DE GRUYTER OPEN

Agriculture (Poľnohospodárstvo), 63, 2017 (4): 128−141

Original paper DOI: 10.1515/agri-2017-0013

THE EFFECTS OF FOLIAR FEEDING OF COMPATIBLE ORGANIC SOLUTES ON AGRONOMIC TRAITS OF SAFFLOWER

MOHSEN JANMOHAMMADI*, FARZANEH ASADI, NASER SABAGHNIA, AMIN ABBASI, MOJTABA NOURAEIN, FARIBORZ SHEKARI

University of Maragheh, Iran

JANMOHAMMADI, M. – ASADI, F. – SABAGHNIA, N. –ABBASI, A. – NOURAEIN, M. – SHEKARI, F.: The effect of foliar feeding of compatible organic solutes on agronomic traits of safflower. Agriculture (Poľnohospodárstvo), vol. *63*, 2017, no. 4, pp. 128–141.

Safflower is originated from Iran and is tolerant against water deficit stress. However, in semi-arid Mediterranean climate terminal drought and heat stress adversely affect the safflower production. In order to investigate the influence of foliar application of proline (Pr) (10 and 20 mM) and glycinebetaine (GB) (2 and 4 mM) under well and deficit irrigation (37.23° N, 46.16° E). Foliar spray of compatible organic solutes started from middle vegetative growth and continued till seed filling stage. Comparison of well irrigated and stress conditions revealed that severity of water deficit stress (SI) was 0.25. Evaluation of growth-related morphological characteristics such as plant height, leaf area, canopy spread and percent ground cover showed that they considerably reduced by water deficit stress. However, foliar application of compatible solutes could somewhat increase growth related parameters. Results showed that water deficit stress noticeably reduced the chlorophyll content, while foliar spray could alleviate the water deficit stress effects when compared with intact plant (non-sprayed plants). The beneficial effect of GB was more prominent than Pr, especially under deficit irrigation condition. Principal component analysis (PCA) indicated that the best performance under well irrigated condition was obtained by application of 4 mM GB while under deficit irrigation condition the best performance was recorded for plants treated with 2 and 4 mM GB and 20 mM Pr. Overall, results of current experiments showed that foliar spray with high concentration of GB may can significantly alleviate the adverse effects of water deficit stress.

Key words: compatible solutes, foliar spray, osmoregulators, osmotic adjustments, drought tolerance, yield components

Safflower (*Carthamus tinctorius* L.) is one of the oldest cultivated crops, usually grown at a small scale. Safflower naturally is an oil seed crop but also is grown for flowers used for coloring, flavoring foods, dyes, medicinal properties, and livestock feed (Hussain *et al.* 2016). Safflower has an acceptable oil profile for straight vegetable oil. Safflower, a strongly tap-rooted annual plant from the family Asteraceae, is native to the Middle East that and it has moderate tolerance to drought (Eslam *et al.* 2010). Safflower is also a potential biofuel crop but, like sunflower it competes directly with human consumption (Hamanci *et al.* 2011). Being a relatively short-season crop, it fits well into crop rotations. Also, it is a valuable forage for Mediterranean areas since it remains green and has a higher feed value under dry conditions. Safflower is a valuable forage provided it is harvested from mid-budding to early blooming stage (Landau *et al.* 2004).

Furthermore, safflower is a potential oilseed crop for semi-arid region that is well suited to dryland production. In this regards it is necessary to note that between the different environmental stresses, water deficit is one the main constraint that significantly affect the crop productivity and food security. According to an estimate, one third of the world's

Dr. Mohsen Janmohammadi (*Corresponding author), Eng. Farzaneh Asadi, Dr. Naser Sabaghnia, Dr. Amin Abbasi, Dr. Mojtaba Nouraein, Dr. Fariborz Shekari, Department of Plant Production and Genetic, Agriculture College, University of Maragheh, P.O. Box 55181-83111, Iran. E-mail: jmohamad@alumni.ut.ac.ir

population live in areas where water is scarce (FAO 2003). Due to population growth and development of economic sectors, the competition for water resources will also grow (Laraus 2004). However, safflower is a neglected oil seed crop in Asian semi-arid regions. It has been used as source of dye, folk medicine and food but it has not got any significant consideration as oil seed crop. The major problems associated with cultivation of this crop are its mechanical harvesting which is quite laborious accompanied by low yields. In the context of increasing demand on crops for food and biofuel production, breeding for drought tolerance holds promises for enhanced yield and extended cultivation seasons.

Compatible solutes are small solutes that are used by cells to maintain cell volume under water stress. They also may involve in scavenging of reactive oxygen species, osmoregulation and stabilization of cell membrane proteins and lipids (Kanwal *et al.* 2013; Hoang *et al.* 2016). Some important osmoprotectants are glycinebetaine (GB), trehalose, soluble sugars, proline, etc. (Raza *et al.* 2017). It is known that plants increase their proline levels under environmental stress. Drought-induced proline accumulation recorded in many plant species has led to the hypothesis that further increases in proline accumulation would improve drought tolerance (Bhaskara *et al.* 2015). When plants are relieved from the stress, the gathered proline is metabolized to produce reducing agents which maintenance mitochondrial oxidative phosphorylation and generation of ATP, for retrieval and repair of stress-induced modifications (Hare *et al.* 1999; Wani *et al.* 2016).

Exogenous proline application as a foliar spray has been demonstrated to decrease the detrimental effects of various abiotic stresses in plants (Ali *et al.* 2007). Foliar application of proline on two Indian mustard cultivars under mild salinity stress could mitigate the negative effect of salt stress by increasing the antioxidative capacity of the plants (Wani *et al.* 2016). Exogenous application of proline under stress condition can improve gas exchange parameters, up-regulate stress protective protein expression, reduce lipid peroxidation and increase activities of antioxidant enzymes (Hayat *et al.* 2012).

Beside, GB is the most well-known quaternary ammonium compounds in higher plants that have critical roles in protection of photosynthesis machinery and ROS detoxification during abiotic stress (Giri 2011). However, at lower concentrations, GB effectively contributes in stabilizing the quaternary structures of enzymes and complex proteins (Chen & Murata 2008). It has been revealed that exogenous application of GB under drought stress conditions (35% soil field capacity), on maize plants improved the leaf area, 100 kernels weight biological yield/plant, grain yield/plant and harvest index (Anjum *et al.* 2012). However the response to osmoregulators significantly was different between the cultivars. Also the review of the literature indicate that the effect of the osmoregulatores considerably depended on plant species, applied concentration, plant developmental stage, severity of environmental stress and other factors (Dawood 2016).

Water efficiency is a key concept to solve water-shortage problems in semiarid areas. Deficit irrigation in many crops has frequently proved to be an efficient tool to optimize water-use efficiency (Tejero *et al.* 2011). Under deficit irrigation can save water and also induce plant physiological regulations such as stomatal opening and reproductive and vegetative growth (Du *et al.* 2015). However, the number of capitula per plant and the number of filled seeds per plant in safflower were shown to be linearly correlated with each other (Eslam *et al.* 2010). However, the time of drought stress or application of deficit irrigation is very important. To date, little is known about the capacities of compatible organic solutes to cope with water deficit condition and develop drought resistance.

With the above facts in mind, the present work was designed to determine whether foliar application of proline and GB ameliorate the water deficit stress in safflower plants.

MATERIAL AND METHODS

This study was conducted in the Research Farm of the Faculty of Agriculture, University of Maragheh, Iran (Latitude 37.23° N, Longitude 46.16° E, Altitude 1,485 m above sea level) during the 2014–2015 growing season. The experiment was designed as a split-split plot (using randomized complete block design) with 3 replications. Maragheh is a representative of highland semi-arid zone and, according to the updated classification of Köppen and Geiger, its climate is classified as BSk; cold semi-arid climate with cold winter, temperate spring and hot summer (Peel *et al.* 2007), with an average annual precipitation of 353 mm, consisting of 73% of rain and 27% of snow.

Irrigation regimes (W: well irrigated and S: deficit water stress; treatments was irrigated after 60, 110 mm evaporation from class A pan, respectively) were located in main plots and foliar spray compatible solutes (control, 10 and 20 mM of proline and 2 and 4 mM of glycinebetaine) were allocated to sub plots. Glycine betaine (GB; CHEBI: 17750) and L-proline ($C_5H_9NO_2$; CHEBI: 26271) were obtained from Merck (Darmstadt, Germany). The concentrations were selected based on the results of preliminary tests and the results of other studies in the literatures (Ali *et al.* 2013; Dawood 2016; Janmohammadi *et al.* 2017). Foliar spray treatments were initiated 40 days after planting and repeated once each 10 days until grain filling stage. Compatible solutes sprayed over the foliage to point of run-off (until every leaf is wetted, but not dripping). Spraying was done by Calibrated backpack sprayer. Considering the similar role of the compounds, the comparison between them and the control was carried out through Fisher's *LSD* (Least Significant Difference). During the irrigations, the plots were irrigated to up to 70% of field capacity. Foliar treatment carried out at stem elongation stages and repeated at intervals of fifteen days. Check plants sprayed with distilled water. Experimental fields were ploughed once in early fall and harrowed twice to bring the soil to fine tilth one week before planting. The recommended dose of fertiliser (100 kg N and 70 kg P_2O_5/ha) was applied in the form of urea and triple superphosphate at the time of seed bed preparation.

A thorn-less variety of safflower, locally referred to as 'Golestan', was used in the present experiment. Before the start of the experiment seed were propagated in isolated fields under full irrigated condition, according to Sabaghnia *et al.* (2015), in northwest of Iran. Seeds of safflower were treated with 2 g/kg Mancozeb and then were sown by hand in 5 cm depth of soil. The soil type was a clay loam, low in organic carbon (0.43%) with a pH value of 6.85 and total nitrogen and $CaCO_3$ contents of 0.17% and 19%, respectively. Electrical conductivity (EC) and iron, manganese, copper, zinc, and potassium contents of the soil were measured at 0.84 ds/m, 1.62 ppm, 6.37 ppm, 0.49 ppm, 0.73 ppm, and 627 ppm, respectively. Safflower seeds planted on 9 April 2015 and harvested at full maturity stage. Each plot included sixteen rows, 4 m long and 25 cm apart. Seeds were sown 5 cm apart at 5 cm depth. The small terraces of 1 m in the interspaces was considered to prevent contamination by surface runoff. Plants were thinned to a spacing of 10 cm within rows 4 weeks after sowing. All plots were irrigated immediately after sowing, and also two irrigations were applied (by 60 mm evaporation from class A pan) till stem elongation stem then deficit irrigation was carried out for half of plats. During the growing season all plots were irrigated 6 or 7 times and weeded manually. No fungicides were applied.

Chlorophyll index was measured on 10 fully expanded leafs of a plant at each plot using a portable chlorophyll meter (SPAD) at capitulum development stage (BBCH scale: 71; according to Flemmer *et al.* 2015).

At maturity in July, plants were cut at ground level from two middle rows of plots and then oven dried at 80°C until a constant weight. Seeds were separated from straw by crushing. Seed and straw (stem plus leaf) were weighted by a balance and yields were determined per unit of area for different treatments. The total biomass was also determined by summation of safflower seed and straw. Agronomic components of the specified plants were determined after harvest. Yield components were number of capitulum per plant, number of seeds per capitulum, number of branches per plant and 1,000-seeds weight. Harvest index (HI) was calculated according to the following formula: Harvest index [%] = (Grain yield / Biological yield) × 100.

Contents of palmitic acid, arachidic acid and myristic acid were evaluated by gas chromatography according to Rudolphi *et al.* (2012).

Principal component analysis (PCA) was used to evaluate the behaviors of agronomic traits against to foliar application of compatible organic solutes and drought stress. Data were subjected to One-way ANOVA (analysis of variance) with SAS software (SAS Institute, Cary, NC, USA). Where F tests were significant ($P < 0.05$), means were separated by least significant difference test (*LSD*).

RESULTS

One-way analysis of variance (ANOVA) showed that phenological development affected by irrigation regimes. Plant grown under well irrigated condition had longer vegetative growth and the emergence of capitulum was later than plant under deficit irrigation (Table 1). Foliar spray of compatible solutes, regardless of concentration, postponed the initiation of the reproductive growth. The mean comparison under deficit irrigation showed that the plant treated with GB had a longer vegetative period (Figure 1).

Evaluation of the chlorophyll indicated that both main effects of foliar spray and irrigation levels and also the interaction effect of foliar spray × irrigation level on chlorophyll concentration was statistically significant at 99% confidence interval ($P < 0.01$). Deficit irrigation decreased the chlorophyll concentration by 25% when compared with plants grown under well irrigated condition. The highest chlorophyll concentration was recorded in plants grown under well irrigated condition and treated with 10 mM proline or 2 mM GB (Figure 2). Foliar spray with low concentration of compatible solutes considerably improved the chlorophyll concentration under deficit irrigation. Leaf fresh weight noticeably decreased by water shortage by 32% in comparison with well irrigated plants. Beside, foliar spray of GB could increase the leaf fresh weight and this was more pronounced at high concentrations (Table 1). A similar trend observed for RWC. Assessment of ground cover showed that application deficit irrigation caused a 14% decrease in ground cover. Foliar spray with proline and GB improved the ground cover, however, the effect of high concentrations of GB was more prominent (28%).

Evaluation of canopy width showed that both main effects of foliar spray and irrigation levels and also the interaction effect of foliar spray × irrigation level were significant. The largest canopy width under well irrigated condition was recorded for plants treated with 2 mM GB while under deficit irrigation condition the best performance obtained by high concentration of GB (4 mM). Assessment of leaf area revealed that deficit irrigation decreased this traits by 37% in comparison with well irrigated condition. On the other hand, foliar spray of GB at both concentration significantly induced the leaf growth and increased the leaf area. The height of plants grown under favorable moisture conditions considerably was higher than plants under deficit irrigation (by 22%). Moreover, foliar spray of com-

T a b l e 1

Effect of foliar spray of compatible organic solutes on some morphological traits of safflower (*Carthamus tinctorius* L.) under well-watered and deficit irrigation condition

Irrigation (I)	DC	CHL	FLW	RWC	GC	CW	LA	PH
	++	++	++	++	++	++	++	++
W	71.13^{a}	55.24^{a}	20.24^{a}	71.20^{a}	80.13^{a}	30.13^{a}	639.50^{a}	64.61^{a}
S	54.93^{b}	41.36^{b}	13.65^{b}	60.32^{b}	69.93^{b}	18.30^{b}	423.10^{b}	50.91^{b}
Foliar spray of compatible organic solutes (F)								
	+	++	++	+	+	+	++	+
Control	54.16^{c}	38.49^{c}	10.57^{d}	59.35^{d}	66.50^{c}	20.21^{b}	483.03^{b}	49.19^{b}
Pr-10 mM	64.33^{ab}	53.37^{a}	15.35^{c}	62.55^{c}	73.00^{b}	23.31^{ab}	518.56^{b}	59.32^{a}
Pr-20 mM	61.33^{b}	44.92^{b}	14.68^{c}	65.36^{bc}	74.33^{b}	24.06^{ab}	516.60^{b}	56.81^{ab}
GB-2 mM	68.00^{a}	51.64^{b}	19.74^{b}	68.35^{b}	76.50^{b}	26.96^{a}	569.58^{a}	58.09^{ab}
GB-4 mM	67.33^{a}	53.08^{a}	24.29^{a}	73.18^{a}	84.83^{a}	26.53^{a}	591.99^{a}	65.04^{a}
I×S	+	++	NS	NS	+	+	NS	NS

CHL – chlorophyll content [SPAD unit]; DC – number of days to capitulum emergence; FLW – leaves fresh weight [g]; RWC – relative water content; GC – ground cover [%]; CW – canopy width [cm]; LA – leaf area [cm^2]; PH – plant height at maturity [cm]; W – well-watered; S – deficit irrigation; Pr – proline; GB – glycinebetaine; NS – Not significant; $^{+}$ – Significant at 5% level of probability, $^{++}$ – Significant at 1% level of probability. Mean values of the same category followed by different letters are significant at $P \leq 0.05$ level.

patible solutes induced the plant elongation and the tallest plants obtained by foliar application of 4 mM GB (Table 1).

Assessment of biological yield showed that the highest value was obtained for plant grown under well irrigated condition along with foliar application GB while the lowest amount was recorded for plants grown under deficit irrigation without foliar treatment or treated with 20 mM proline (Table 2). Application of GB in both moisture regimes could improve the biological yield.

Both main effects of foliar spray and irrigation levels and also the interaction effect of foliar spray × irrigation level were significant on capitulum diameter ($P < 0.05$). The largest capitulum was recorded in plant grown under well irrigated condition with foliar application of 10 mM proline or 2 mM GB (Figure 3). Interestingly, the low concentration of both compatible solutes could significantly increase capitulum diameter under deficit irrigation. Number of the capitulum per plant was not affected by foliar treatments. However, water shortage reduced the number of the capitulum by 24% in comparison with well irrigated plants (Table 2).

Evaluation of seed weight showed that the interaction effect of foliar spray × irrigation level were significant on this trait ($P < 0.05$). The effect of compatible solutes on seed weight under well irrigated condition was more prominent than deficit irrigation condition. The highest seed weight was recorded for plants grown under well irrigated condition and treated with GB. Seed number per capitulum as one of the most important yield component considerably affected ($P < 0.01$) by irrigation level so that water shortage decreased this trait by 25% when compared with well irrigated condition.

