt, D. J., Stevens, J., & Dixon, K. (2017). Seed coating: science or marketing spin? *Trends in Plant Science*, *22*(2), 106–116. doi:10.1016/j.tplants.2016.11.002

Radi, A. F., Shaddad, M. A. K., El-Enany, A. E., & Omran, F. M. (2001). Interactive effects of plant hormones (GA3 or ABA) and salinity on growth and some metabolites of wheat seedlings. In W. J. Horst, M. K. Schenk, A. Urkert , N. Claassen, H. Flessa, W. B. Frommer, H. Goldbach, H. W. Olfs, & V. Romheld (Eds.), *Plant nutrition, food security and sustainability of agro-ecosystems through basic and applied research. 14th international plant nutrition colloquium* (pp. 436–437). Hannover, Germany. doi:10.1007/0-306-47624-X_211

Rahman, M. U., Gul, S., & Ahmad, I. (2004). Effects of water stress on growth and photosynthetic pigments of corn (*Zea mays* L.) cultivars. *International Journal of Agriculture & Biology*, *6*(4), 652–655.

Rahman, M., Ali, J., & Masood, M. (2015). Seed priming and *Trichoderma* application: a method for improving seedling establishment and yield of dry direct seeded boro (winter) rice in Bangladesh. *Universal Journal of Agricultural Research*, *3*(2), 59–67.

Ramamurthy, V., Gajbhiye, K. S., Venugopalan, M. V., & Parhad, V. N. (2005). On-farm evaluation of seed priming technology in sorghum (*Sorghum bicolor* L.). *Agricultura Tropica et Subtropica*, *38*(1), 34–41.

Rakshit, A., Pal, S., Rai, S., Rai, A., Bhowmick, M. K., & Singh, H. B. (2013). Micronutrient seed priming: a potential tool in integrated nutrient management. *Satsa Mukkhapatra- Annual Technical Issue*, *17*, 77–89.

Rakshit, A., Sunita, K., Pal, S., Singh, A. & Singh, H. B. (2015). Bio-priming mediated nutrient use efficiency of crop species. In A. Rakshit, H. B. Singh, & A. Sen (Eds.), *Nutrient use efficiency: from basics to advances* (pp. 181–191). Springer, India. doi:10.1007/978-81-322-2169-2_12

Rashid, A., Harris, D., Hollington, P. A., & Khattak, R. A. (2002). On-farm seed priming: a key technology for improving the livelihoods of resource-poor farmers on saline lands. In R. Ahmad & K. A. Malik (Eds.), *Prospects for saline agriculture* (pp. 423–431). Springer, Dordrecht. doi:10.1007/978-94-017-0067-2_44

Rashid, A., Hollington, P. A., Harris, D., & Khan, P. (2006). On-farm seed priming for barley on normal, saline and saline–sodic soils in North West Frontier Province, Pakistan. *European Journal of Agronomy*, *24*, 276–281. doi:10.1016/j.eja.2005.10.006

Rawat, L., Singh, Y., Shukla, N., & Kumar, J. (2012). Seed biopriming with salinity tolerant isolates of *Trichoderma harzianum* alleviates salt stress in rice: growth, physiological and biochemical characteristics. *Journal of Plant Pathology*, *94*(2), 353–365.

Reddy, P. P. (2013). Bio-priming of seeds. In P. P. Reddy (Ed.), *Recent advances in crop protection* (pp. 83-90). Springer, India. doi:10.1007/978-81-322-0723-8

Reddy, C. S., & Smith, J. D. (1978). Effects of delayed post treatment of gamma-irradiated seed with cysteine on the growth of *Sorghum bicolor* seedlings. *Environmental and Experimental Botany*, *18*, 241–243. doi:10.1016/0098-8472(78)90050-3

Rejeb, I. B., Pastor, V., & Mauch-Mani, B. (2014). Plant responses to simultaneous biotic and abiotic stress: molecular mechanisms. *Plants*, *3*, 458–475. doi:10.3390/plants3040458

Rock, C. D. (2000). Pathways to abscisic acid regulated gene expression. *New Phytologist*, *148*, 357–384. doi:10.1046/j.1469-8137.2000.00769.x

Sadeghi, H., Khazaei, F., Yari, L., & Sheidaei, S. (2011). Effect of seed osmopriming on seed germination behavior and vigor of soybean (*Glycine max* L.). *ARPN Journal of Agricultural and Biological Science*, *6*(1), 39–43.