Furthermore, foliar treatment significantly affected the seed number per capitulum and the highest number was recorded for plant treated with GB or high concentration of proline (Table 2). Assessment of seed yield revealed that interaction effect of foliar spray × irrigation level were significant on this trait ($P < 0.05$). Mean comparison of seed yield between combined treatments showed that the highest value

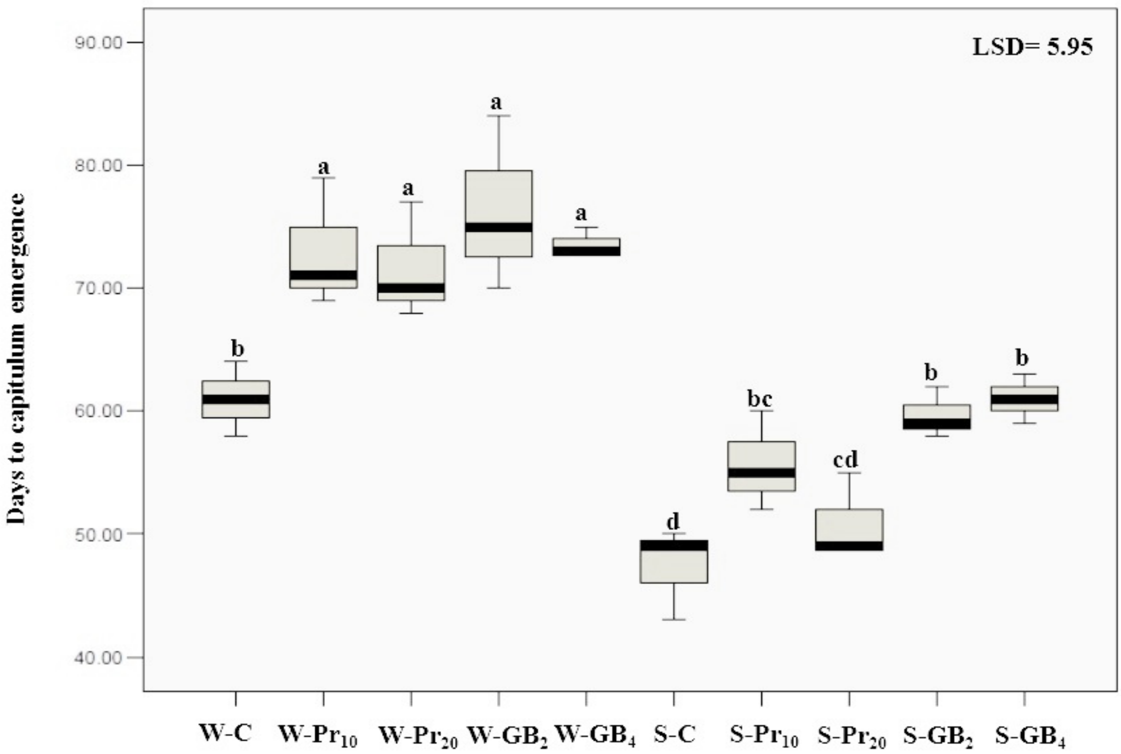


Figure 1. The effects of foliar spray of proline and glycinebetaine on phenological development of safflower under well irrigated (W) and deficit irrigation (S) condition. C – control plants that treated with distilled water; Pr – proline; GB – glycinebetaine. The numbers along with the compatible compounds refer to the applied concentrations (mM). Different letters within columns indicate statistically significant differences ($P < 0.05$).

T a b l e 2

Impact of foliar spray of proline and glycinebetaine on some yield components of safflower (*Carthamus tinctorius* L.) under well-watered and deficit irrigation condition

Irrigation (I)	BY	CD	CNP	TSW	SNC	SY	HI
	++	+	+	++	++	++	++
W	5,159[a]	28.33[a]	9.36[a]	35.87[a]	28.79[a]	1,136.41[a]	24.37[a]
S	3,648[b]	24.05[b]	7.12[b]	30.73[b]	21.57[b]	883.95[b]	22.10[b]
Foliar spray of compatible organic solutes (F)							
	++	+	NS	+	+	+	NS
Control	4,154[c]	23.66[c]	7.33[b]	31.55[c]	22.99[c]	946.50[c]	23.17[a]
Pr-10mM	4,363[bc]	31.68[a]	7.90[ab]	33.03[bc]	23.80[bc]	996.34[bc]	23.04[a]
Pr-20 mM	4,185[c]	25.77[bc]	7.50[b]	32.84[b]	25.42[abc]	976.78[bc]	23.68[a]
GB-2 mM	4,556[ab]	30.22[ab]	9.16[a]	34.05[ab]	25.92[ab]	1,011.73[b]	22.45[a]
GB-4 mM	4,758[a]	25.63[bc]	9.33[a]	35.01[a]	27.76[a]	1,119.55[a]	23.81[a]
I×S	+	+	NS	+	NS	+	NS

BY – biological yield [kg/ha]; CD – capitulum diameters [mm]; CNP – capitulum numbers per plants; TSW – 1,000-seeds weight [g]; SNC – seed number per capitulum; SY – seed yield [kg/ha]; HI: harvest index [%]. W – well-watered; S – deficit irrigation; Pr – proline; GB – glycinebetaine; NS – Not significant; + – Significant at 5% level of probability; ++ – Significant at 1% level of probability. Mean values of the same category followed by different letters are significant at $P \leq 0.05$ level.

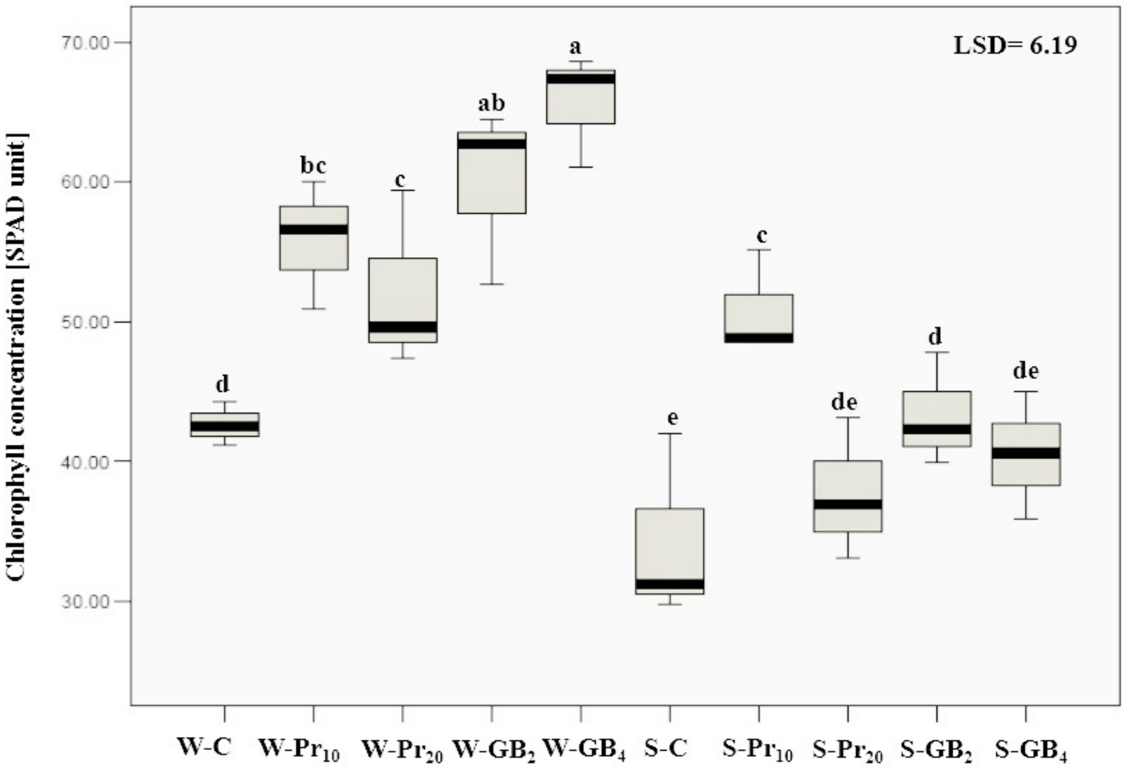


Figure 2. Impacts of foliar spray of compatible organic solutes (proline and glycinebetaine) on chlorophyll concentration of safflower under well irrigated (W) and deficit irrigation (S) condition. C – control plants that treated with distilled water; Pr – proline; GB – glycinebetaine.

patible solutes induced the plant elongation and the tallest plants obtained by foliar application of 4 mM GB (Table 1).

Assessment of biological yield showed that the highest value was obtained for plant grown under well irrigated condition along with foliar application GB while the lowest amount was recorded for plants grown under deficit irrigation without foliar treatment or treated with 20 mM proline (Table 2). Application of GB in both moisture regimes could improve the biological yield.

Both main effects of foliar spray and irrigation levels and also the interaction effect of foliar spray × irrigation level were significant on capitulum diameter ($P < 0.05$). The largest capitulum was recorded in plant grown under well irrigated condition with foliar application of 10 mM proline or 2 mM GB (Figure 3). Interestingly, the low concentration of both compatible solutes could significantly increase capitulum diameter under deficit irrigation. Number of the capitulum per plant was not affected by foliar treatments. However, water shortage reduced the number of the capitulum by 24% in comparison with well irrigated plants (Table 2).

Evaluation of seed weight showed that the interaction effect of foliar spray × irrigation level were significant on this trait ($P < 0.05$). The effect of compatible solutes on seed weight under well irrigated condition was more prominent than deficit irrigation condition. The highest seed weight was recorded for plants grown under well irrigated condition and treated with GB. Seed number per capitulum as one of the most important yield component considerably affected ($P < 0.01$) by irrigation level so that water shortage decreased this trait by 25% when compared with well irrigated condition.

Furthermore, foliar treatment significantly affected the seed number per capitulum and the highest number was recorded for plant treated with GB or high concentration of proline (Table 2). Assessment of seed yield revealed that interaction effect of foliar spray × irrigation level were significant on this trait ($P < 0.05$). Mean comparison of seed yield between combined treatments showed that the highest value was recorded for plant grown under well irrigated condition and treated with 4 mM GB. Remarkably, differences between other compatible solutes in well irrigated conditions were not significant. Utilization of high concentrations of GB under deficit irrigation conditions had more promising effects (Figure 4).

Evaluation of fatty acid profile in safflower oil showed that the interaction effect of foliar spray × irrigation level were significant on content of palmitic acid, arachidic acid and myristic acid ($P < 0.05$). The content of palmitic acid and arachidic acid significantly reduced by water shortage (Table 3). Mean comparision for palmitic acid showed that although foliar application of compatible solutes increased the palmitic acid content, the highest amount was recorded in plants grown under irrigated condition and treated with 4 mM GB (Table 3). The effects of foliar spray under well irrigated condition was more prominent than deficit irrigation condition. The highest content of arachidic acid recorded for plants grown under well irrigated condition and sprayed with proline while the highest content under deficit irrigation was obtained by foliar spray of 4 mM GB. The content of myristic acid less affected by water shortage compared to other fatty acids. Under both irrigation level the highest content of myristic acid was recorded for plant treated with 4 mM GB (Table 3).

Principal component analysis (PCA) was employed to provide an overview of the capacity to distinguish combined treatments. First principal component clearly separated the irrigation levels (Figure 5). PCA could separate the foliar treatments by second component. PCA evidently separated the high concentration of proline and both concentrations of GB from other treatments under deficit irrigation. Also second principal component segregated the 4 mM GB from other treatment under well irrigated condition (Figure 5). Correlation between all possible characters are presented in Table 4. Correlations between seed yield and chlorophyll concentration, relative water content (RWC), ground cover, canopy width, leaf area, capitulum diameter, seed number per capitulum and 1,000-seed weight were positive and significant. High correlation between seed yield and RWC refers to the importance of water status in semi-arid conditions. A positive significant correlation observed between seed number per capitulum (SNC) and canopy growth parameter (canopy width, ground cover, leaf area). Also there was a positive correlation between SNC and number of the days to capitulum emergence (Table 4).

T a b l e 3

Effect of foliar spray of proline and glycinebetaine on fatty acids content of safflower (*Carthamus tinctorius* L.) under well-watered and deficit irrigation condition

Combined treatments	Palmitic acid	Arachidic acid	Myristic acid
W-Control	8.17^{c}	0.35^{c}	0.24^{d}
W-Pr-10 mM	9.04^{bc}	0.43^{ab}	0.293^{c}
W-Pr-20 mM	9.17^{b}	0.576^{a}	0.316^{bc}
W-GB-2 mM	9.75^{b}	0.286^{d}	0.263^{d}
W-GB-4 mM	10.86^{a}	0.413^{b}	0.459^{a}
S-Control	6.46	0.176^{ef}	0.156^{ef}
S-Pr-10 mM	6.97^{de}	0.196^{e}	0.273^{cd}
S-Pr-20 mM	7.44^{d}	0.29^{d}	0.196^{e}
S-GB-2 mM	7.6^{cd}	0.23^{d}	0.30^{c}
S-GB-4 mM	8.24^{c}	0.303^{c}	0.396^{b}

W – well irrigated and deficit irrigation (S) condition; C – control plants that treated with distilled water; Pr – proline; B – glycinebetaine. The numbers along with the compatible compounds refer to the applied concentrations (mM). Different letters within columns indicate statistically significant differences ($P < 0.05$).

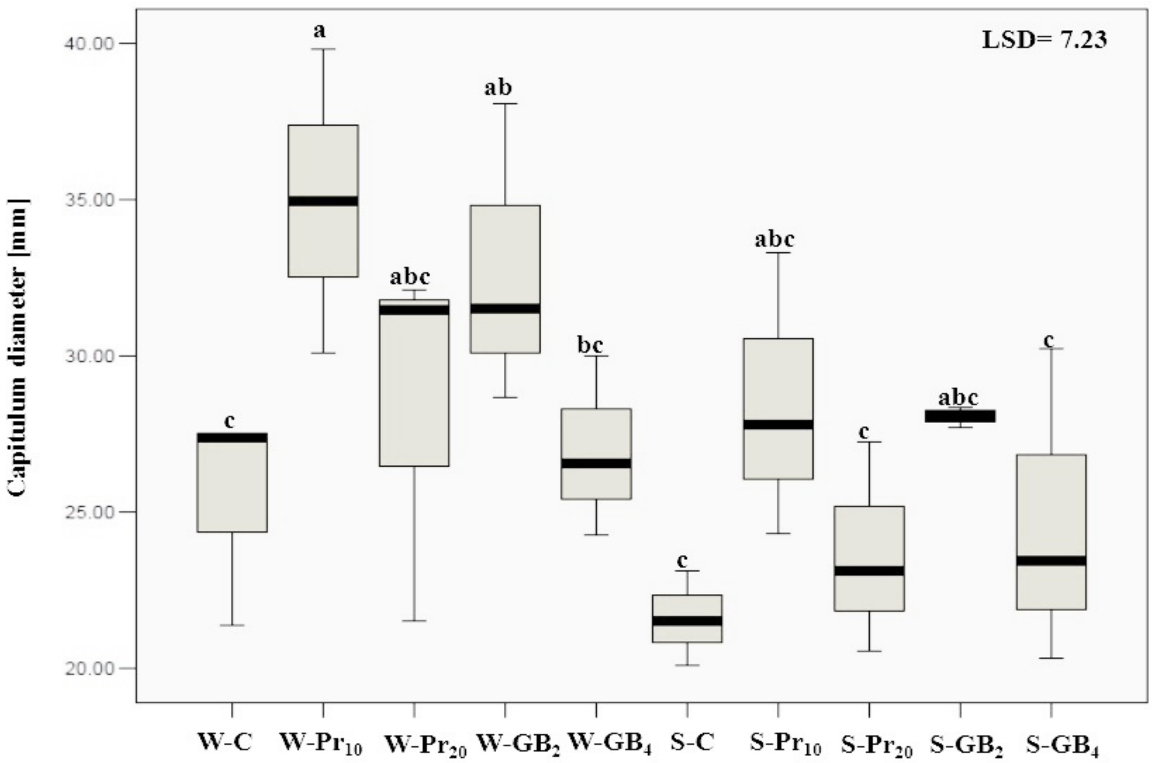


Figure 3. The effects of foliar spry of proline and glycinebetaine on capitulum diameter safflower under well irrigated (W) and deficit irrigation (S) condition. C – control plants that treated with distilled water; Pro – proline, GB – glycinebetaine.

T a b l e 4

Correlation coefficient for morphological and agronomical traits of Safflower (*Carthamus tinctorius* L.) under different irrigation level and foliar treatments

	CHL	DC	FLW	RWC	GC	CW	LA	PH	BY	CD	CNP	HSW	SNC	SY
DC	0.88^{++}													
FLW	0.82^{+}	0.83^{+}												
RWC	0.71^{+}	0.92^{++}	0.93^{++}											
GC	0.77^{+}	0.88^{++}	0.74^{+}	0.97^{++}										
CW	0.87^{+}	0.96^{++}	0.60	0.91^{++}	0.92^{++}									
LA	0.83^{+}	0.94^{++}	0.71^{+}	0.92^{++}	0.85^{+}	0.97^{++}								
PH	0.59	0.91^{++}	0.46	0.60	0.73^{++}	0.56	0.59							
BY	0.85^{+}	0.72^{+}	0.77^{+}	0.88^{++}	0.81^{+}	0.96^{++}	0.99^{++}	0.84^{+}						
CD	0.72^{+}	0.77^{+}	0.47	0.49	0.47	0.45	0.61	0.65	0.59					
CNP	0.55	0.53	0.56	0.86^{++}	0.86^{++}	0.71^{+}	0.77^{+}	0.81^{+}	0.72^{+}	0.79^{+}				
HSW	0.89^{++}	0.95^{++}	0.76^{+}	0.92^{++}	0.86^{++}	0.71^{+}	0.98^{++}	0.90^{++}	0.97^{++}	0.61	0.78^{+}			
SNC	0.76^{+}	0.90^{++}	0.63	0.93^{++}	0.87^{++}	0.93^{+}	0.97^{++}	0.62	0.94^{++}	0.52	0.68	0.96^{++}		
SY	0.82^{+}	0.60	0.63	0.92^{++}	0.89^{++}	0.82^{+}	0.78^{+}	0.59	0.97^{++}	0.73^{+}	0.79^{+}	0.96^{++}	0.96^{++}	
HI	–0.43	$–0.76^{+}$	–0.44	–0.58	–0.45	$–0.83^{+}$	–0.58	–0.50	$–0.84^{+}$	–0.63	–0.38	$–0.76^{+}$	–0.65	$–0.70^{+}$

CHL – chlorophyll content; DC – number of days to capitulum emergence; FLW – leaves fresh weight; RWC – relative water content; GC – ground cover; CW – canopy width; LA – leaf area; PH – plant height at maturity; BY – biological yield; CD – capitulum diameters; CNP – capitulum numbers per plants; TSW – 1,000-seeds weight; SNC – seed number per capitulum; SY – seed yield; HI – harvest index. $^{+}$, $^{++}$Significant at the 0.05 and 0.01 probability levels, respectively.

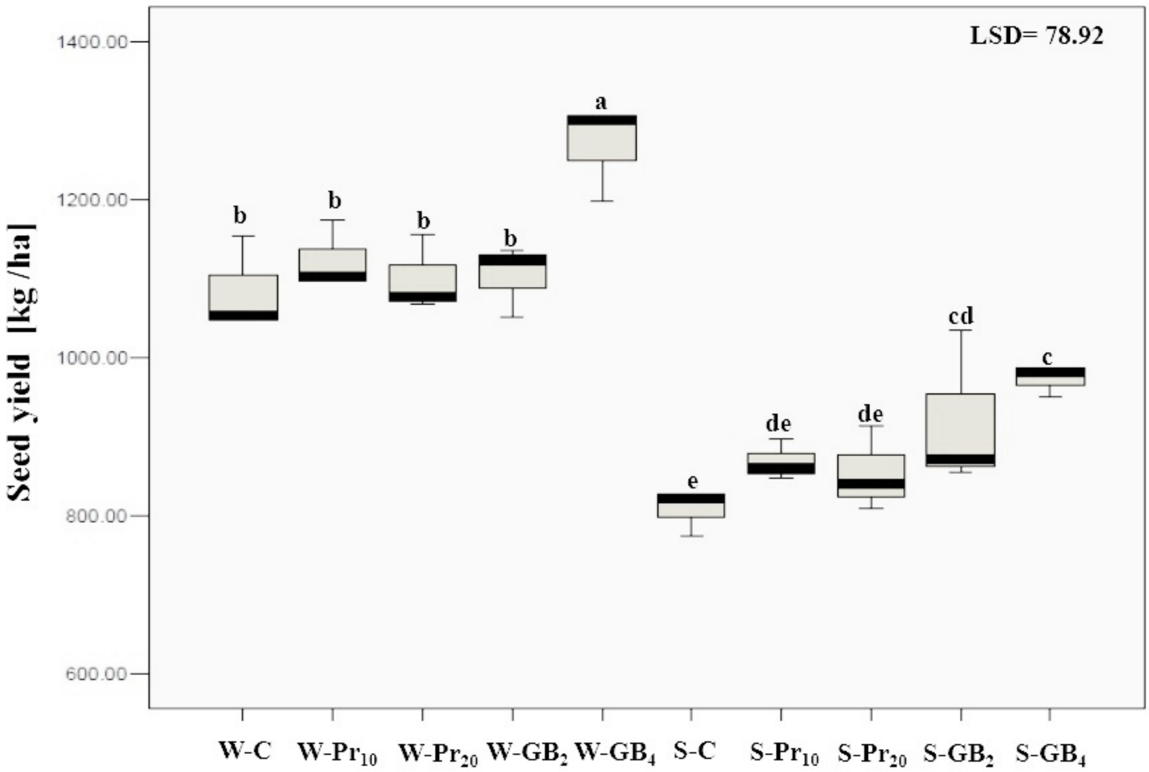


Figure 4. Seed yield of safflower affected by foliar spray of compatible organic solutes (proline and glycinebetaine). C – control plants that treated with distilled water; Pr – proline; GB – glycinebetaine. The numbers along with the compatible compounds refer to the applied concentrations (mM). Different letters within columns indicate statistically significant differences (*P < 0.05*).

In addition cluster analysis was used for categorizing the evaluated traits according to the similarities in change trends (Figure 6). Cluster analysis could divided the all evaluated traits in four group. Group I included trait such as chlorophyll concentration, days to capitulum emergence, canopy width, 1,000-seed weight, leaf area, biological yield, seed yield and number of the capitulum per plant. In this cluster the best performance was obtained by application of GB. However application of low concentration of proline under deficit irrigation could improve the mentioned traits. Group II included harvest index, seed number per plants, RWC, ground cover and plant height that showed the best performance by application of 4 mM GB. Group III included traits such as capitulum diameter that the best performance was recorded by application of low concentration proline or GB. Group IV included leaf fresh weight that induced by both compatible solutes. However the effects of GB application was more prominent.

DISCUSSION

In the changing climate, plants are constantly exposed to abiotic stress, such as drought, which is one of the most serious problems associated with plant growth and development affecting agricultural demands (Overpeck & Cole 2006). When plants are subjected to environmental stress may show a wide range of behaviors or strategies of coping, and accordingly them can be divided from very sustainable to high tolerant. Although drought resistance is largely controlled by plant genetics, it appears that some agronomic strategies can also improve plant resistance to environmental stress (Raza *et al.* 2017). The results of present study revealed that in general, deficit irrigation (irrigation after 60 mm evaporation) applied at vegetative and reproductive growth had strong negative effects on the growth safflower plants. The main objective of deficit irrigation is to increase the water use efficiency (WUE) of a crop by eliminating irrigations that have little

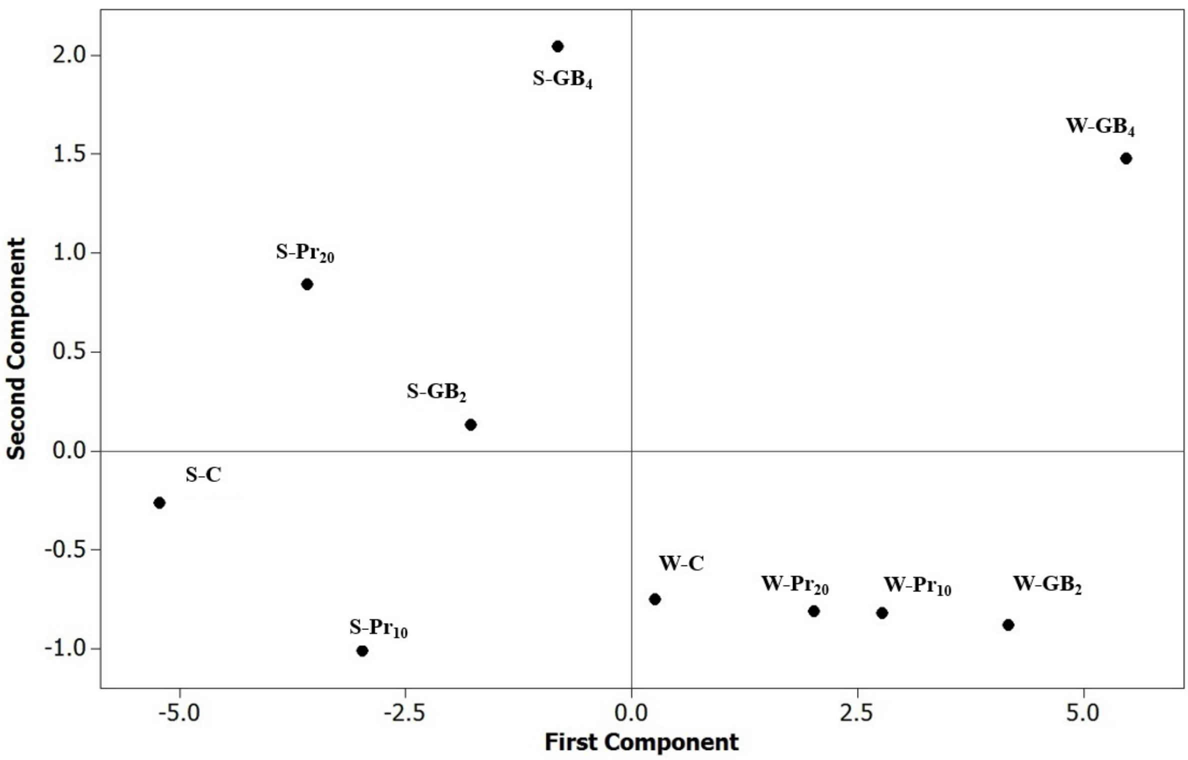


Figure 5. Bi-plot of the first two principal components (PC1 and PC2) for combined treatments of foliar spray of compatible solutes and irrigation levels. W – well irrigated and S – deficit irrigation; C – control plants that treated with distilled water, Pr – proline; GB – glycinebetaine. The numbers along with the compatible compounds refer to the applied concentrations (mM).

impact on yield. The resulting yield reduction may be small compared with the benefits gained through diverting the saved water to irrigate other crops for which water would normally be insufficient under traditional irrigation practices. However, due to the severe shortage of water in the northwest of Iran, high water prices, the lack of adequate water distribution structures, the lack of proper and the lack of proper irrigation planning to distribute available water between crops during the spring deficit irrigation should continue to be considered as a solution. It can also be associated with other agronomic measures such as varying tillage practices, mulching and anti-transpirants that reduce the demand for irrigation water. It seems with eliminating some irrigation during the vegetative and increasing the amount of water supply at reproductive stage partially can improve the seed yield.

Altogether, application of long-term deficit irrigation can change the sink-source relationship and allocation of nutrients. Under long-term exposure to water deficit conditions both sink and source size reduced and this is interpretable by reduced the canopy size and decreased the yield components. On the other hand, a significant decrease in chlorophyll content of leaves can have a negative effect on the activity of the source. However, our result showed that foliar application of compatible organic solute improved plant sink-source relationship and water statue and RWC. Reduction in relative water content (RWC) and leaf water potential caused decrease in photosynthesis of higher plants (Lawlor & Cornic 2002). In accordance to these results, Blum (1996) and Shahbaz *et al.* (2012) concluded that osmolytes accumulation in plant cells results in a decrease of the cell osmotic potential and thus in maintenance of water absorption and cell turgor pressure, which might contribute to sustain physiological processes, such as stomatal opening, photosynthesis, and growth expansion. These results were in a good conformity with those obtained by Aldesuquy *et al.* (2013) who reported that foliar application of

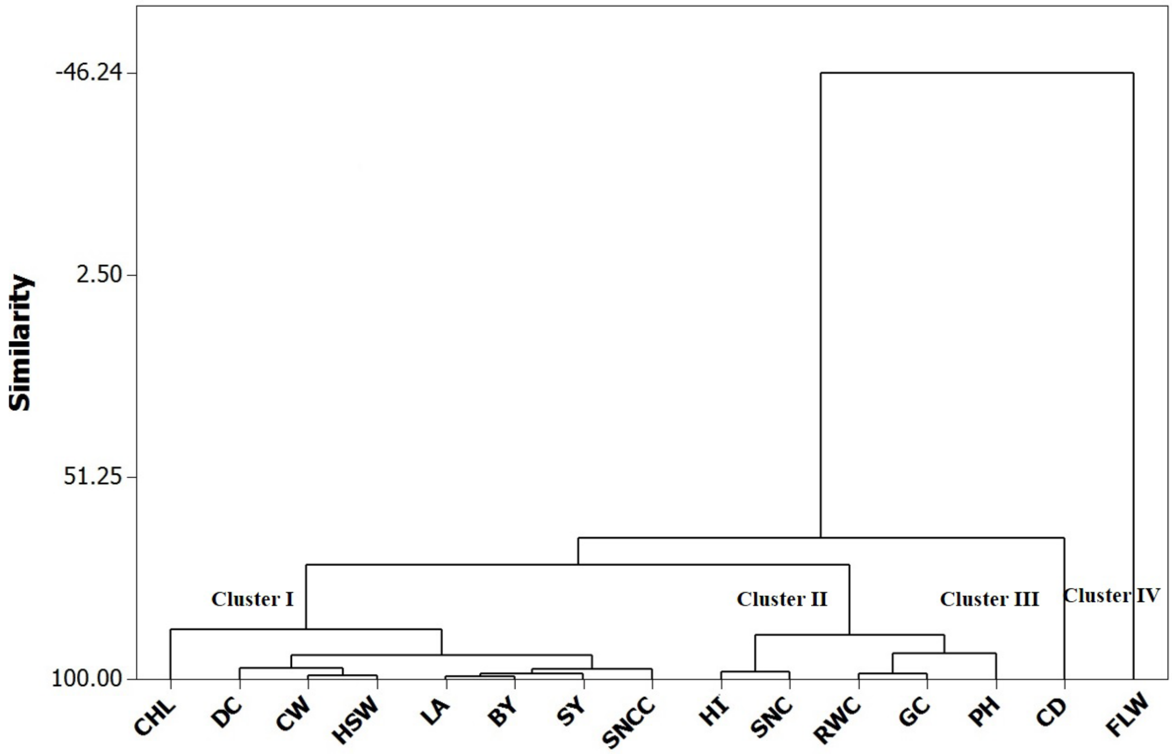


Figure 6. Cluster analysis of agronomic traits of Safflower (*Carthamus tinctorius* L.) grown in highland semi-arid region of Maragheh. CHL – chlorophyll concentration; DC – days to capitulum emergence; HSW – 1,000-seeds weight; LA – leaf area; BY – biological yield; SY – seed yield; SNCC – seed number per capitulum; HI – harvest index; SNC – percentage of unfilled seeds; RWC – relative water content; GC – ground cover; PH – plant height; CD – capitulum diameter; FLW – leaves fresh weigh.

compatible solute appeared to mitigate the effect of water stress on wheat yield and the effect was more pronounced with (GB + salicylic acid) treatment. This improvement would result from the beneficial effect of the provided chemicals on growth and metabolism of wheat plants under water deficit conditions. This finding also is in agreement with Iqbal *et al.* (2008) findings which showed that foliar application of GB at the vegetative or reproductive growth stage, however, increased leaf water and turgor potentials to some extent in both sunflower lines when grown under water stress.

Beside, another important finding was a strong correlation between seed number per capitulum and canopy width. This finding is in agreement with our previous finding (Janmohammadi *et al.* 2014) which showed some foliar treatment can accelerate the canopy growth and ground covering and then significantly reduce the evaporation for soil surface. The maintenance of soil moisture can support seed yield production in the end stages.

Amino acid profiling revealed that deficit irrigation negatively affect the amount of palmitic acid and arachidic acid, however, the foliar application of compatible organic solutes had significant ameliorative effect. However, the findings of the current study do not support the previous research where the foliar application of GB and proline could not increase the oil content in sunflower seeds (Janmohammadi *et al.* 2017). This may be due to the concentration used or the poor response of sunflower as a plant species. The results of the present study are consistent with those of Hussain *et al.* (2009), who found that exogenous application of GB and salicylic acid improved oil content of sunflower (*Helianthus annuus* L.) under different irrigation regimes.

Although the best performance was obtained by GB application, proline was also useful in some cases especially under deficit irrigation condition. In this regards, Ali *et al.* (2013) reported that exogenous application of proline has ameliorating effects of on seed composition, seed oil quality and oil antioxidant activity of maize (*Zea mays* L.) under drought stress. The concentrations of antioxidant compounds namely phenolics, carotenoids, flavonoids and tocopherols estimated in the seed oil increased due to foliar-applied proline under water deficit conditions that was positively correlated with the enhanced free radical scavenging activity. It has been revealed that exogenous application of proline can induce stress tolerance by maintaining cell turgor or osmotic balance; stabilizing membranes thereby preventing electrolyte leakage; and bringing concentrations of reactive oxygen species within normal ranges, thus preventing oxidative burst in plants (Hayat *et al.* 2012). However, the low efficacy of proline in comparison to GB can be attributed to applied concentrations.

Although drought-stressed plants accumulate various compatible molecules such as proline, GB etc., our result showed that foliar application of GB significantly mitigate the negative effects of drought stress. This may be due to this reason that the accumulation of a compatible solute is an energy-consuming process in addition to the already existing metabolic costs. These findings further support the previous researches that revealed the positive effects of exogenous application of GB on plant growth and final crop yield under drought stress in different plants such as tobacco, wheat, barley, sorghum, soya bean and common beans (reviewed by Ashraf & Follad 2007).

CONCLUSIONS

Results revealed that applied deficit irrigation, cannot be a suitable method to save water and improving the water use efficiency. Water deficit significantly decreased the canopy growth, leaf area and yield components. However foliar application of glycinebetaine and proline especially in high concentration could mitigate the negative effects of water deficit stress. Foliar application of glycinebetaine much more effective than proline. Foliar application high concentration proline showed some ameliorative effect under water deficit stress. Among the evaluated traits a positive significant correlation was recorded between seed yield and RWC. Hence the study of plant water statue can be introduced as valuable marker for evaluating the efficiency of spraying treatments.

Acknowledgments. This work was funded by University of Maragheh and the Ministry of Science, Research and Technology of Iran. The authors express their deepest gratitude to S. Soltani and A. Ezati for their contribution to field experiment. They also thank A. Ebadi for his advice on English corrections to paper.

REFERENCES

ALDESUQUY, H.S. – ABBAS, M.A. – ABO-HAMED, S.A. – ELHAKEM, A.H. 2013. Does glycine betaine and salicylic acid ameliorate the negative effect of drought on wheat by regulating osmotic adjustment through solutes accumulation? In *Journal of Stress Physiology & Biochemistry*, vol. *9,* no. 3, pp. 5–22. DOI: 10.1177/0741713611402046

ALI, Q. – ANWAR, F. – ASHRAF, M. – SAARI, N. – PERVEEN, R. 2013. Ameliorating effects of exogenously applied proline on seed composition, seed oil quality and oil antioxidant activity of maize (*Zea mays* L.) under drought stress. In *International Journal of Molecular Sciences*, vol. *14*, no.1, pp. 818–835. DOI: 10.3390/ijms14010818

ANJUM, S.A. – SALEEM, M.F. – WANG, L.C. – BILAL, M.F. – SAEED, A. 2012. Protective role of glycinebetaine in maize against drought-induced lipid peroxidation by enhancing capacity of antioxidative system. In *Australian Journal of Crop Science*, vol. *6,* no. 4, pp. 576–583. DOI: ielhss;dn/260696456888352

ASHRAF, M. – FOOLAD, M. 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. In *Environmental and Experimental Botany*, vol. *59,* no. 2, pp. 206–216. DOI: 10.1016/j.envexpbot.2005.12.006

BHASKARA, G.B. – YANG, T.H. – VERSLUES, P.E. 2015. Dynamic proline metabolism: importance and regulation in water limited environments. In *Frontiers in Plant Science*, vol. *6*. DOI: 10.3389/fpls.2015.00484

BLUM, A. 1996. Crop responses to drought and the interpretation of adaptation. In *Plant Growth Regulation*, vol. *20*, no. 2, pp. 135–148. DOI: 10.1007/BF00024010

CHEN, T.H. – MURATA, N. 2008. Glycinebetaine: an effective protectant against abiotic stress in plants. In *Trends in Plant Science*, vol. *13*, no. 9, pp. 499–505. DOI: 10.1016/j.tplants.2008.06.007

DAWOOD, M.J. 2016. Influence of osmoregulators on plant tolerance to water stress. In *Scientia Agricola*, vol. *13*, no. 1, pp. 42–58.