Saha, P., Chatterjee, P., & Biswas, A. K. (2010). NaCl pretreatment alleviates salt stress by enhancement of antioxidant defense system and osmolyte accumulation in mungbean (*Vigna radiata* L. Wilczek). Indian Journal of Experimental Biology *48*, 593–600.

Sakhabutdinova, A. R., Fatkhutdinova, D. R., Beazrukova, M. V., & Shakirova, F. M. (2003). Salicylic acid prevents the damaging action of stress factor of wheat plants. *Bulgarian Journal of Plant Physiology*, Special Issue, 314–319.

Salama, K. H. A., Mansour, M. M. F., & Hassan, N. S. (2011). Choline priming improves salt tolerance in wheat (*Triticum aestivum* L.). *Australian Journal of Basic and Applied Sciences*, *5*(11), 126–132.

Sarkar, D., Pal, S., Singh, H. B., Yadav, R. S., & Rakshit, A. (2017). Harnessing bio-priming for integrated resource management under changing climate. In H.B. Singh, B.K. Sarma, & C. Keswani (Eds.), *Advances in PGPR Research* (pp. 349–363). CAB International, UK.

Schaefer, F. Q., & Buettner, G. R. (2001). Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radical Biology and Medicine*, *30*(11), 1191–1212. doi:10.1016/S0891-5849(01)00480-4

Shakirova, F. M., Sakhabutdinova, A. R., Bezrokuva, M. V., Fatkhutdinova, R. A., & Fatkhutdinova, D. R. (2003). Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. *Plant Science*, *164*, 317–322. doi:10.1016/S0168-9452(02)00415-6

Sharma, K. K., Singh, U. S., Sharma, P., Kumar, A., & Sharma, L. (2015). Seed treatments for sustainable agriculture-a review. *Journal of Applied and Natural Science*, *7*(1), 521–539. doi:10.31018/jans.v7i1.641

Shirinzadeh, A., Soleimanzadeh, H., & Shirinzadeh, Z. (2013). Effect of seed priming with plant growth promoting rhizobacteria (PGPR) on agronomic traits and yield of barley cultivars. *World Applied Sciences Journal*, *21*(5), 727–731.

Shukla, N., Kuntal, H., Shanker, A., & Sharma, S. (2018). Hydro-priming methods for initiation of metabolic process and synchronization of germination in mung bean (*Vigna radiata* L.) Seeds. *Journal of Crop Science and Biotechnology*, *21*(2), 137–146. doi:10.1007/s12892-018-0017-0

Singh, H. B. (2016). Seed biopriming: a comprehensive approach towards agricultural sustainability. *Indian Phytopathology*, *69*(3), 203–209.

Singh, H. P., Sharma, K. D., Reddy, G. S., & Sharma, K. L. (2004). Dryland agriculture in India. In S. C. Rao & J. Ryan (Eds.), *Challenges and strategies of dryland agriculture* (pp. 67–92). Madison, WI (USA), Crop Science Society of America, American Society of Agronomy.

Sneideris, L. C., Gavassi, M. A., Campos, M. L., D'Amico-Damiao, V., & Carvalho, R. F. (2015). Effects of hormonal priming on seed germination of pigeon pea under cadmium stress. *Anais da Academia Brasileira Ciências*, *87*(3), 1847–1852. doi:10.1590/0001-3765201520140332

Soleimanzadeh, H. (2013). Effect of seed priming on germination and yield of corn. *International Journal of Agriculture and Crop Sciences*, *5*(4), 366–369.

Srivastava, A. K., Lokhande, V. H., Patade, V. Y., Suprasanna, P., Sjahril, R., & D'Souza, S. F. (2010a). Comparative evaluation of hydro-, chemo-, and hormonal priming methods for imparting salt and PEG stress tolerance in Indian mustard (*Brassica juncea* L.). *Acta Physiologiae Plantarum*, *32*, 1135–1144. doi:10.1007/s11738-010-0505-y

Srivastava, A.K., Ramaswamy, N. K., Mukopadhyaya, R., Chiramal Jincy, M. G., & D'Souza, S. F. (2009). Thiourea modulates the expression and activity profile of mtATPase under salinity stress in seeds of *Brassica juncea* L. *Annals of Botany*, *103*, 403–410. doi:10.1093/aob/mcn229