DU, T. – KANG, S. – ZHANG, J. – DAVIES, W.J. 2015. Deficit irrigation and sustainable water-resource strategies in agriculture for China's food security. In *Journal of Experimental Botany*, vol. *66*, no. 8, pp. 2253–2269. DOI: 10.1093/jxb/erv034

ESLAM, B.P. – MONIRIFAR, H. – GHASSEMI, M.T. 2010. Evaluation of late season drought effects on seed and oil yields in spring safflower genotypes. In *Turkish Journal of Agriculture and Forestry*, vol. *34,* no. 5, pp. 373–380. DOI: ajcs.2012.72.79

FAO, 2003. U*nlocking the water potential of agriculture*. Food and Agriculture Organization, United Nation, Rome. www.fao.org/docrep/006/y4525e/y4525e00.

FLEMMER, A.C. – FRANCHINI, M.C. – LINDSTRÖM, L.I. 2015. Description of safflower (*Carthamus tinctorius*) phenological growth stages according to the extended BBCH scale. In *Annals of Applied Biology*, vol. *166,* no. 2, pp. 331–339. DOI: 10.1111/aab.12186

GIRI, J. 2011. Glycinebetaine and abiotic stress tolerance in plants. In *Plant signaling & behavior*, vol. *6*, no. 11, pp. 1746–1751. DOI: 10.4161/psb.6.11.17801

HAMAMCI, C. – SAYDUT, A. – TONBUL, Y. – KAYA, C. – KAFADAR, A.B. 2011. Biodiesel production via transesterification from safflower (*Carthamus tinctorius* L.) seed oil. In *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*, vol. *33*, no. 6, pp. 512–520. DOI: 10.1080/15567030903096964

HARE, P.D. – CRESS, W.A. – Van STADEN, J. 1999. Proline synthesis and degradation: a model system for elucidating stress-related signal transduction. In *Journal of Experimental Botany*, vol. *50,* no. 333, pp. 413–434. DOI: https://doi.org/10.1093/jxb/50.333.413

HAYAT, S. – HAYAT, Q. – ALYEMENI, M.N. – WANI, A.S. – PICHTEL, J. – AHMAD, A. 2012. Role of proline under changing environments: a review. In *Plant Signaling & Behavior*, vol. *7*, no. 11, pp.1456–1466. DOI: 10.4161/psb.21949

HOANG, T.M.L. – TRAN, T.N. – NGUYEN, T.K.T. – WILLIAMS, B. – WURM, P. – BELLAIRS, S. – MUNDREE, S. 2016. Improvement of salinity stress tolerance in rice: challenges and opportunities. In *Agronomy*, vol. *6*, no. 4, pp. 54. DOI: 10.3390/agronomy6040054

HUSSAIN, M.I. – LYRA, D.A. – FAROOQ, M. – NIKOLOUDAKIS, N. – KHALID, N. 2016. Salt and drought stresses in safflower: a review. In *Agronomy for sustainable development*, vol. *36,* no. 1, pp. 1–31. DOI: 10.1007/s13593-015-0344-8

IQBAL, N. – ASHRAF, M. – ASHRAF, M.Y. 2008. Glycinebetaine, an osmolyte of interest to improve water stress tolerance in sunflower (*Helianthus annuus* L.): water relations and yield. In *South African Journal of Botany*, vol. *74*, no. 2, pp. 274–281. DOI: 10.1016/j.sajb.2007.11.016

JANMOHAMMADI, M. – NASIRI, Y. – ZANDI, H. – KOR-ABDALI, M. – SABAGHNIA, N. 2014. Effect of manure and foliar application of growth regulators on lentil (*Lens culinaris*) performance in semi-arid highland environment. In *Botanica Lithuanica*, vol. *20*, no. 2, pp. 99–108. DOI: 10.3923/pjbs.2013.51.58

JANMOHAMMADI, M. – YOUSEFZADEH, S. – DASHTI, S. – SABAGHNIA, N. 2017. Effects of exogenous application of nano particles and compatible organic solutes on sunflower (*Helianthus annuus* L.). In *Botanic Serbica*, vol. *14*, no. 2, pp. 37–46. DOI: 10.5281/zenodo.453554

KANWAL, H. – ASHRAF, M. – HAMEED, M. 2013. Water relations and ionic composition in the seedlings of some newly developed and candidate cultivars of wheat (*Triticum aestivum* L.) under saline conditions. In *Pakistan Journal of Botany*, vol. *45*, no. 4, pp.1221–1227.

LANDAU, S. – FRIEDMAN, S. – BRENNER, S. – BRUCKENTAL, I. – WEINBERG, Z.G. – ASHBELL, G. – HEN, Y. – DVASH, L. – LESHEM, Y. 2004. The value of safflower (*Carthamus tinctorius*) hay and silage grown under Mediterranean conditions as forage for dairy cattle. In *Livestock Production Science*, vol. *88*, no. 3, pp. 263–271. DOI: 10.1016/j.livprodsci.2003.11.011

LARAUS, J. 2004. The problems of sustainable water use in the Mediterranean and research requirements for agriculture. In *Annals of Applied Biology,* vol. *144*, no. 3, pp. 259–272. DOI:10.1111/j.1744-7348.2004. tb00342.x

LAWLOR, D.W. – CORNIC, G. 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. In *Plant, cell & environment*, vol. *25*, no. 2, pp. 275–294. DOI: 10.1046/j.0016-8025.2001.00814.x

OVERPECK, J.T. – COLE, J.E. 2006. Abrupt change in Earth's climate system. In *Annual Review of Environment and Resources*, vol. *31*, pp. 1–31. DOI: 10.1146/annurev.energy.30.050504.144308

PEEL, M.C. – FINLAYSON, B.L. – MCMAHON, T.A. 2007. Updated world map of the Köppen-Geiger climate classification. In *Hydrology and Earth System Sciences Discussions*, vol. *4*, no. 2, pp. 439–473. DOI: 10.5194/hess-11-1633-2007

RAZA, M.A.S. – SHAHID, A.M. – SALEEM, M.F. – KHAN, I.H. – AHMAD, S. – ALI, M. – IQBAL, R. 2017. Effects and management strategies to mitigate drought stress in oilseed rape (*Brassica napus* L.): a review. In *Žemdirbystė (Agriculture)*, vol. *104*, no. 1, pp. 85–94. DOI: 0.13080/z-a.2017.104.012

RUDOLPHI, S. – BECKER, H.C. – SCHIERHOLT, A. – VON WITZKE-EHBRECHT, S. 2012. Improved estimation of oil, linoleic and oleic acid and seed hull fractions in safflower by NIRS. In *Journal of the American Oil Chemists' Society*, vol. *89*, no. 3, pp. 363–369. DOI: 10.1007/s11746-011-1920-y

SABAGHNIA, N. – AHADNEZHAD, A. – JANMOHAMMDI, M. 2015. Genetic variation in garden cress (*Lepidium sativum* L.) germplasm as assessed by some morphological traits. in *Genetic Resources and Crop Evolution*, vol. *5*, no. 62, pp. 733–745. DOI: 10.1007/s10722-014-0192-4

SHAHBAZ, M. – MASOOD, Y. – PERVEEN, S. – ASHRAF, M. 2012. Is foliar-applied glycinebetaine effective in mitigating the adverse effects of drought stress on wheat (*Triticum aestivum* L.)? In *Journal of Applied Botany and Food Quality*, vol. *84*, no. 2, pp. 192–199. DOI: 10/1094/ CM-2003-0804-02-RS

TEJERO, I.G. – ZUAZO, V.H.D. – BOCANEGRA, J.A.J. – FERNÁNDEZ, J.L.M. 2011. Improved water-use efficiency by deficit-irrigation programmes: Implications for saving water in citrus orchards. In *Scientia Horticulturae*, vol. *128*, no. 3, pp. 274–282. https://doi.org/10.1016/j.scienta.2011.01.035

WANI, A.S. – AHMAD, A. – HAYAT, S. – TAHIR, I. 2016. Is foliar spray of proline sufficient for mitigation of salt stress in *Brassica juncea*. In *Environmental Science and Pollution Research*, vol. *23*, no. 13, pp. 13413–13423. DOI: 10.1007/s11356-016-6533-4

Received: August 31, 2017

DE GRUYTER OPEN

Agriculture (Poľnohospodárstvo), 63, 2017 (4): 142−150

Original paper DOI: 10.1515/agri-2017-0014

PHENOTYPING ROOT SYSTEM ARCHITECTURE OF COTTON (*GOSSYPIUM BARBADENSE* L.) GROWN UNDER SALINITY

SHADY A. MOTTALEB[1*], ESSAM DARWISH[1], MENNA MOSTAFA[2], AND GEHAN SAFWAT[2]

[1]Cairo University, Egypt
[2]October University for Modern Sciences and Arts, Egypt

MOTTALEB, S.A. – DARWISH, E. – MOSTAFA, M. – SAFWAT, G.: Phenotypic root system architecture of cotton (*Gossypium barbadense* L.) grown under salinity. Agriculture (Poľnohospodárstvo), vol. *63*, 2017, no. 4, pp. 142–150.

Soil salinity causes an annual deep negative impact to the global agricultural economy. In this study, the effects of salinity on early seedling physiology of two Egyptian cotton (*Gossypium barbadense* L.) cultivars differing in their salinity tolerance were examined. Also the potential use of a low cost mini-rhizotron system to measure variation in root system architecture (RSA) traits existing in both cultivars was assessed. Salt tolerant cotton cultivar 'Giza 90' produced significantly higher root and shoot biomass, accumulated lower Na^+/K^+ ratio through a higher Na^+ exclusion from both roots and leaves as well as synthesized higher proline contents compared to salt sensitive 'Giza 45' cultivar. Measuring RSA in mini-rhizotrons containing solid MS nutrient medium as substrate proved to be more precise and efficient than peat moss/sand mixture. We report superior values of main root growth rate, total root system size, main root length, higher number of lateral roots and average lateral root length in 'Giza 90' under salinity. Higher lateral root density and length together with higher root tissue tolerance of Na^+ ions in 'Giza 90' give it an advantage to be used as donor genotype for desirable root traits to other elite cultivars.

Key words: *Gossypium barbadense* L., salinity stress, phenotyping, root system architecture

Soil salinity is estimated to cause losses in crop production of about 27.3 billion US dollars annually (Qadir *et al.* 2014). The effects that excess Na^+ cations present in saline soils have on plant physiology are devastating, ranging from ion toxicity and physiological drought to reactive oxygen species (ROS) formation and cell death (Munns & Tester 2008). Although plants have developed a set of strategies to tolerate salinity stress (Roy *et al.* 2014), the majority of economically important crop plants are considered glycophytes and are severely affected by high Na^+ concentration with an evident trade-off between yield and salinity tolerance.

Salinity tolerance among cotton germplasm varies widely, both intra- and interspecifically where, for example, *Gossypium barbadense* varieties were reported to be more tolerant to salinity than *Gossypium hirsutum* or *Gossypium arboreum* cottons (Abul-Naas & Omran 1974). Phenotypic variability of cotton root traits was reported to be present for root weight, length, volume, total dry matter, and shoot-to-root ratio in *G. hirsutum* germplasm (Basal *et al.* 2003; Aboukheir *et al.* 2008). However, not only very little is known about phenotypic variability of other important RSA traits such as lateral root length and density but also assessing its available variability in Egyptian cotton (*G. barbadense*), an economically valuable species, is lacking.

Plant root system architecture (RSA), the spatial distribution of the root system within the rooting volume, controls the fate of the plant through its efficiency of anchorage to the soil as well as water,

Shady A. Mottaleb (*Corresponding author), Essam Darwish, Agricultural Botany Department – Plant Physiology Division, Faculty of Agriculture, Cairo University, Egypt. E-mail: shady.osama@agr.cu.edu.eg
Menna Mostafa, Gehan Safwat, Faculty of Biotechnology, October University for Modern Sciences and Arts, Egypt

nutrients uptake and abiotic stress tolerance. Modifying RSA traits was reported to contribute in improving grain yield and drought tolerance (Steele *et al.* 2013; Uga *et al.* 2013). Nevertheless, studying RSA is difficult regarding root sampling measurement, and plasticity in response to various environmental stimuli (Julkowska & Testerink 2015). Several methods have been proposed to study RSA, including hydroponics (Tuberosa *et al.* 2002), rhizotrons (Devienne-Barret *et al.* 2006), mini-lysimeters (Udayakumar *et al.* 1998), and PVC tubes (Taylor *et al.* 1991).

The present study aims to investigate the existence of variability in RSA under salinity stress in Egyptian cotton as well as identify potential new traits beneficial for salinity tolerance. Using a morphological and physiological approach to study two Egyptian cotton cultivars differing in salinity tolerance, cv. 'Giza 45' (salt sensitive) and cv. 'Giza 90' (salt tolerant), evidence will be provided that phenotypic variability in RSA is present in Egyptian cotton cultivars. Also, the possibility of phenotyping this variability will be described using a simple mini-rhizotron system. Finally some recommendations on the optimum type of substrates and conditions to be used for an accurate phenotyping of Egyptian cotton roots at seedling stage are given.

MATERIAL AND METHODS

The present study was carried out during January–July 2016 in the Plant Physiology division, Department of Agricultural Botany, Faculty of Agriculture, Cairo University, Egypt.

Plant material

Egyptian cotton (*Gossypium barbadense* L.) cultivars 'Giza 90' (salt tolerant) and 'Giza 45'(salt sensitive) were used in our experiments, both obtained from the Cotton Research Institute, Agricultural Research Centre, Giza, Egypt.

Mini-rhizotron description

Mini-rhizotron system allows a non-destructive study of root development during early stages of seedling growth. It usually contains a thin layer of substrate that directs the roots to grow in 2D conditions, facilitating the monitoring and measurement of root system morphology and architecture. The mini-rhizotron used in our experiments consisted of two glass sheets of 30 × 30 cm separated by a 3 mm wide glass separator. The inner space available for culture substrate was approximately 250 cm^3.

Experimental design and plant growth conditions

Experiments were arranged as a randomized design with 3–5 replicates, and were repeated two times to confirm results. Two different experiments were performed using this mini-rhizotron in the present research work as follows:

Experiment 1

The substrate of this experiment included peat moss and sieved fine sand mixture (2:1). After the mini-rhizotron was filled with the substrate, the two glass sheets were placed over each other and sealed from the corners and below with paper clips and sellotape to keep substrate from being lost and was perforated at the bottom to allow drainage. Mini-rhizotrons were kept in a growth chamber under the following conditions: 25°C, 60% humidity, photon irradiance of 100 $\mu E/m^2/s$ and 16/8h light/dark cycle). Mini-rhizotrons were placed vertically under the angle of 70° in the growth chamber and covered with black plastic bags to provide dark conditions for roots.

Experiment 2

The substrate placed in mini-rhizotrons of this experiment consisted of sucrose-free ¼ strength MS medium (Murashige & Skoog 1962) supplemented with agarose gel 1.2% and pH was adjusted to 5.8. The same growing conditions were maintained as experiment 1.

Seed sterilization and cultivation

Seeds were sterilized in commercial sodium hypochlorite solution 1% for 10 min and then rinsed three times with tap water, and then left to germinate for two days in Petri dishes lined with water-soaked filter paper. In both experiments, one germinated seed from cultivar Giza 90 was planted at approximately equal spacing from the other cultivar Giza 45 per mini-rhizotron.

Salinity stress treatment

In Experiment 1, two days old seedlings were transferred to mini-rhizotrons and randomly divided into two groups. The first group was the control and was watered with 100 ml of ¼ MS medium every two days during the two weeks. The second group was treated with ¼ MS medium containing 150 mM NaCl every two days also for two weeks.

In Experiment 2, using mini-rhizotron containing solid MS nutrient medium, salinity stress was imposed from the beginning using solid ¼ strength MS medium containing 150 mM NaCl, where 2 days old seedlings of both cotton cultivars were transferred to four mini-rhizotrons. In the case of control conditions, another four seedlings of both cultivars were transferred to NaCl free solid ¼ strength MS medium.

Salinity tolerance physiological traits

Leaf relative water content (RWC) was calculated according to Weatherly (1950). Fresh and dry weights were determined with the accuracy of 0.001 g on the regular lab scale. Dry weight of root and shoot tissues were measured after drying the material for 48 h at 70°C. Free proline concentration in leaves was determined according to Bates *et al.* (1973) using 0.5 g dry weight samples. Na^+ and K^+ cation contents of shoot or root dry weight samples were extracted by 0.1 M HCl solution (Garciadeblas *et al.* 2003). Determination of Na^+ and K^+ cation contents was realized using a flame photometer (Jenway PFP-7, Bibby Scientific Limited, UK).

Image capturing and analysis of RSA

Mini-rhizotrons were scanned with a Canon MG2400 series Scanner at 200 dpi at 14 days plant age. Scanned images RSA parameters were processed and quantified using EZ-Rhizo software (Armengaud *et al.* 2009). Data were collected from 3 individual seedlings per treatment per experiment. RSA parameters of control conditions were not quantified, since the roots were too entangled for the EZ-Rhizo software to detect. All data were cleared from outliers.

Statistical analysis of data

Differences among means were tested by a two-way ANOVA followed by Duncan *post hoc* test. In the case of RSA parameters, differences between means were tested by *t*-test. Differences were considered statistically significant at $P < 0.05$ in all analyses. Both statistical analyses were carried out using IBM SPSS Statistics V. 20 (IBM, USA).

RESULTS

Cv. 'Giza 90' accumulates lower Na^+ and higher proline contents than 'Giza 45'

RWC was significantly decreased by almost 10% under 150 mM NaCl treatment in both cultivars but with no differences between them (Table 1). Nevertheless, salinity treatment significantly increased the content of Na^+ but not K^+ in both shoots and roots as compared to plants under control conditions. These increases in Na^+ concentrations were significantly higher in cv. 'Giza 45' making it less efficient than cv. 'Giza 90' in eliminating Na^+ to the outside of root cells. This was reflected by a significantly less decrease in K^+ / Na^+ ratio in cv. 'Giza 90' roots under salinity. However, cv. 'Giza 90' showed similar values to "Giza 45" under control conditions. In addition, the ability to synthesize proline under salinity stress in cv. 'Giza 45' was found to be significantly lower than cv. 'Giza 90'. Leaf proline contents of cv. 'Giza 90' were one-fold higher than cv. 'Giza 45' under salinity stress, being *ca.* 1.93 and 4.17 mg/g leaf dry weight basis in cvs. 'Giza 45' and 'Giza 90', respectively. Surprisingly, the synthesis of proline in cv. 'Giza 90' leaves under control conditions was similar to the value of cv. 'Giza 45' leaf proline under salinity conditions.

Salinity stress severely hinders root growth rate of cv. 'Giza 45'

Hidden lateral roots in the thin layer of substrate (Figure 1) made possible to only examine and measure growth of the main root of both cotton cultivars. Root growth rate of both cultivars was significantly hindered under 150 mM NaCl treatment compared with control (Table 1). Nevertheless, root system of cv. 'Giza 45' was apparently more sensitive to salinity as the decrease in its main root growth rate was more significant than cv. 'Giza 90' under 150 mM NaCl, giving values of approximately 11.1 and 16.6 mm/day, respectively.

Cv. 'Giza 90' gives higher root and shoots biomass under salinity

Harvesting intact root system from peat moss/sand substrate was difficult to achieve in both treatments of experiment 1, because significant root biomass was lost in harvesting. However, in mini-rhizotron containing solid MS medium, the whole intact root system was easily harvested and washed. The effect of 150 mM NaCl was detrimental to shoots of both cultivars (Table 1) being more significantly pronounced in the case of cv. 'Giza 45'.

Root system architecture (RSA) traits under salinity of cv. 'Giza 90' is highly superior than 'Giza 45'

Solid MS media in mini-rhizotrons permitted a clear and accurate measurement of the whole root system of both cotton cultivars (Figure 2). Nonetheless, the vigorous growth under control treatment led to overlapping of lateral roots of both cultivars which prevented its analysis using EZ-Rhizo root image analysis software (Figure 2 and Supplementary Figure 1). This, however, was not the case under 150 mM NaCl, where root growth was slower and less dense. Results in Table 2 show the effect of salinity on RSA of both cultivars. Overall, the morphology of cv. 'Giza 90' was considerably superior under both control and 150 mM NaCl conditions (Figure 2). The total root system size of cv. 'Giza 90' was 2.5 folds over cv. 'Giza 45' under 150 mM NaCl. This was attributed to the significantly longer main root length of cv. 'Giza 90' as compared to cv. 'Giza 45' as well as almost a 2.5 folds bigger cumulative lateral roots length. This might imply that the effect of 150 mM NaCl on lateral roots of cv. 'Giza 45' was more profound and more detrimental. This was also reflected in cv. 'Giza 45' as a longer main root as a ratio of total root size of *ca.* 45%, which clearly demonstrates an extreme effect on lateral root growth than on main root comparing with 'Giza 90' (*ca.* 19%). Finally, this was also evident in 'Giza 90' giving higher number of lateral roots and average lateral root length values, which was almost the double in size than that of cv. 'Giza 45' in both cases.

DISCUSSION

Assessing salinity tolerance of 'Giza 90' and 'Giza 45' cotton cultivars

Egyptian cotton varieties are classified according to their salinity tolerance into three groups; salt sensitive, moderate salt tolerant and salt tolerant (Ashour & Abd-El'Hamid 1970). Curiously, early reports considered cv. 'Giza 45' salt tolerant, (El-Zahab 1971) while more recent reports classify it as salt sensitive (El-Kadi *et al.* 2006). Thus, it was important in our work to assess the degree of seedling stage salinity tolerance of 'Giza 45' and 'Giza 90'

T a b l e 1

Salinity tolerance traits studied in cvs. 'Giza 90' and 'Giza 45' cotton plants (14 days age) under control and 150 mM NaCl treatment (Experiments 1 and 2)

Experiment	Trait	Control		150 mM NaCl	
		'Giza 90'	'Giza 45'	'Giza 90'	'Giza 45'
1	Leaf RWC [%]	82.1 ± 1.8^a	83.2 ± 1.5^a	73.5 ± 1.3^b	77.5 ± 0.5^b
	Root growth rate [mm/d]	28.0 ± 2.0^a	19.0 ± 1.9^b	16.6 ± 2.5^b	11.1 ± 1.0^c
	Leaf proline [mg/g]	2.22 ± 0.12^b	1.53 ± 0.20^c	4.17 ± 0.32^a	1.93 ± 0.03^{bc}
	Leaf Na^+ [nmol/mg]	89.8 ± 9.4	60.6 ± 7.1^a	430.8 ± 37.2^b	648.4 ± 44.4^c
	Root Na^+ [nmo/mg]	310.5 ± 21.6^a	438.1 ± 61.5^a	716.6 ± 35.9^b	891.3 ± 57.3^c
	Leaf K^+ [nmol/mg]	282.2 ± 44.5^a	247.5 ± 53.8^a	302.2 ± 28.5^a	301.4 ± 29.2^a
	Root K^+ [nmol/mg]	251.1 ± 40.0^a	300.5 ±16.2^a	394.4 ± 61.0^a	343.4 ± 5.8^a
	Leaf K^+/Na^+	3.3 ± 0.9^a	4.2 ± 1.0^a	0.7 ± 0.0^b	0.5 ± 0.0^b
	Root K^+/Na^+	0.8 ± 0.1^a	0.7 ± 0.1^{ab}	0.5 ± 0.1^{bc}	0.4 ± 0.0^c
2	Shoot DWT [mg]	186.5 ± 2.6^a	92.7 ± 4.2^b	68.2 ± 10.3^b	25.3 ± 6.9^c
	Root DWT [mg]	126.2 ± 1.0^a	20.7 ± 1.4^b	104.7 ± 11.4^a	14.1 ± 2.7^b
	Shoot/Root	1.5 ± 0.7^b	4.5 ± 0.5^a	1.3 ± 0.6^b	2.4 ± 0.6^b

Each value represents the mean ± standard error of 3 replicates. Means with identical letters in the same row are not significantly different ($P > 0.05$) according to Duncan test. (Abbreviations: RWC – relative water content; DWT – dry weight) cultivars using simple physiological measurements before starting root phenotyping.

High salinity reduces vegetative and reproductive growth of cotton (Gorham *et al.* 2010). Both plant height and leaf expansion are negatively affected in saline soils where the differentiation of nodes is suppressed (Ahmed 1994). These effects are however less accentuated in tolerant as in the case 'Giza 90' where both its shoot and root biomass are significantly higher than cv. 'Giza 45'. Salinity level of 150 mM NaCl was reported to reduce the elongation of the taproot of cotton plants by 60% over control plants (Zhong & Lauchli 1993). The severity of this level of salinity on the water relations of both cultivars was assessed by measuring the relative water content (RWC) in leaves (Table 1), a trait that measures of water deficit in the leaf that reflecting the dynamic water balance between water flow into and out of the tissue (Sinclair & Ludlow 1985). It is clear that under this moderate stress, the stomata are compelled to adjust their conductance to maintain more or less stable water balance in the leaves and prevent further water losses to maintain cell and tissue turgor, and this effect was similar on both cultivars.

The apparent higher efficiency of cv. 'Giza 90' in Na^+ exclusion or sequestration inside the cell vacuole might depend on the level of transcription of transporters and activity of responsible transporters such as *SOS1* and *NHX*, respectively. Also, significantly lower foliar Na^+ content accumulated in cv. 'Giza 90' was detected. This is an important trait to protect the leaves photosynthetic machinery from any damage induced by excessive Na^+ involving several mechanisms such as Na^+ xylem loading, Na^+ retrieval from the xylem and Na^+ retrieval from the shoots (Karley *et al.* 2000; Davenport *et al.* 2007). A lower Na^+ concentration in the leaves is usually expressed in more salinity tolerance. Furthermore, cell depolarization occurs under salinity makes K^+ uptake more problematic, causing a massive K^+ efflux resulting in a depletion of the cytosolic K^+ pool (Shabala & Munns 2012). Nevertheless, our results do not show any significant perturbation in K^+ levels under 150 mM NaCl treatment neither in roots nor in shoots, indicating that stress level imposed was not very severe nor extended in time (Table 1). Cytosolic K^+/Na^+ ratio, and not the absolute quantity of Na^+ *per se*, seems to determine cell metabolic competence and, ultimately, the ability of a plant to survive in saline environments (Shabala & Cuin 2008) and, thus, higher K^+/Na^+ ratio could reflect more salinity tolerance, which might seem to be the case in cv. 'Giza 90'. Nevertheless, its higher K^+/Na^+ ratio is attributed to a higher Na^+ efflux and not from higher K^+ retention (Table 1).

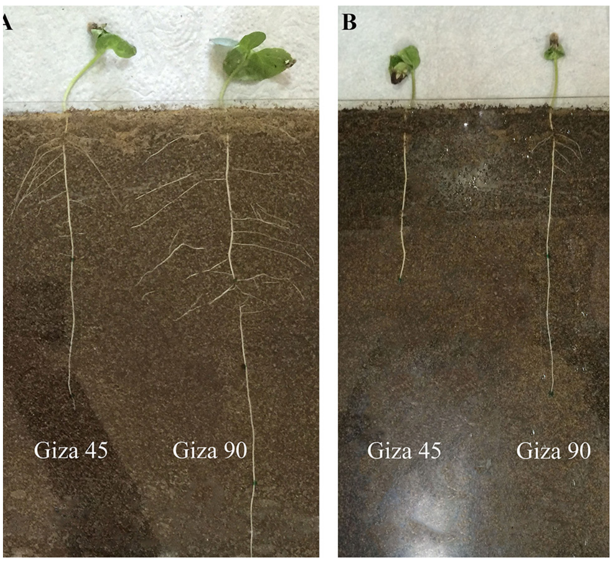


Figure 1. Root system morphology of cvs. 'Giza 90' and 'Giza 45' cotton plants (14 days age) under A) control and B) 150 mM NaCl conditions (Experiment 1)

Under salinity, cells adjust their osmotic potential by accumulating many compatible solutes which also perform many other important functions. Oosterhuis and Wullschleger (1988) reported that cotton has more osmotic adjustment capabilities than other major crops. Moreover, significant differences among cotton cultivars for osmotic potential exist, suggesting that genotypic variation for osmoregulation in cotton is wide (Quisenberry *et al.* 1982). All in all, it is extensively reported that proline concentration increases in cotton with increasing soil salinity (He *et al.* 2007). It is apparent that under our experimental conditions, proline content of cv. 'Giza 90' is significantly higher than cv. 'Giza 45', giving it a superior ability to maintain its turgor under salinity, results similarly reported by El-Kadi *et al.* (2006).

Challenges in studying RSA of cotton

Root phenotype of plant seedling can be a sound predictor of later stages of plant development (Tuberosa *et al.* 2002). However, a problem we faced in studying roots of early stage cotton plants in

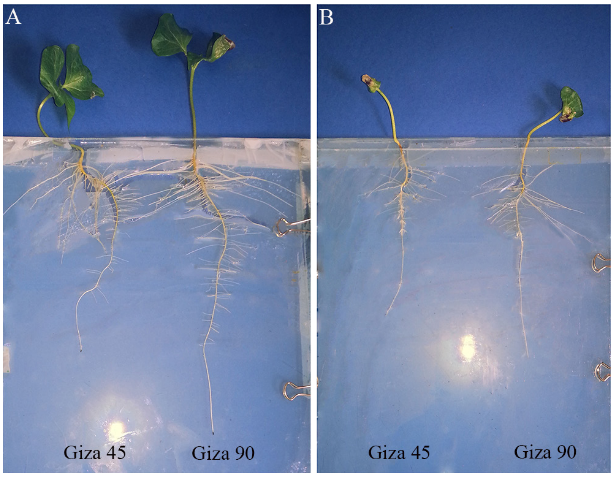
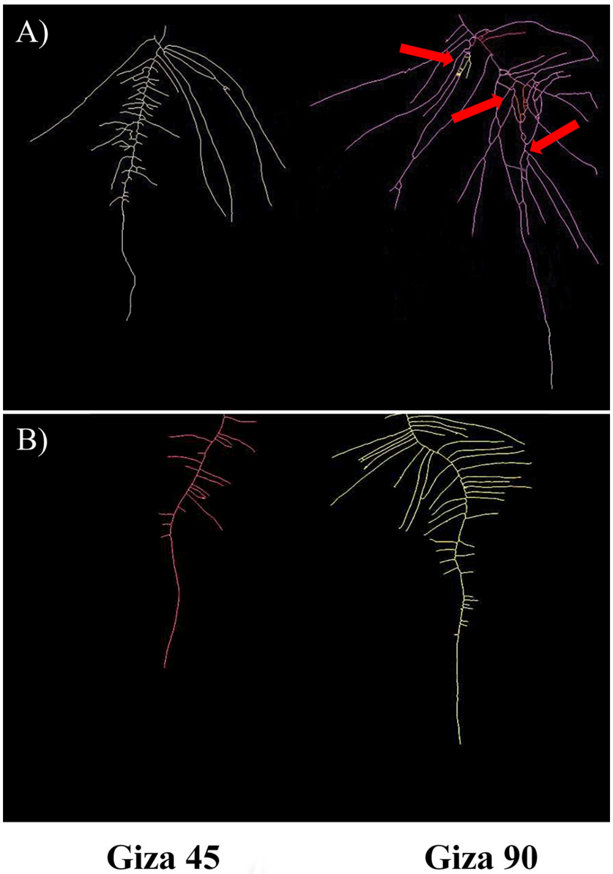


Figure 2. Root system morphology of cvs. 'Giza 45' and 'Giza 90' cotton plants (14 days age) under A) 150 mM NaCl and B) control conditions (Experiment 2)



Suppl. Figure 1. Processed image by EZ-Rhizo software of root system morphology of cvs. 'Giza 90' and 'Giza 45' cotton plants (14 days age) under A) control and B) 150 mM NaCl conditions (Experiment 2). Red arrows indicate entangled regions erroneously detected by the software

mini-rhizotron, in contrast to other crops such as tomato (Darwish *et al.* 2016), was that some lateral roots were either hidden into the soil layer and not showing over neither the front nor back glass plates of the mini-rhizotron to be scanned for subsequent image analysis. Also, some lateral roots showed at back side only. The main cause of this problem is that the lateral roots of cotton emerge on the main root in a 3D manner, which decreases the efficiency of studying RSA in 2D mini-rhizotrons, as in the case of our experiment. A possible solution to avoid this problem is to force the growth of lateral roots in an even more 2D growth by decreasing the spacing between the two mini-rhizotron glass plates even lower than 3 mm. This, however, will probably put a mechanical strain which might affect the main root growth making its growth pattern not be reliable to study. Thus, solid MS medium was used to provide a translucent environment needed to detect all lateral roots and study their RSA. Another problem we faced was that, under control conditions, the root growth was very vigorous and led to the overlapping and entanglement of lateral roots in many zones (Figure 2). This problem leads to a faulty detection of roots in Ez-Rhizo software and gave erroneous results (Supplementary Figure 1). This could be overcome by studying RSA under control conditions at earlier stages of growth (e.g. 10 days age). For this reason, it was only possible for us to study the RSA of cvs. 'Giza 45' and 'Giza 90' only under salinity stress.

Identifying potential root traits in Egyptian cotton desirable for salinity tolerance

A significant phenotypic variability in *G. hirsutum* cotton, i.e. root length, root fresh weight, root dry weight, lateral root number, lateral root dry weight, total root dry weight, root volume, and root-to-shoot ratio was reported in previous studies (Basal *et al.* 2003; AbouKheir *et al.* 2008). This variability, however, seems to be much lower in genotypes adapted to humid and high-rainfall conditions (Quisenberry *et al.* 1981). In the case of our experiments, substantial variation in root traits was detected between cvs. 'Giza 90' and 'Giza 45'. The analyzed data of mini-rhizotron root system images (Experiment 2) with EZ-Rhizo software (Table 2) shows that cv. 'Giza 90' root system architecture under salinity conditions was significantly higher in various parameters including total root size, main root length, cumulative lateral root length, average lateral root length, number of lateral roots, length of basal and branched zones, and depth. This was also the case with main root growth rate. On the other hand, several other parameters did not show any significant difference such as length of apical zone and number of lateral roots per cm of main root. These impressive root system characteristics of the salinity tolerant cultivar cv. 'Giza 90' suggest that the allocation of photosynthate from the source to the roots is more effective than in cv. 'Giza 45', which is finally translated as a higher root biomass as shown earlier (Table 1). The body of literature published on the effect of salinity on root traits of cotton in general, and RSA in particular, is very limited (Gorham *et al.* 2010). However, a number of different root morpho-physiological traits have been proposed to be implicated as important mechanisms that impart drought tolerance in cotton, which might be beneficial in salinity tolerance as well. These include distance from transition zone to the first main lateral root, taproot weight, number of lateral roots, seedling vigour, rapidity of root system development, and root to shoot ratio and longer taproot length (Pace *et al.* 1999). Our results show that cv. 'Giza 90' possesses several of the aforementioned traits that are beneficial under drought and probably under salinity stress as well. For example, the production of significantly denser and longer lateral roots in the top soil is desired traits and especially in saline soils because salinity is lower at these areas and becomes more concentrated in deeper layers. This high density of lateral roots permits a more efficient extraction of less salinised water from topsoil and consequently the plants become less susceptible to dehydration. This trait present in salt tolerant cv. 'Giza 90' cotton suggests its advantage as a donor genotype for this particular desirable root trait to other elite cotton cultivars in any of the ongoing breeding programs for salinity and /or drought tolerance.