Srivastava, A.K., Suprasanna, P., Srivastava, S., & D'Souza, S.F. (2010b). Thiourea mediated regulation in the expression profile of aquaporins and its impact on water homeostasis under salinity stress in *Brassica juncea* roots. *Plant Science*, *178*, 517–522. doi:10.1016/j.plantsci.2010.02.015

Sun, C. -X., Cao, H. -X., Shao, H. -B., Lei, X. –T., & Xiao, Y. (2011). Growth and physiological responses to water and nutrient stress in oil palm. *African Journal of Biotechnology*, *10*(51), 10465–10471. doi:10.5897/AJB11.463

Suzuki, N., Rivero, R. M., Shulaev, V., Blumwald, E., & Mittler, R. (2014). Abiotic and biotic stress combinations. *New Phytologist*, *203*, 32–43. doi:10.1111/nph.12797

Taylor, A. G., Allen, P. S., Bennett, M. A., Bradford, K. J., Burris, J. S., & Misra, M. K. (1998). Seed enhancements. *Seed Science Research*, *8*, 245–256. doi:10.1017/S0960258500004141

Varier, A., Vari, A.K., & Dadlani, M. (2010). The subcellular basis of seed priming. *Current Science*, *99*(4), 450–456.

Vaz Mondo, V. H., Nascente, A. S., Neves, P. D. C. F., Taillebois, J. E., & Oliveira, F. H. S. (2016). Seed hydropriming in upland rice improves germination and seed vigor and has no effects on crop cycle and grain yield. *Australian Journal of Crop Science*, *10*(11), 1534–1542. doi:10.21475/ajcs.2016.10.11.PNE70

Verma, V., Ravindran, P., & Kumar, P. P. (2016). Plant hormone-mediated regulation of stress responses. *BMC Plant Biology*, *16*(1), 86. doi:10.1186/s12870-016-0771-y

Wahid, A., Perveen, M., Gelani, S., & Basra, S. M. A. (2007). Pretreatment of seed with $H_2O_2$ improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins. *Journal of Plant Physiology*, *164*, 283–294. doi:10.1016/j.jplph.2006.01.005

Wang, W., Chen, Q., Hussain, S., Mei, J., Dong, H., Peng, S, Huang, J., Cui, K., & Nie, L. (2016a). Pre-sowing seed treatments in direct-seeded early rice: consequences for emergence, seedling growth and associated metabolic events under chilling stress. *Scientific Reports*, *6*, 19637. doi:10.1038/srep19637

Wang, W., Peng, S., Chen, Q., Mei, J., Dong, H., & Nie, L. (2016b). Effects of pre-sowing seed treatments on establishment of dry direct-seeded early rice under chilling stress. *AoB Plants*, *8*, plw074. doi:10.1093/aobpla/plw074

Wojtyla, Ł., Lechowska, K., Kubala, S., & Garnczarska, M. (2016). Molecular processes induced in primed seeds—increasing the potential to stabilize crop yields under drought conditions. *Journal of Plant Physiology*, *203*, 116–126. doi:10.1016/j.jplph.2016.04.008

Waqar, K., Surriya, O., Afzal, F., Kubra, G., Iram, S., Ashraf, M., & Kazi, A. G. (2014). Modern tools for enhancing crop adaptation to climatic changes. In P. Ahmad (Ed.), *Emerging technologies and management of crop stress tolerance, volume 1* (pp. 143–157). Elsevier Inc. doi:10.1016/B978-0-12-800876-8.00007-2

Welch, R. M. (1986). Effects of nutrient deficiencies on seed production and quality. *Advances in Plant Nutrition*, *2*, 205–247.

Xiong, L., & Zhu, J.K. (2002). Molecular and genetic aspects of plant responses to osmotic stress. *Plant, Cell & Environment*, 25, 131–139. doi:10.1046/j.1365-3040.2002.00782.x

Yadav, P. V., Kumari, M., & Ahmed, Z. (2011). Seed priming mediated germination improvement and tolerance to subsequent exposure to cold and salt stress in capsicum. *Research Journal of Seed Science*, *4*(3), 125–136. doi:10.3923/rjss.2011.125.136

Yağmur, M., & Kaydan, D. (2008). Alleviation of osmotic stress of water and salt in germination and seedling growth of triticale with seed priming treatments. *African Journal of Biotechnology*, *7*(13), 2156–2162.

Yilmaz, A., Ekiz, H., Gultekin, I., Torun, B., Barut, H., Karanlik, S., & Cakmak, I. (1998). Effect of seed zinc content on grain yield and zinc concentration of wheat grown in zinc-deficient calcareous soils. *Journal of Plant Nutrition*, *21*, 2257–2264. doi:10.1080/01904169809365559

Yuan-Yuan, S., Yong-Jian, S., Ming-Tian, W., Xu-Yi, L., Xiang, G., Rong, H., & Jun, M. (2010). Effects of seed priming on germination and seedling growth under water stress in rice. *Acta Agronomica Sinica*, *36*(11), 1931–1940. doi:10.1016/S1875-2780(09)60085-7

Zheng, M., Tao, Y., Hussain, S., Jiang, Q., Peng, S., Huang, J., Cui, K., & Nie, L. (2016). Seed priming in dry direct-seeded rice: consequences for emergence, seedling growth and associated metabolic events under drought stress. *Plant Growth Regulation*, *78*, 167–178. doi:10.1007/s10725-015-0083-5

# In Memoriam - Prof. dr. Julija Smole
(1930–2018)

V jeseni, ko je sadjar poplačan za ves trud z obilico plodov raznolikih oblik in barv, nas je 27. septembra 2018, v 89. letu starosti zapustila naša cenjena pedagoginja, spoštovana sodelavka in kolegica profesorica dr. Julije Smole.

Prof. dr. Julija Smole se je rodila 25. aprila 1930 v Ljubljani. Osnovno šolo je obiskovala v Šmarju pri Ljubljani (1937-1942), gimnazijo pa v Ljubljani. Leta 1949 se je vpisala na tedanjo Agronomsko in gozdarsko fakulteto, kjer je junija 1955 diplomirala. 1. avgusta 1955 se je zaposlila v Sadni drevesnici in vrtnariji v Kamniku. Tu je ob nastajajočih nasadih uspešno opravila prakso in se v štirih letih usposobila za vodenje pridelave ter leta 1959 postala direktor obrata. Leta 1961 je bila izvoljena na razpisano mesto asistenta na Katedri za sadjarstvo Biotehniške fakultete. S tem imenovanjem se je začela njena uspešna, hitra akademska kariera ter pot med vodilne univerzitetne učitelje na fakulteti in med vrhunske strokovnjake v pomoloških vedah, v teoriji in praksi.

Prevzela je naloge iz teme proučevanja in selekcije češenj in marelic v goriškem in istrskem sadnem okolišu. Njeni dosežki so zbrani v letnih poročilih in zbirkah Pomološke raziskave slovenskega ozemlja ter v veliko objavljenih razpravah v domačih in tujih publikacijah. Leta 1971 je pridobila akademski naziv magistra, 1973 pa je bila promovirana za doktorico agronomskih znanosti. Njeno magistrsko delo, disertacija in druge razprave predstavljajo znanstvene temelje sodobnega izbora češenj in višenj za goriški sadni okoliš ter priporočilo za vseslovenski perspektivni sadni izbor. Na osnovi raziskovalnih dosežkov je bila prof. dr. Julija Smole leta 1974 habilitirana, leta 1976 izvoljena v naziv docentke, leta 1978 v naziv izredne profesorice in leta 1983 v naziv redne profesorice za področje sadjarstva.

Delovno področje prof. dr. Julije Smole je bila tudi hibridizacija, selekcija in introdukcija ter vzgoja novih sort breskev in češenj ter proučevanje in uvajanje marelic v posavskem in goriškem sadnem okolišu. Uspešno je bilo križanje in vzgoja novih sort breskev. V sedemdesetih letih je opravila s sodelavci križanje sorte 'Slovenija' z ameriškimi sortami ter s testiranjem in odbiro dobila osem pozitivnih križancev. Posebej uspešno je bilo tudi križanje češenj in kot rezultat je nova slovenska sorta 'Vigred'. Rezultati njenega dela so objavljeni v knjigah Naš sadni izbor, Razmnoževanje sadnih rastlin, Češnje in višnje. Sodelovala je na mnogih domačih in tujih konferencah in znanstvenih srečanjih, bila je mentor številnim diplomantom, pod njenim mentorstvom pa je magistriralo in doktoriralo 6 kandidatov.