CONCLUSIONS

Salt tolerant 'Giza 90' cotton cultivar showed superior shoot/root biomass, higher K^+/Na^+ ratio and proline content. This superiority also holds true regarding the majority of root system architecture (RSA) parameters. The possibility of phenotyping of cotton RSA at early stage could be predictor for later developmental stages, using a mini-rhizotron system which was demonstrated being more accurate using solid MS media than peat moss/sand as substrate. Phenotypic variation in potential beneficial root traits for salinity tolerance, such as a longer and denser lateral roots in branched zone, in the

T a b l e 2

Root system architecture (RSA) parameters of cvs. 'Giza 45' and 'Giza 90' cotton plants (14 days age) measured using EZ-Rhizo software under 150 mM NaCl salinity stress (Experiment 2)

Root system architecture (RSA) parameter	'Giza 45'	'Giza 90'
Main root "MR" length [cm]	14.47 ± 0.12	19.01 ± 0.72*
Lateral roots "LR" cumulative length [cm]	19.64 ± 4.66	97.56 ± 3.98*
Total root size "cumulative length of LR and MR" [cm]	35.38 ± 6.05	120.43 ± 8.56*
Number of lateral roots per main root (#)	19 ± 0	43 ± 4*
Average length of lateral roots [cm]	0.93 ± 0.18	1.94 ± 0.17*
Average lateral root length as ratio of main root length [%]	6 ± 0	9 ± 0*
Main root length as ratio of total root size [%]	45 ± 4	19 ± 2*
Length of basal zone [cm]	1.04 ± 0.92	0.74 ± 0.54
Length of branched zone [cm]	8.42 ± 0.22	18.63 ± 1.87*
Length of apical zone [cm]	6.29 ± 2.10	7.30 ± 0.55

Each value represents the mean ± standard error of 3 replicates. Means with asterisk (*) in the same row are significantly different ($P \leq 0.05$) according to t-test.

case of 'Giza 90' cultivar, was identified. This low-cost approach using inexpensive material and open source software will allow a rapid and cost effective phenotyping of root systems present in cotton germplasm available in developing countries. The obtained results in this work will hopefully open the door for future studies including additional accessions and salinity levels allowing performing accurate correlation studies between each of the RSA and salinity tolerance parameters.

Acknowledgments. The authors would like to thank Professor Dr. Saeed Hamouda for providing seeds of 'Giza 90' and 'Giza 45' cotton cultivars and for his constructive comments on the experiments. The help of Mohamed Saad and Reham Naguib in plant analyses is deeply acknowledged. The financial support for this work was provided by the Faculty of Agriculture, Cairo University, Egypt.

REFERENCES

ABOUKHEIR, E. – SHESHSHAYEE, M.S. – UDAYAKUMAR, M. 2008. *AAB International Conference on Resource Capture by Crops: Integrated Approach*, 14–16 September 2008, University of Nottingham at Sutton Bonington.

ABUL-NAAS, A.A. – OMRAN, M.S. 1974. Salt tolerance of seventeen cotton cultivars during germination and early seedling development. In *Zeitschrift für Acker- und Pflanzenbau*, vol. *140*, pp. 229–236.

AHMED, F.M. 1994. Effect of saline water irrigation at different stages of growth on cotton plant. In *Assiut Journal of Agricultural Sciences*, vol. *25*, pp. 63–74.

ARMENGAUD, P. – ZAMBAUX, K. – HILLS, A. – SULPICE, R. – PATTISON, R.J. – BLATT, M.R. – AMTMANN, A. 2009. EZ–Rhizo: integrated software for the fast and accurate measurement of root system architecture. In *Plant Journal*, vol. 57, pp. 945–956. DOI: 10.1111/j.1365-313X.2008.03739.x

ASHOUR, N.I. – ABD-EL'HAMID, A.E.H.M. 1970. Relative salt tolerance of Egyptian cotton varieties during germination and early seedlings development. In *Plant and Soil*, vol. *3*, pp. 493–495. DOI: 10.1007/BF01378240

BASAL, H. – BEBELI, P. – SMITH, C.W. – THAXTON, P. 2003. Root growth parameters of converted race stocks of upland cotton and two BC_2F_2 populations. In *Crop Science*, vol. *43*, pp. 1983–1988. DOI:10.2135/cropsci2003.1983

BATES, L.S. – WALDEEN, R.P. – TEARE, I.D. 1973. Rapid determination of free proline for water-stress studies. In *Plant and Soil*, vol. *39*, pp. 205–207. DOI: 10.1007/BF00018060

DARWISH, E. – MOTTALEB, S.A. – OMARA, M. – SAFWAT, G. 2016. Effect of salt stress on root plasticity and expression of ion transporter genes in tomato plants. In *International Journal of Botany and Research* (IJBR), vol. *6*, pp. 13–26. Available from: https://www.researchgate.net/profile/Heba_Ibrahim4/publication/299289414_EFFECT_OF_SALT_STRESS_ON_ROOT_PLASTICITY_AND_EXPRESSION_OF_ION_TRANSPORTER_GENES_IN_TOMATO_PLANTS/links/570d581a08ae2b772e43200e/EFFECT-OF-SALT-STRESS-ON-ROOT-PLASTICITY-AND-EXPRESSION-OF-ION-TRANSPORTER-GENES-IN-TOMATO-PLANTS.pdf

DAVENPORT, R.J. – MUNOZ-MAYOR, A. – JHA, D. – ESSAH, P.A. – RUS, A. – TESTER, M. 2007. The Na^+ transporter AtHKT1;1 controls retrieval of Na^+ from the xylem in Arabidopsis. In *Plant, Cell and Environment*, vol. *30*, pp. 497–507. DOI: 10.1111/j.1365-3040.2007.01637.x

DEVIENNE-BARRET, F. – RICHARD-MOLARD, C. – CHELLE, M. – MAURY, O. – NEY, B. 2006. Ara-rhizotron: An effective culture system to study simultaneously root and shoot development of Arabidopsis. In *Plant and Soil*, vol. *280*, pp. 253–266. DOI: 10.1007/s11104-005-3224-1

EL-KADI, D.A. – AFIAH, S.A. – ALY, M.A. – BADRAN, A.E. 2006. Bulked segregant analysis to develop molecular markers for salt tolerance in Egyptian cotton. In *Arab Journal of Biotechnology*, vol. *9*, pp. 129–142. Available from: https://www.researchgate.net/profile/Mohammed_Aly2/publication/228936120_Bulked_segregant_analysis_to_develop_molecular_markers_for_salt_tolerance_in_Egyptian_cotton/links/0c96052de7b9fcfc03000000.pdf

EL-ZAHAB, A.A.A. 1971. Salt tolerance of eight Egyptian cotton varieties. Part II. At the seedling stage. In *Zeitschrift für Acker- und Pflanzenbau*, vol. *133*, pp. 308–314.

GARCIADEBLAS, B. – SENN, M.E. – BANUELOS, M.A. – RODRÍGUEZ-NAVARRO, A. 2003. Sodium transport and HKT transporters: the rice model. In *Plant Journal*, vol. *34*, pp. 788–801. DOI: 10.1046/j.1365-313X.2003.01764.x

GORHAM, J. – LAUCHLI, A. – LEIDI, E.O. 2010. Plant responses to salinity. In STEWART, J.M. – OOSTERHUIS, D.M. – HEITHOLT, J.J. – MAUNEY, J.R. (Eds.) *Physiology of Cotton*. London : Springer, pp. 129–141. DOI: 10.1007/978-90-481-3195-2_13

HE, G. – SHEN, G. – PASAPULA, V. – LUO, J. – VENKATARAMANI, S. – QIU, X. – KUPPU, S. – KORNYEYEV, D. – HOLADAY, A.S. – AULD, D. – BLUMWALD, E. – ZHANG, H. 2007. Ectopic expression of *AtNHX1* in cotton (*Gossypium hirsutum* L.) increases proline content and enhances photosynthesis under salt stress conditions. In *Journal of Cotton Science*, vol. *11*, pp. 266–274. Available from: http://www.cotton.org/journal/2007-11/4/upload/jcs11-266.pdf

JULKOWSKA, M.M. – TESTERINK, C. 2015. Tuning plant signaling and growth to survive salt. In *Trends in Plant Science*, vol. *20*, pp. 586–594. DOI: http://dx.doi.org/10.1016/j.tplants.2015.06.008

KARLEY, A.J. – LEIGH, R.A. – SANDERS, D. 2000. Differential ion accumulation and ion fluxes in the mesophyll and epidermis of barley. In *Plant Physiology*, vol. *122*, pp. 835–844. DOI: 10.1104/pp.122.3.835.

MUNNS, R. – TESTER, M. 2008. Mechanisms of salinity tolerance. In *Annual Review of Plant Biology*, vol. *59*, pp. 651–681. DOI: 10.1146/annurev.arplant.59.032607.092911

MURASHIGE, T. – SKOOG, F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. In *Physiologiae Plantarum*, vol. *15*, pp. 473–497. DOI: 10.1111/j.1399-3054.1962.tb08052.x

OOSTERHUIS, D.M. – WULLSCHLEGER, S.D. 1988. Drought tolerance and osmotic adjustment of various crops in response to water stress. In *Arkansas Farm Research*, vol. *37*, pp. 12.

PACE, P.F. – CRALLE, H.T. – EL-HALAWANY, S.H. – COTHREN, J.T. – SENSEMAN, S.A. 1999. Drought-induced changes in shoot and root growth of young cotton plants. In

Journal of Cotton Science, vol. *3*, pp. 183–187. Available from https://www.cotton.org/journal/1999-03/4/upload/jcs03-183.pdf. [accessed 23 July 2016].

QADIR, M. – QUILLÉROU, E. – NANGIA, V. – MURTAZA, G. – SINGH, M. – THOMAS, R.J. – DRECHSEL, P. – NOBLE, A.D. 2014. Economics of salt-induced land degradation and restoration. In *Natural Resources Forum*, vol. *38*, pp. 282–295. DOI: 10.1111/1477-8947.12054

QUISENBERRY, J.E. – JORDAN, W.R. – ROARK, B.A. – FRYREAR, D.W. 1981. Exotic cottons as genetic sources for drought resistance. In *Crop Science*, vol. *21*, pp. 889–895. DOI:10.2135/cropsci1981.0011183X002100060022x

QUISENBERRY, J.E. – ROARK, B.A. – McMICHAEL, B.L. 1982. Use of transpiration decline curves to identify drought-tolerant cotton germplasm. In *Crop Science*, vol. *22*, pp. 918–922. DOI:10.2135/cropsci1982.0011183X002200050004x

ROY, S.J. – NEGRÃO, S. – TESTER, M. 2014. Salt resistant crop plants. In *Current Opinion in Biotechnology*, vol. *26*, pp. 115–124. DOI: 10.1016/j.copbio.2013.12.004

SHABALA, S. – CUIN, T.A. 2008. Potassium transport and plant salt tolerance. In *Physiologiae Plantarum*, vol. *133*, pp. 651–669. DOI: 10.1111/j.1399-3054.2007.01008.x

SHABALA, S. – MUNNS, R. 2012. Salinity stress: physiological constraints and adaptive mechanisms. In SHABALA, S. (Ed.) *Plant Stress Physiology*. Oxfod : CAB International, pp. 59–93. DOI: 10.1079/9781845939953.0059

SINCLAIR, T.R. – LUDLOW, M.M. 1985. Who taught plants thermodynamics? The unfulfilled potential of plant water potential. In *Australian Journal of Plant Physiology*, vol. *12*, pp. 213–218. DOI: 10.1071/PP9850213

STEELE, K.A. – PRICE. A.H. – WITCOMBE, J.R. – SHRESTHA, R. – SINGH, B.N. – GIBBONS, J.M. – VIRK, D.S. 2013. QTLs associated with root traits increase yield in upland rice when transferred through marker-assisted selection. In *Theoretical and Applied Genetics*, vol. *126*, pp. 101–108. DOI: 10.1007/s00122-012-1963-y

TAYLOR, H.M. – UPCHURCH, D.R. – BROWN, J.M. – ROGERS, H.H. 1991. Some methods of root investigation. In McMICHAEL, B.L. – PERSSON, H. (Eds.) *Plant roots and Their Environment*. New York : Elsevier Science Publishers, Inc., pp. 553–564. DOI:10.1016/B978-0-444-89104-4.50075-X

TUBEROSA, R. – SANGUINETI, M.C. – LANDI, P. – GIULIANI, M.M. – SALVI, S. – CONTI, S. 2002. Identification of QTLs for root characteristics in maize grown in hydroponics and analysis of their overlap with QTLs for grain yield in the field at two water regimes. In *Plant Molecular Biology*, vol. *48*, pp. 697–712. DOI: 10.1023/A:1014897607670

UDAYAKUMAR, M. – RAO, R.C.N. – WRIGHT, G.C. – RAMASWAMY, G.C. – ASHOK, R.S. – GANGADHAR, G.C. – AFTAB HUSSAIN, I.S. 1998. Measurement of transpiration efficiency in field conditions. In *Journal of Plant Physiology and Biochemistry*, vol. *1*, pp. 69–75.

UGA, Y. – SUGIMOTO, K. – OGAWA, S. – RANE, J. – ISHITANI, M. – HARA, N. – KITOMI, Y. – INUKAI, Y. – ONO, K. – KANNO, N. – INOUE, H. 2013. Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. In *Nature Genetics*, vol. *45*, pp. 1097–1102. DOI:10.1038/ng.2725

WEATHERLY, P.E. 1950. Studies in the water relations of the cotton plant. The field measurement of water deficits in leaves. In *New Phytologist*, vol. *49*, pp. 81–97. DOI: 10.1111/j.1469-8137.1950.tb05146.x

ZHONG, H. – LAUCHLI, A. 1993. Spatial and temporal aspects of growth in the primary root of cotton seedlings: Effects of NaCl and $CaCl_2$. In *Journal of Experimental Botany*, vol. *44*, pp. 763–771. DOI: 10.1093/jxb/44.4.763

Received: April 24, 2017

DE GRUYTER OPEN

Agriculture (Poľnohospodárstvo), 63, 2017 (4): 151−162

Original paper DOI: 10.1515/agri-2017-0015

SOIL ORGANIC-MATTER IN WATER-STABLE AGGREGATES UNDER DIFFERENT SOIL-MANAGEMENT PRACTICES

VLADIMÍR ŠIMANSKÝ [1]*, JÁN HORÁK[1], BRENT CLOTHIER[2], NATALYA BUCHKINA[3], DUŠAN IGAZ[1]

[1]Slovak University of Agriculture in Nitra, Slovak Republic
[2]Food Industry Science Centre, New Zealand
[3]Agrophysical Research Institute of the Russian Academy of Agricultural Sciences, Russia

ŠIMANSKÝ, V. – HORÁK, J. – CLOTHIER, B. – BUCHKINA, N. – IGAZ, D.: Soil organic-matter in water-stable aggregates under different soil-management practices. Agriculture (Poľnohospodárstvo), vol. *63*, 2017, no. 4, pp. 151–162.

An experiment of different management practices in a commercial vineyard, which was established in 2006 in the locality of Nitra-Dražovce, Slovakia on Rendzic Leptosol, was used to evaluate the dynamics of soil organic-matter parameters during the years 2008–2015. The following treatments were established: 1. G (grass without fertilisation as control), 2. T (tillage), 3. T+FYM (tillage + farmyard manure), 4. G+NPK3 (grass + 3rd intensity of fertilisation for vineyards: it means 125 kg/ha N, 50 kg/ha P, 185 kg/ha K), and 5. G+NPK1 (grass + 1st intensity of fertilisation for vineyards: it means 100 kg/ha N, 30 kg/ha P, 120 kg/ha K). The results showed that the soil-management practices in the vineyard significantly influenced the soil organic carbon in water-stable aggregates (SOC in WSA). The content of SOC in WSA_{ma} increased on average in the following order: T < G < G+NPK1 < G+NPK3 < T+FYM. Intensive soil cultivation in the T treatment resulted in a statistically significant build-up of SOC in WSA_{ma} at an average rate of 1.33, 1.18, 0.97, 1.22 and 0.76 g/kg/y across the size fractions > 5 mm, 5–3 mm, 2–1 mm, 1–0.5 mm and 0.5–0.25 mm, respectively. The content of non-labile carbon reflected the contents of SOC in WSA. The highest labile carbon (C_L) in WSA_{ma}, as compared to others, was found in T+FYM. Overall, application of higher NPK doses resulted in higher content of C_L in WSA_{ma} compared with the lower applications of NPK. On the other hand, lower applications of NPK to soil increased the content of C_L in WSA_{mi}, as compared to G+NPK3.

Key words: soil organic carbon, labile carbon, non-labile carbon, soil structure, soil fertility

Soil organic-matter (SOM) plays an important role in plant-nutrient cycling, increasing yields and improving the physical, chemical and biological properties of soils (Bhattacharyya *et al.* 2010; Gaida *et al.* 2013) and the impact varies across space and time and is a result of the influence of many environmental and anthropogenic factors (Jonczak 2014). For example, intensive cultivation leads to loss of SOM (Khorramdel *et al.* 2013) while reduced tillage increases SOM (Šimanský *et al.* 2008). The results of Tong *et al.* (2014) indicated that application of manure had the highest sequestration rate of total SOC in comparison to mineral fertilisers. Triberti *et al.* (2008) found that continuous additions of organ-

doc. Ing. Vladimír Šimanský, PhD. (*Corresponding author), Department of Soil Science, FAFR – SUA Nitra, 949 76 Nitra, Tr. A. Hlinku 2, Slovak Republic. E-mail: Vladimir.Simansky@uniag.sk
Ing. Ján Horák, PhD., Department of Biometeorology and Hydrology, HLEF – SUA Nitra, 949 76 Nitra, Hospodárska 7, Slovak Republic. E-mail: jan.horak@uniag.sk
Dr. Brent E. Clothier, Plant and Food Research, Food Industry Science Centre, Bachelor Road, PO Box 11-600, Palmerston North 4442, New Zealand. E-mail: Brent.Clothier@plantandfood.co.nz
Dr. Natalya P. Buchkina, Agrophysical Research Institute of the Russian Academy of Agricultural Sciences, 14 Grazhdansky prospect, St. Petersburg, Russia. E-mail: buchkina_natalya@mail.ru
doc. Ing. Dušan Igaz, PhD., Department of Biometeorology and Hydrology, HLEF – SUA Nitra, 949 76 Nitra, Hospodárska 7, Slovak Republic. E-mail: dusan.igaz@uniag.sk

ic material to the soil led to a SOC build up at rates 0.16–0.26 t C/ha/y over a 22 year period. Purakayastha *et al.* (2008) reported the rates of SOC build-up in the soil up to a level of 1.0 Mg/ha/y due to NPK + farmyard application under a maize-wheat-cowpea cropping system. Abdollahi *et al.* (2014) showed the rate of SOC due to added organic fertilisers to be between 220–240 kg C/ha/y. All the information about the increase or decline of C is from different soils. However, the determination of carbon pools in aggregates provides important information on soil C sequestration processes and mineralization mechanisms in aggregate size fractions that could be used to protect SOC by using appropriate soil and crop management practices (Whalen & Chang 2002). Water-stable aggregates may protect the carbon inside of aggregates more intensively than aggregates with low resistance against destructive influence of water (Šimanský & Bajčan 2014) which is also an effective strategy to mitigate global climate change (Paustian *et al.* 1997), whilst sustaining soil health. Changes in land use significantly influence the carbon cycle. An important factor of SOM stabilization is a favourable soil structure (Berhe & Kleber 2013; Chaplot & Cooper 2015). This is because SOM is one of the most significant binding agents which is responsible for the association of mineral particles within the aggregates (Rabbi *et al.* 2015).