Prof. dr. Julija Smole je prejela številna priznanja in odlikovanja. Za uspehe pri pedagoškem in znanstvenem delu, za prizadevanje pri razvoju stroke in fakultete je leta 1975 prejela Priznanje Biotehniške fakultete. Za ekološke in fiziološke raziskave pri češnjah je prejela leta 1978 Nagrado sklada Borisa Kidriča. Za uresničevanje delavskih interesov z dolgoletnim in požrtvovalnim delom v sindikalni organizaciji in za pomembne uspehe v njenem uveljavljanju in razvoju je prejela leta 1978 Srebrni znak sindikatov Slovenije, leta 1979 pa od takratnega predsednika SFRJ Red dela s srebrnim vencem.

Bila je namestnica predstojnika za agronomijo in dolgoletna predstojnica Katedre za sadjarstvo in kasneje Inštituta za sadjarstvo, vinogradništvo in vrtnarstvo, kjer je vzgojila precej diplomantov in mladih raziskovalcev. Z veseljem je priskočila na pomoč z idejami, komentarji, popravki, sadjarskimi nasveti in nasveti za vsakdanje življenje. 15. 10. 1994 se je upokojila.

Prof. dr. Julija Smole je bila dejavna v strokovnih organizacijah, združenjih in društvih, v odborih Poslovne skupnosti za sadjarstvo Republike Slovenije in delovnih skupinah Alpe – Jadran. Bila je član oddelčnih in fakultetnih teles, Raziskovalne skupnosti Slovenije, Strokovnega sadjarskega društva Slovenije in Hortikulturnega društva, uredniškega odbora glasila Naš vrt in revije SAD in drugih upravnih, poslovnih in družbenih teles. Bila je ustanovna članica Strokovnega sadjarskega društva Slovenije in v njem delovala že od vsega začetka – zavidljivih 48 let.

Predavanja prof. Smoletove niso bila obvezna, pa je bila predavalnica vedno polna. Njena predavanja so bila izredno zanimiva in so vedno pritegnila študente. Snov je podajala na zelo razumljiv in zanimiv način, vzbujala je vedoželjnost. Mnogo študentov ima shranjene zapiske njenih predavanj in velikokrat pridejo prav še danes.

Bila je odličen pedagog. V odnosu z nami študenti je bila vedno prijateljska, izkazovala je pomoč pri delu in si zavzeto prizadevala za boljši učni uspeh. Svoje znanje je nesebično razdajala številnim generacijam sadjarjev, povedala kakšno modrost in življenjski nasvet.

Prof. dr. Julija Smole je pustila pomemben pečat v slovenskem sadjarstvu s svojim raziskovalnim, strokovnim in pedagoškim delom ter s svojim znanjem zaznamovala slovenske sadjarje.

# In memoriam Prof. Dr. Julija Smole
(1930–2018)

Fall is the time, when fruit grower's work is rewarded with bountiful and vibrant fruit. But as the earth gives it also takes away and we are sad to say goodbye to our esteemed colleague, teacher and friend, prof. dr. Julija Smole, who passed away on 27th of September 2018.

Prof. dr. Julija Smole was born on 25th of April 1930 in Ljubljana. She attended primary school in Šmarje near Ljubljana (1937-1942) and high school in Ljubljana. In 1949 she enrolled to the former Faculty for Agronomy and Forestry in Ljubljana and graduated in June 1955. She started work at a fruit nursery in Kamnik on August 1st 1955, where she acquired practical experience by setting up and managing new orchards. After four years, in 1959, she became the director of the company. In 1961 she started work as an assistant at the Chair for Fruit Growing at Biotechnical Faculty. Her fast and fruitful academic carrier launched her among the leading university professors at the Faculty. Moreover, she became a skilled professional in pomological sciences, both in theoretical and practical aspects.

She was involved in cherry and apricot selection set up in Gorica and Istria fruit-growing regions. Her achievements were described in Pomological yearbooks and other Slovenian pomological publications. She was also very active in disseminating her work in national and international periodicals. In 1971 she became Master of Science and defended her PhD in agronomy in 1973. Her MSc and doctoral thesis represented the scientific basis for modern sweet cherry and sour cherry selection suitable for the Gorica fruit-growing region. Moreover, these works paved the path for Slovenian Fruit selection today. Research achievements enabled prof. dr. Julija Smole to acquire university habilitation in 1974, promotion to assistant professor for Fruit Growing in 1976, associate professor in 1978 and full professor in 1983.

The work field of prof. dr. Julija Smole extended to hybridization, selection and introduction of new peach and sweet cherry cultivars as well as to the study and introduction of apricot cultivars in the Posavje and Gorica fruit-growing regions. She was successful in crossing and cultivation of new peach cultivars. In 1970's she and her colleagues succeeded in crossing the cultivar 'Slovenia' with several American cultivars with an outcome of 8 new promising crosses. She was also successful in sweet cherry breeding and is most known for the 'Vigred' cultivar. She published her breeding activities in the following publications: Naš sadni izbor (Our fruit selection), Razmnoževanje sadnih rastlin (Propagation of fruit plants) and Češnje in višnje (Sweet and sour cherries). She actively participated in many national and international conferences and research meetings, and mentored numerous graduate and MSc theses. She was a mentor to no less than 6 doctoral students.

Prof. dr. Julija Smole was the recipient of several awards and honours. Among others, she received the award of Biotechnical Faculty for her teaching and research work in 1975 as well as the Boris Kidič fund award for her environmental and physiological research on cherries in 1978. She received the Silver token of the labour union in 1978 as she devoted many years and energy for recognition and progress of this organization and particularly, worker's rights. In 1979 the former president of SFRY awarded prof. dr. Julija Smole the Silver wreath order.

She was deputy head of the Agronomy Department and long-time head of the Chair for Fruit Growing, Viticulture and Vegetable Growing. She was always eager to help, advise, comment, direct and lead young undergraduate students, PhD students, researchers and colleagues. Her professional knowledge was also complemented with day-to-day advice on life itself. Prof. dr. Julija Smole retired on 15th of October 1994 but she remained active in professional society as well as warmly greeted by her co-workers on different social occasions.

Prof. dr. Julija Smole actively participated in professional organizations, associations and societies, took part in the Slovenian Business Community for Fruit Growing and in Alpe – Jadran working groups. She was a member of different committees at the Department and Faculty levels, Slovenian Research society, Professional Fruit Growing Society, Horticultural Society, she was on editorial board of Naš vrt and SAD newsletters and attended several other administrative, legal and social groups. She was one of the founding members of Professional Fruit Growing Society in Slovenia and has been active in it since the very beginnings – for 48 years!

Her classes were not obligatory but nevertheless, the classroom was always full. She was a compelling lecturer and always knew how to appeal to students. She presented the topics coherently and intrigued curiosity. Many students still have notes from her lectures and these remain relevant today. Prof. dr. Julija Smole was simply an amazing professor. She was always friendly with the students, helpful and eagerly trying to promote their learning success. She unselfishly distributed her knowledge to many generations of fruit growers, shared her wisdom and advice.

Prof. dr. Julija Smole assigned a significant mark on Slovenian fruit growing both in research and professional fields but mostly left a lasting impression of her knowledge on many generations of Slovenian fruit growers.

prof. dr. Metka Hudina
Biotehniška fakulteta / Biotehnical Faculty
Oddelek za agronomijo / Department of Agronomy

# NAVODILA AVTORJEM

## UVOD

Acta agriculturae Slovenica je četrtletna odprtodostopna znanstvena revija z recenzentskim sistemom, ki jo izdaja Biotehniška fakulteta Univerze v Ljubljani. Revija sprejema izvirne in še neobjavljene znanstvene članke v slovenskem ali angleškem jeziku, ki se vsebinsko nanašajo na širše področje rastlinske pridelave in živalske prireje in predelave. Pokritost zajema širok razpon tem, kot so agronomija, hortikultura, biotehnologija, fiziologija rastlin in živali, pedologija, ekologija in okoljske študije, agrarna ekonomika in politika, razvoj podeželja, sociologija podeželja, genetika, mikrobiologija, imunologija, etologija, mlekarstvo, živilska tehnologija, prehrana, bioinformatika, informacijske znanosti in ostala področja, povezana s kmetijstvom. Pregledne znanstvene članke sprejemamo v objavo samo po poprejšnjem dogovoru z uredniškim odborom. 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. Uredništvo revije zagotovi prevode izbranih bibliografskih elementov (naslova, izvlečka, opomb in ključnih besed) v primeru tujih avtorjev. Prispevke sprejemamo skozi celo leto.