Vineyard soils are strongly influenced by anthropogenic activities. Before establishing a vineyard, the original soil type is transformed. There is a change in the structural conditions and capacity of the soil to retain the organic carbon. Therefore, understanding the dynamics and mechanism of carbon sequestration in the water-stable aggregates under different management practices in vineyard soils could be a primary way to improve the soil fertility. This will sustain the grape yield production around the world, and in Slovakia in particular.

The objectives of our study were: (i) to determine C changes in the size fractions of water-stable aggregates under the different management practices in a vineyard; (ii) to determine the rate of C input into water-stable aggregates under different management practices over the period of 8 years.

MATERIAL AND METHODS

The study was carried out in a commercial vineyard located in Nitra-Dražovce (48°21’6.16”N; 18°3’37.33”E) in Nitra wine-growing region of Slovakia. The climate is temperate with an average annual rainfall of 550 mm and with the mean annual temperature being ≥ 10°C. The soil had developed on limestone and dolomite and is classified as Rendzic Leptosol (WRB 2014). The soil was analysed prior the vineyard establishment (spring 2000) and contained 17.0±1.6 g/kg of soil organic carbon, 1867±103 mg/kg of total nitrogen and had a pH_{H2O} value of 7.18±0.08, with the base saturation percentage being 99.3±0.01%. The content of

T a b l e 1

The various treatments imposed in the vineyard (Nitra-Drážovce)

Treatment	Description
Control (G)	Sown grass in the rows and between vine rows, without fertilisation.
Tillage (T)	Every year medium tilth to the depth of 25 cm with intensive cultivation (three times on average, without fertilisation) between vine rows during the growing season.
Tillage + application of farmyard manure (T+FYM)	Medium tilth to the depth of 25 cm with application of farmyard manure (FYM) in a dose of 40 t/ha applied in autumn 2005, 2009 and 2012 with intensive cultivation between vine rows during growing season.
Application of fertilisers in 3rd intensity for vineyards according to Fecenko and Ložek (2000) (G+NPK3)	There was used Duslofert Extra 14-10-20-7 fertiliser with the real doses of nutrients applied in the treatment being 125 kg/ha N, 50 kg/ha P, 185 kg/ha K. Split application of fertilisers was applied with 2/3 of fertilisers applied in the spring (bud burst – on March) and 1/3 during the flowering stage (on May). The grass was sown in and between the vine rows.
Application of fertilisers in 1st intensity for vineyards according to Fecenko and Ložek (2000) (G+NPK1)	There was used Duslofert Extra 14-10-20-7 fertiliser with the real doses of nutrients applied in the treatment being 100 kg/ha N, 30 kg/ha P, 120 kg/ha K. The doses of nutrients were split with 1/2 applied in the spring (bud burst – on March) and 1/2 during flowering (on May). The grass was sown in and between the vine rows.

sand, silt and clay was 57%, 33% and 10%, respectively.

A variety of grasses (*Lolium perenne* 50% + *Poa pratensis* 20% + *Festuca rubra commutata* 25% + *Trifolium repens* 5%) were sown as grass strip between the vineyard rows in 2003. The experiment with different soil management practices (5 treatments) was initiated in 2006, and laid out on a randomized complete block design with four replicates. The investigated treatments are presented in Table 1. Soils samples (0–0.25 m) were taken from 4 random locations within each treatment every spring during years 2008–2015. Soil samples were then mixed together to form an average sample for each treatment. Large clods were gently broken up along natural fracture lines, and then air-dried in the laboratory to achieve undisturbed soil samples for the determination of the individual size fractions of aggregates. The Baksheev method for aggregate separation was adopted from Vadjunina and Korchagina (1986). Seven aggregate-size fractions were separated by wet-sieving of the soil through the series of six sieves. Briefly, the soil sample (30 g) was covered with distilled water with the water level 1 cm above aggregates. Two hours later, the sample was transferred to the top sieve (>5 mm) in a cylindrical container (Baksheev device), which had been filled with distilled water. The cylinder was hermetically closed and the sample was sieved for 12 minutes. The size fractions of WSA were the following: >5, 5–3, 3–2, 2–1, 1–0.5, 0.5–0.25 mm as water-stable macro-aggregates (WSA_{ma}) and <0.25 mm as micro-aggregates (WSA_{mi}). The material retained was quantified in each sieve, except for the micro-aggregates (<0.25 mm). Their content was calculated as difference between total weight of soil sample and sums of the macro-aggregates (>0.25 mm). The content of soil organic carbon (SOC) in the individual size fractions of water-stable aggregates (WSA) was determined using the wet combustion method by oxidation of organic matter using a mixture of 0.07 M H_2SO_4 and $K_2Cr_2O_7$ with titration using 0.01 M Mohr's salt. This is described in Dziadowiec and Gonet (1999). The labile carbon content (C_L) was extracted from samples containing 1 g of individual size fractions of WSA by shaking in 50 mL of 0.005 M $KMnO_4$ for 2 h. After centrifugation, the C_L was determined with titration using 0.05 M Mohr's salt (Loginow *et al.* 1987). Non-labile carbon (C_{NL}) was calculated according to equation (1):

$$C_{NL} = SOC - C_L \quad (1)$$

where: SOC (in g/kg) is the organic carbon content and C_L (in g/kg) is the labile carbon content

The statistical processing of the data included checking of the data for normality and later by using one-way analysis of variance (ANOVA) using Statgraphics Centurion XV.I statistical software (Statpoint Technologies, Inc., USA). Significant differences between the treatment means of the three replicates were identified using a least significant difference (*LSD*) at $p < 0.05$. The correlations between SOM in WSA and WSA contents were then determined. A linear model was used to evaluate the trends of SOC, C_{NL} and C_L under the different soil-management practices of vineyard during the period of 8 years.

RESULTS AND DISCUSSION

Water-stable aggregates

The distribution of aggregate sizes under the different soil-management practices of the vineyard is shown in Figure 1. If the results of WSA were evaluated as an average of WSA_{mi} and WSA_{ma} there would not be any significant differences between management practices. However, if they were evaluated by individual aggregate sizes of $WSA_{ma,}$ the one-way ANOVA analysis showed significant differences between treatments, for size fractions of WSA_{ma} >5mm, 5–3 mm, 1–0.5 mm and 0.5–0.25 mm. The lowest average content of WSA_{ma} was determined in T treatment. There have been reported a negative impacts of intensive soil cultivation on the content of water-stable aggregates (Wang *et al.* 2015). Tillage was held responsible for the disruption of soil aggregates (Plante & McGill 2002). On the other hand, the highest average content of WSA_{ma} was found in the G+NPK3 treatment and then with G > T+FYM > G+NPK1. The complexity of the chemical and physical effects of fertilisers has resulted in variable effects of fertilisation on the aggregates. There have been reported negative impacts of the use mineral fertilisers on aggregate resistance (Czachor *et al.* 2015). However, the fertiliser application generally improves the soil aggregation (Haynes & Naidu 1998) due to increasing macro-aggregation and enhanced resistance to slaking (Whalen & Chang 2002).

Soil organic carbon in water-stable aggregates

Our study has shown that soil-management practices significantly influenced SOC in WSA (Table 2). The content of SOC was lower in WSA_{mi} than in WSA_{ma} across all treatments. Six *et al.* (2004) pointed out that at the lowest depletion of C content in small macro-aggregates and micro-aggregates, could be due to a better protection of C in these aggregate sizes (Rabbi *et al.* 2015). The lowest content of SOC in WSA_{mi} was found here in T and the highest in G+NPK1 treatment. There was observed an increase of the C concentration for larger size fractions of WSA_{ma}, which is in line with other studies where the linear increase of C concentration was found to be in larger aggregates size classes (Biswas *et al.* 2009). Overall, the highest average SOC content in WSA_{ma} was in T+FYM, while the lowest ones were in the T treatment. The SOC content in WSA_{ma} increased in the following order: T < G < G+NPK1 < G+NPK3 < T+FYM. Several authors have also confirmed a loss of C in the soil (Khorramdel *et al.* 2013), and in the aggregates (Abdollahi *et al.* 2014), under intensive tillage systems. This is logical because the tillage disrupts the soil and supports mineralization processes in the soils (Polidori *et al.* 2008). Application of organic and mineral fertilisers increased the average content of SOC in WSA_{ma} by 22%, 9% and 6% in T+FYM, G+NPK3 and G+NPK1, respectively, as compared to control (Table 2).

Table 3 provides the dynamics of SOC at the individual aggregate sizes of $WSA_{ma,}$ under the different soil-management practices during the whole studied period. Intensive cultivation of the soil in the T treatment significantly increased the SOC in WSA_{ma} at an average rate of 1.33, 1.18, 0.97, 1.22 and 0.78 g/kg/y across the size fractions > 5 mm, 5–3 mm, 2–1 mm, 1–0.5 mm and 0.5–0.25 mm, respectively. Expressed as percentage this is an in-

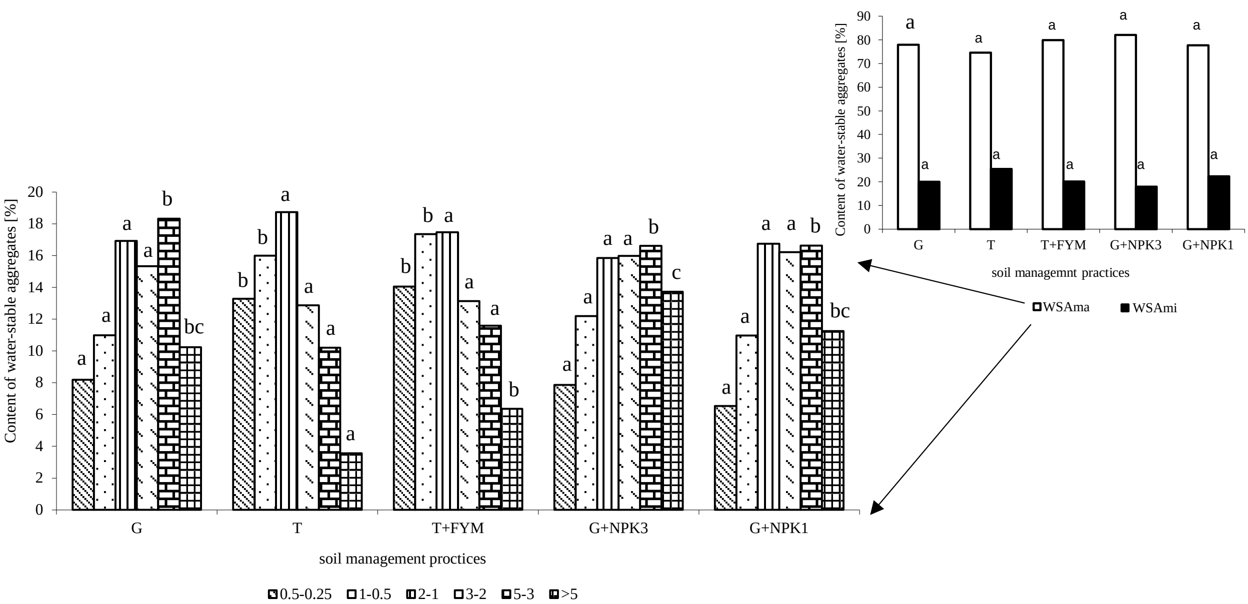


Figure 1. Statistical evaluation of water-stable aggregates contents under different soil-management practices

where: G – control; T – tillage; T+FYM – tillage+farmyard manure; G+NPK3 – doses of NPK fertilisers in 3-rd intensity for vineyards; G+NPK1 – doses of NPK fertilisers in 1st intensity for vineyards; WSA_{ma} – water-stable macro-aggregates; WSA_{mi} – water-stable micro-aggergates.
Different letters between columns (a, b, c) indicate that treatment means are significantly different at $P < 0.05$ according to *LSD* multiple-range test.

crease of 90, 82, 81, 148 and 130% of SOC in these size fractions of WSA_{ma} over the period of 8 years. As mentioned above, C in the soil is reduced due to cultivation. However, its content in the size fractions of WSA_{ma} was increased, which means that part of C after the soil disturbances is sequestered inside of the WSA_{ma}, especially in the two smallest size fractions (1–0.5 and 0.5–0.25 mm). There was not found to be any significant effect on the build-up of SOC in the individual aggregate sizes of WSA in the case of the application of NPK in the 1st and 3rd intensities of vineyard fertilisation (Table 3). Fertilisers can influence SOM in a different ways. Their use improves residue quantity and quality. But this does not necessarily increase the SOC pool. Tong *et al.* (2014) reported that the soils under NPK and NP treatments significantly increased SOC stocks. However, fertilisers may also decrease C content

T a b l e 2

Statistical evaluation of organic and labile carbon contents in size fractions of water-stable aggregates

Parameters	Size fractions of water-stable aggregates in mm		Treatments				
			G	T	T+FYM	G+NPK3	G+NPK1
SOC [g /kg]	WSA_{mi}	<0.25	12.2^{b}	10.0^{a}	12.1^{b}	12.6^{b}	12.7^{b}
	WSA_{ma}	0.25–0.5	14.0^{ab}	12.4^{a}	16.0^{b}	15.0^{ab}	15.0^{ab}
		0.5–1	16.8^{ab}	14.5^{a}	18.8^{b}	16.3^{ab}	17.2^{ab}
		1–2	16.4^{a}	15.5^{a}	19.3^{b}	17.2^{ab}	17.4^{ab}
		2–3	14.9^{ab}	14.8^{a}	17.8^{c}	17.4^{bc}	16.3^{abc}
		3–5	14.9^{a}	15.9^{ab}	19.6^{c}	17.7^{bc}	16.7^{ab}
		>5	16.9^{a}	17.5^{a}	23.3^{b}	18.9^{a}	17.1^{a}
	Mean WSA_{ma}		15.7^{a}	15.1^{a}	19.1^{c}	17.1^{b}	16.6^{b}
C_{NL} [g /kg]	WSA_{mi}	<0.25	10.6^{b}	8.37^{a}	10.3^{b}	10.6^{b}	11.0^{b}
	WSA_{ma}	0.25–0.5	12.2^{ab}	10.5^{a}	13.8^{b}	12.9^{ab}	13.0^{ab}
		0.5–1	14.8^{ab}	12.8^{a}	16.3^{b}	13.9^{ab}	14.8^{ab}
		1–2	14.5^{ab}	13.0^{a}	16.6^{b}	15.0^{ab}	15.4^{b}
		2–3	13.2^{ab}	12.8^{a}	15.5^{c}	15.1^{bc}	14.2^{abc}
		3–5	13.0^{a}	13.8	17.1^{b}	15.1^{ab}	14.7^{a}
		>5	14.9^{a}	15.0^{a}	20.1^{b}	16.2^{a}	15.1^{a}
	Mean WSA_{ma}		13.8^{a}	13.0^{a}	16.6^{c}	14.7^{b}	14.5^{ab}
C_{L} [g /kg]	WSA_{mi}	<0.25	1.57^{a}	1.67^{ab}	1.80^{ab}	2.00^{b}	1.68^{ab}
	WSA_{ma}	0.25–0.5	1.76^{a}	1.93^{ab}	2.42^{b}	2.09^{ab}	2.01^{ab}
		0.5–1	2.08^{a}	1.93^{a}	2.44^{a}	2.42^{a}	2.43^{a}
		1–2	1.91^{a}	2.43^{bc}	2.66^{c}	2.20^{ab}	1.99^{a}
		2–3	1.73^{a}	2.11^{ab}	2.32^{b}	2.23^{b}	2.03^{ab}
		3–5	1.81^{a}	2.17^{ab}	2.56^{b}	2.54^{b}	1.97^{a}
		>5	2.08^{a}	1.93^{a}	2.44^{a}	2.42^{a}	2.43^{a}
	Mean WSA_{ma}		1.90^{a}	2.08^{ab}	2.47^{b}	2.32^{b}	2.14^{ab}

where: SOC – organic carbon in water-stable aggregates; C_{NL} – non-labile carbon in water-stable aggregates; C_{L} – labile carbon in water-stable aggregates; WSA_{mi} – water-stable macro-aggregates; WSA_{mi} – water-stable micro-aggregates; G – control; T – tillage; T+FYM – tillage+farmyard manure; G+NPK3 – doses of NPK fertilisers in 3rd intensity for vineyards; G+NPK1 – doses of NPK fertilisers in 1st intensity for vineyards. Different letters between columns (a, b, c) indicate that treatment means are significantly different at $P < 0.05$ according to *LSD* multiple-range test.