## PROCES ODDAJE PRISPEVKA

Avtorji lektorirane prispevke oddajo v elektronski obliki na spletni strani OJS Acta agriculturae Slovenica. Pred oddajo prispevka se mora avtor na spletni strani najprej prijaviti oziroma registrirati, če prvič vstopa v sistem (potrebno je klikniti na Registracija in izpolniti obrazec za registracijo). Bodite pozorni, da na dnu regis-

# AUTHOR GUIDELINES

## INTRODUCTION

Acta agriculturae Slovenica is an open access peer-reviewed scientific journal published quarterly by the Biotechnical Faculty of the University of Ljubljana, Slovenia. The Journal accepts original scientific articles from the fields of plant production (agronomy, horticulture, plant biotechnology, plant-related food-and-nutrition research, agricultural economics, information-science, ecology, environmental studies, plant physiology & ecology, rural development & sociology, soil sciences, genetics, microbiology, food processing) and animal production (genetics, microbiology, immunology, nutrition, physiology, ecology, ethology, dairy science, economics, bioinformatics, animal production and food processing, technology and information science) in Slovenian or English language. Review articles are published upon agreement with the editor. Reports presented on conferences that were not published entirely in the conference reports can be published. 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 paper is part of BSc, MSc or PhD thesis, this should be indicated together with the name of the mentor at the bottom of the front page and will appear as foot note. All notes should be written in Slovenian and English language. Slovenian-language translation of selected bibliographic elements, for example the title, abstract, notes and keywords, will be provided by the editorial board. Manuscripts are accepted throughout the year.

## SUBMISSION PROCESS

Manuscripts should be submitted to the Acta agriculturae Slovenica OJS site. The submitting author should be registered to the site. Click Register and fill in the registration form. Be sure to check in the Author tracijskega obrazca ne pozabite odkljukati potrditvenega polja »Avtor«, sicer oddaja prispevka ne bo mogoča.

Proces oddaje prispevka poteka v petih korakih. Priporočljivo je, da se avtor pred oddajo najprej seznani s postopkom in se na oddajo prispevka pripravi:

**Korak 1: Začetek oddaje prispevka**
- izbrati je potrebno eno od sekcij,
- pri rubriki »Pogoji za oddajo prispevka« morate potrditi vsa potrditvena polja,
- dodatna pojasnila uredniku je mogoče vpisati v ustrezno polje.

**Korak 2: Oddaja prispevka**
- Naložite prispevek v formatu Microsoft Word (.doc ali .docx).

**Korak 3: Vpis metapodatkov**
- Podatki o avtorjih: ime, priimek, elektronski naslovi in ustanove vseh avtorjev v ustreznem vrstnem redu. Korespondenčni avtor mora biti posebej označen.
- Vpišite naslov in izvleček prispevka,
- Vpišite ključne besede (največ 8, ločeno s podpičjem) in označite jezik besedila,
- Vnesete lahko tudi podatke o financerjih,
- V ustrezno besedilno polje vnesite reference (med posameznimi referencami naj bo prazna vrstica).

**Korak 4: Dodajanje morebitnih dodatnih datotek**
- Grafično gradivo naj bo naloženo v eni ZIP datoteki. Grafične slike imenujte Slika1.jpg, Slika2.eps, in podobno,
- Za vsako dodatno naloženo datoteko je potrebno zagotoviti predvidene metapodatke.

**Korak 5: Potrditev**
- Potrebna je končna potrditev.

check box on the form. We advise you to check in also the Reader check box.

Submission process consists of 5 steps. Before submission, authors should go through the checklist and prepare for submission:

**Step 1: Starting the submission**
- Choose one of the journal sections.
- Confirm all the requirements of the Submission Preparation Checklist.
- Additional plain text comments for the editor can be provided in the relevant text field.

**Step 2: Upload submission**
- Upload full manuscript in the form of the Microsoft Word document file format (.doc or .docx).

**Step 3: Enter metadata**
- First name, last name, contact e-mail and affiliation for all authors, in relevant order, must be provided. Corresponding author has to be selected.
- Title and abstract must be provided in plain text.
- Key words must be provided (max. 8, separated by semicolons) and enter the language of the text.
- Data about contributors and supporting agencies may be entered.
- References in plain text must be provided in the relevant text filed (between each reference should be a blank line).

**Step 4: Upload supplementary files**
- All graphic have to be uploaded in a single ZIP file. Graphics should be named Figure1.jpg, Figure2.eps, etc.
- For each uploaded file the author is asked for additional metadata which may be provided.

**Step 5: Confirmation**
- Final confirmation is required.

## PODROBNEJŠA NAVODILA / DETAILED INSTRUCTIONS

http://ojs.aas.bf.uni-lj.si/index.php/AAS/about/submissions#authorGuidelines