Table 3

Trends of the SOC distribution in the WSA during the 2008–2015 (y = SOC content) with time (x = years)

Treatments	Equations	Probability	Trend
SOC in WSA_{ma} >5 mm			
G	y = 0.0071x + 2.5071	n.s.	increase
T	y = 1.3333x – 2664.5	++	increase
T+FYM	y = 1.2929x – 2577.3	n.s.	increase
G + NPK3	y = 0.9298x – 1851.3	n.s.	increase
G + NPK1	y = 0.506x – 1000.6	n.s.	increase
SOC in WSA_{ma} 5–3 mm			
G	y = –0.1143x + 244.74	n.s.	decrease
T	y = 1.175x – 2347.6	+	increase
T+FYM	y = 0.9774x – 1946.4	+	increase
G + NPK3	y = 0.2643x – 513.94	n.s.	increase
G + NPK1	y = –0.5917x + 1206.8	n.s.	decrease
SOC in WSA_{ma} 3–2 mm			
G	y = –0.275x + 568.1	n.s.	decrease
T	y = 0.9298x – 1855.3	n.s.	increase
T+FYM	y = 0.6762x – 1342.4	+	increase
G + NPK3	y = 0.1964x – 377.75	n.s.	increase
G + NPK1	y = –0.456x + 933.4	n.s.	decrease
SOC in WSA_{ma} 2–1 mm			
G	y = 0.0536x – 91.346	n.s.	increase
T	y = 0.9738x – 1943.4	+	increase
T+FYM	y = 0.8905x – 1771.9	n.s.	increase
G + NPK3	y = 0.0321x – 47.468	n.s.	increase
G + NPK1	y = –0.2536x + 527.47	n.s.	decrease
SOC in WSA_{ma} 1–0.5 mm			
G	y = 0.056x – 95.786	n.s.	increase
T	y = 1.2167x – 2432.8	+	increase
T+FYM	y = 0.9083x – 1808.3	n.s.	increase
G + NPK3	y = –0.2238x + 466.49	n.s.	decrease
G + NPK1	y = 0.9369x – 1867.4	n.s.	increase
SOC in WSA_{ma} 0.5–0.25 mm			
G	y = –0.5381x + 1096.4	n.s.	decrease
T	y = 0.775x – 1546.5	+	increase
T+FYM	y = 0.394x – 776.61	n.s.	increase
G + NPK3	y = –0.6095x + 1241	n.s.	decrease
G + NPK1	y = 0.3405x + 699.87	n.s.	decrease
SOC in WSA_{mi} <0.25 mm			
G	y = –0.1488x + 311.52	n.s.	decrease
T	y = 0.4155x – 825.69	n.s.	increase
T+FYM	y = 0.1488x – 287.19	n.s.	increase
G + NPK3	y = 0.3762x – 744.11	n.s.	increase
G + NPK1	y = 0.0881x – 164.55	n.s.	increase

where: G – control; FYM – farmyard manure; G + NPK3 – doses of NPK fertilisers in 3rd intensity for vineyards; G + NPK1 – doses of NPK fertilisers in 1st intensity for vineyards; SOC – soil organic carbon content; WSA_{ma} – water-stable macroaggregates, WSA_{mi} – water-stable micro aggregates. $^{++}P \leq 0.01$; $^{+}P \leq 0.05$; n.s. – non-significant.

T a b l e 4

Trends in the C_{NL} distribution in the WSA during the 2008–2015 (y = C_{NL} content) with time (x = years)

Treatments	Equations	Probability	Trend
C_{NL} in WSA_{ma} >5 mm			
G	y = 44.917x – 75412	n.s.	increase
T	y = 1085.5x – 2E+06	+	increase
T+FYM	y = 1133.5x – 2E+06	n.s.	increase
G + NPK3	y = 736.01x – 1E+06	n.s.	increase
G + NPK1	y = 489.43x – 969377	n.s.	increase
C_{NL} in WSA_{ma} 5–3 mm			
G	y = –50.5x + 114625	n.s.	decrease
T	y = 981.35x – 2E+06	+	increase
T+FYM	y = 921.99x – 2E+06	+	increase
G + NPK3	y = 225x – 437456	n.s.	increase
G + NPK1	y = –500.77x + 1E+06	n.s.	decrease
C_{NL} in WSA_{ma} 3–2 mm			
G	y = –196.46x + 408392	n.s.	decrease
T	y = 751.01x – 1E+06	n.s.	increase
T+FYM	y = 636.33x – 1E+06	++	increase
G + NPK3	y = 174.69x – 336253	n.s.	increase
G + NPK1	y = –453.45x + 926357	n.s.	decrease
C_{NL} in WSA_{ma} 2–1 mm			
G	y = 77.429x – 141240	n.s.	increase
T	y = 862.48x – 2E+06	+	increase
T+FYM	y = 739.76x – 1E+06	n.s.	increase
G + NPK3	y = 16.167x – 17530	n.s.	increase
G + NPK1	y = –203.48x + 424716	n.s.	decrease
C_{NL} in WSA_{ma} 1–0.5 mm			
G	y = 72.071x – 130292	n.s.	increase
T	y = 1199.1x – 2E+06	+	increase
T+FYM	y = 912.48x – 2E+06	n.s.	increase
G + NPK3	y = –223.36x + 463164	n.s.	decrease
G + NPK1	y = 874.26x – 2E+06	n.s.	increase
C_{NL} in WSA_{ma} 0.5–0.25 mm			
G	y = -424.3x + 865686	n.s.	decrease
T	y = 750.8x – 1E+06	+	increase
T+FYM	y = 301.56x – 592793	n.s.	increase
G + NPK3	y = –536.06x + 1E+06	n.s.	decrease
G + NPK1	y = –272.33x + 560788	n.s.	decrease
C_{NL} in WSA_{mi} <0.25 mm			
G	y = 1.5714x + 7461	n.s.	increase
T	y = 433.73x – 864069	n.s.	increase
T+FYM	y = 193x – 377881	n.s.	increase
G + NPK3	y = 443.81x – 882121	n.s.	increase
G + NPK1	y = 159.65x – 310174	n.s.	increase

where: G – control; FYM – farmyard manure; G + NPK3 – doses of NPK fertilisers in 3rd intensity for vineyards; G + NPK1 – doses of NPK fertilisers in 1st intensity for vineyards; C_{NL} – non-labile carbon content; WSA_{ma} – water-stable macroaggregates, WSA_{mi} – water-stable micro aggregates. $^{++}P \leq 0.01$; $^{+}P \leq 0.05$; n.s. – non-significant.

T a b l e 5

Trends of C_L distribution in the WSA during the 2008–2015 (y = C_L content) with time (x = years)

Treatments	Equations	Probability	Trend
C_L in WSA_{ma} >5 mm			
G	y = –37.774x + 77919	n.s.	decrease
T	y = 230.01x – 460149	n.s.	increase
T+FYM	y = 159.33x – 317307	n.s.	increase
G + NPK3	y = 193.75x – 387052	n.s.	increase
G + NPK1	y = 15.571x – 29333	n.s.	increase
C_L in WSA_{ma} 5–3 mm			
G	y = –63.744x + 130027	n.s.	decrease
T	y = 193.65x – 387369	$^{++}$	increase
T+FYM	y = 55.393x – 108862	n.s.	increase
G + NPK3	y = 39.327x – 76564	n.s.	increase
G + NPK1	y = -90.893x + 184796	n.s.	decrease
C_L in WSA_{ma} 3–2 mm			
G	y = –78.494x + 159624	n.s.	decrease
T	y = 177.32x – 354572	n.s.	increase
T+FYM	y = 39.905x – 77946	n.s.	increase
G + NPK3	y = 21.78x – 41584	n.s.	increase
G + NPK1	y = –2.5x + 7054	n.s.	decrease
C_L in WSA_{ma} 2–1 mm			
G	y = –23.815x + 49810	n.s.	decrease
T	y = 111.33x – 221516	$^{+}$	increase
T+FYM	y = 150.71x – 300501	n.s.	increase
G + NPK3	y = 15.976x – 29938	n.s.	increase
G + NPK1	y = –50.054x + 102672	n.s.	decrease
C_L in WSA_{ma} 1–0.5 mm			
G	y = –16.077x + 34423	n.s.	decrease
T	y = –7.9762x + 17974	n.s.	decrease
T+FYM	y = –4.1429x + 10769	n.s.	decrease
G + NPK3	y = –0.4107x + 3245,1	n.s.	decrease
G + NPK1	y = 62.66x – 123608	n.s.	increase
C_L in WSA_{ma} 0.5–0.25 mm			
G	y = –113.76x + 230584	n.s.	decrease
T	y = 24.202x – 46758	n.s.	increase
T+FYM	y = 111.54x – 221936	n.s.	increase
G + NPK3	y = –73.423x + 149775	n.s.	decrease
G + NPK1	y = –68.101x + 138996	n.s.	decrease
C_L in WSA_{mi}			
G	y = –150.38x + 304056	$^{+}$	decrease
T	y = –18.25x + 38377	n.s.	decrease
T+FYM	y = –44.19x + 90688	n.s.	decrease
G + NPK3	y = –67.619x + 138014	n.s.	decrease
G + NPK1	y = –71.56x + 145621	n.s.	decrease

where: G – control; FYM – farmyard manure; G + NPK3 – doses of NPK fertilisers in 3rd intensity for vineyards; G + NPK1 – doses of NPK fertilisers in 1st intensity for vineyards; C_L – labile carbon content; WSA_{ma} – water-stable macroaggregates, WSA_{mi} – water-stable micro aggregates. $^{++}P \leq 0.01$; $^{+}P \leq 0.05$; n.s. – non-significant.

as compared to unfertilised soil (Shimizu *et al.* 2009). This indicates C loss which is connected with the higher use of chemical fertilisers – especially nitrogen (Edwards *et al.* 1992; Yang 2011). On the other hand, higher nutrient contents through biomass production can increase SOC in the higher size fractions of WSA_{ma}, especially in the short-term (Šimanský & Polláková 2012). As mentioned above, overall the highest average content of SOC in WSA_{ma} was determined in the T+FYM treatment (Table 2). However, application of FYM (40 t/ha) significantly built-up SOC in WSA_{ma} at an average rate of 0.98 and 0.68 g/kg/y for only the size fractions 5–3 mm and 3–2 mm, respectively (Table 3). Expressed as percentage, this is an increase of 44, and 30% of SOC in these size fractions of WSA_{ma} over the period of 8 years. Results of our study have shown that the highest carbon sequestration after the FYM application is connected with the higher size fractions of WSA_{ma}. Similar results were found in the studies of Kundu *et al.* (2007) and Huang *et al.* (2010). The study of Tong *et al.* (2014) found there to be increased amplitude of stock and sequestration rate in C under FYM, than for mineral fertiliser. Our results confirm this findings (Table 2).

Non-labile carbon in water-stable aggregates

Soil organic-carbon pools can be divided into a labile pool and a recalcitrant fraction (Belay-Tedla *et al.* 2009). Both pools were determined in this study. The content of non-labile carbon (C_{NL}) in WSA, which represents a large sized pool and slow response to soil micro-organism activity, is shown in Table 2. The C_{NL} contents in WSA ranged from 84 to 89% (from SOC) and the effect of soil management practices on C_{NL} in WSA were significant. The C_{NL} contents reflected the contents of SOC in WSA. Overall, the highest average content of C_{NL} in WSA_{ma} was found for the T+FYM treatment, while the lowest ones were in the T treatment. The content of C_{NL} in WSA_{ma} increased on average in the following order: T < G < G+NPK1 < G+NPK3 < T+FYM. The dynamics of C_{NL} at individual aggregate sizes of WSA_{ma} showed that intensive tillage significantly increased C_{NL} in WSA_{ma}. This was especially so in the last two smallest size fractions of WSA_{ma} 1–0.5 and 0.5–0.25 mm, except for the size fraction of 3–2 mm during the whole period (Table 4). The same was found in case of WSA_{mi}. Application of FYM significantly built-up the C_{NL} in WSA_{ma} at an average rate of 0.92 and 0.64 g/kg/y across the size fractions of 5–3 mm and 3–2 mm, respectively. That means increase of 152% and 132% for C_{NL} in WSA_{ma} in 5–3 mm and 3–2 mm over the period. The values of C_{NL} after the application of mineral fertilisers (G+NPK1 a G+NPK3) had great fluctuations during the individual years (2008–2015) and therefore it was not possible to estimate the trend in the change of their values for the individual aggregate size fractions.

Labile carbon in water-stable aggregates

Tillage had no significant effect on the C_L content in WSA_{mi} and the average content of WSA_{ma} (Table 2). However, when evaluating the C_L at different aggregate sizes of WSA_{ma}, there was found to be a significant difference in the C_L content in WSA_{ma} 1–2 mm between T, G and G+NPK1 treatments. In the T treatment, the average content of C_L in WSA_{mi} and C_L in WSA_{ma} was 1.67 g/kg and 2.08 g/kg, respectively (Table 2). The trend line in Table 5 shows that the C_L increase rate in the cultivated treatment ranged from 24 mg/kg/y (WSA_{ma} 0.5–0.25 mm) to 230 mg/kg/y (WSA_{ma} >5 mm). However there was found to be a significant increase of C_L content only in the size fractions of 5–3 mm and 2–1 mm over the period.

The T+FYM treatment was found to have the highest C_L in all individual size fractions of WSA_{ma} (Table 2). The results are consistent with the findings of Purakayastha *et al.* (2008) and Abdollahi *et al.* (2014), where the application of FYM increased the labile C content. Application of FYM decreased C_L in WSA at an average rate of –4 and –44 mg/kg/y in the size fractions of 1–0.5 mm and <0.25 mm, respectively. However there was no statistical significance (Table 5). The built up of C_L in the WSA showed great fluctuations, which was caused by the timing of the FYM application. Overall, the highest C_L content was, however found in the size fraction of WSA_{ma} 2–1 mm, which is in line with the study of Abdollahi *et al.* (2014). Also Degens (1997) found higher C_L contents in the aggregate size of >1 mm. Zhang & Peng (2006) reported an increase of C_L in WSA >5 mm due to FYM application. This is an aggregation effect which creates the space for sequestration of higher C_L contents as found in our study (Table 5).

There were significant differences between the G and G+NPK3 treatments in the C_L in WSA_{mi} and average content of C_L in WSA_{ma}. Generally, the application of higher NPK doses resulted in higher contents of C_L in WSA_{ma} and in WSA_{mi}, as compared to the lower doses of mineral fertiliser (Table 2). The dynamics of the C_L in WSA were influenced by the different NPK doses in the vineyard during the 2008–2015 (Table 5). Application of NPK at the 3rd intensity of fertilisation builds up C_L in WSA at an average rate of 193, 39, 21 and 15 mg/kg/y across the size fractions of > 5 mm, 5–3 mm, 3–2 mm and 2–1 mm, respectively. Again however, there was no statistical significance. With the NPK treatment we observed a decline of C_L in WSA at an average rate of –0.4, –73 and –67 mg/kg/y across the size fractions of 1–0.5 mm, 0.5–0.25 mm and <0.25 mm, respectively, but again with no statistical significance. Our data also showed that in G+NPK1 the most intensive increase of C_L in WSA_{ma} in the size fraction of 1–0.5 mm (63 mg/kg/y). The greatest decline was in the size fraction of 5–3 mm (–91 mg/kg/y) (Table 5). Conteh *et al.* (1999) reported that C_L in the soil is related to the contents of fulvic acids, soil polysaccharides and soil microbial biomass carbon. Variable rhizodeposition, a major source of labile carbon in the soil during the year, as well as microbial activity, cause the fluctuation of C_L production in the soil. This will be influenced by fertilisation (Šimanský 2013). Hence, microbial communities of decomposers in the soil can prefer labile fractions of organic matter as a carbon source. This is positively reflected in aggregation (Shepherd *et al.* 2001) and might result in a better physical protection also of the labile C inside the aggregates (Peth *et al.* 2008).

CONCLUSION

Our study emphasizes the importance of soil-management practices in relation to carbon sequestration, mainly in water-stable aggregates, for a commercial vineyard. Water-stable aggregates are able to protect carbon. So in this regard it is necessary to pay further attention to their stability, especially in relation of intensive cultivation of vineyards. Water-stable micro-aggregates might be responsible for carbon sequestration in the intensive tilled and fertilised vineyard soils. The results of our study indicate that the application of farmyard manure increases the contents of soil organic carbon and both its labile and non-labile forms in water-stable aggregates. Significant differences in the dynamics of carbon in water-stable aggregates indicate the merit of their use as a sensitive indicator of the quality of the soil environment under the different soil management practices. This information is very important for winegrowers. Because on this basis, they can optimize soil-management practices, and avoid environmental degradation of their soils. Based on our findings, we recommend a decrease in the application of high doses of NPK in 3rd intensity of fertilisation for vineyards which means low than 125 kg/ha N, 50 kg/ha P and 185 k/ha K, as well as less intensive vineyard soils cultivation. Application of farmyard manure at rate of 40 t/ha in 3–4 yearly cycles and lower amounts of mineral fertilisers than 100 kg/ha N, 30 kg/ha P and 120 k/ha K (1st intensity of fertilisation for vineyards) would be more suitable for sustainable management with respect to carbon sequestration.

Acknowledgements. The project was supported by the Cultural and Educational Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic (No. 014SPU-4/2016).

REFERENCES

ABDOLLAHI, L. – SCHJØNNING, P. – ELMHOLT, S. – MUNKHOLM, L.J. 2014. The effects of organic matter application and intensive tillage and traffic on soil structure formation and stability. In *Soil & Tillage Research,* vol. *136*, pp. 28–37. DOI: 10.1016/S0167-1987(97)00038-X

BELAY-TEDLA, A. – ZHOU, X. – SU, B. – WAN, S. – LUO, Y. 2009. Labile, recalcitrant, and microbial carbon and nitrogen pools of a tallgrass prairie soil in the US Great Plains subjected to experimental warming and clipping. In *Soil Biology & Biochemistry,* vol. *41* no. 1, pp. 110–116. DOI: 10.1016/j.soilbio.2008.10.003

BERHE, A.A. – KLEBER M. 2013. Erosion, deposition, and the persistence of soil organic matter: mechanistic considerations and problems with terminology. In *Earth Surface Processes and Landforms*, vol. *38*, no. 8, pp. 908–912. DOI: 10.1002/esp.3408

BHATTACHARYYA, R. – VED PRAKASH KUNDU, S. – SRIVASTVA, A.K. – GUPTA, H.S. 2010. Long term effects of fertilisation on carbon and nitrogen sequestration and aggregate associated carbon and nitrogen in the Indian sub-Himalayas. In *Nutrient Cycling in Agroecosystems*, vol. *86*, no. 1, pp. 1–16. DOI: 10.1007/s10705-009-9270-y

BISWAS, A.K. – MOHANTY, M. – HATI, K.M. – MISRA, A.K. 2009. Distillery effluents effect on soil organic carbon and aggregate stability of a Vertisol in India. In *Soil & Tillage Resarch*, vol. *104*, no. 2, pp. 241–246. DOI: 10.1016/j.still.2009.02.012

CHAPLOT, V. – COOPER, M. 2015. Soil aggregate stability to predict organic carbon outputs from soils. In *Geoderma*, vol. *243–244*, pp. 205–213. DOI: 10.1016/j.geoderma.2014.12.013

CONTEH, A. – BLAIR, G.J. – LEFROY, R.D.B. – WHITBREAD, A. 1999. Labile organic carbon determined by permangante oxidation and its relationships to other measurements of soil organic carbon. In *Humic Substances in the Environment,* vol. *1*, pp. 3–15.

CZACHOR, H. – CHARYTANOWICZ, M. – GONET, S. – NIEWCZAS, J. – JOZEFACIUK, G. – LICHNER, L. 2015. Impact of long-term mineral and organic fertilizer application on the water stability, wettability and porosity of aggregates obtained from two loamy soils. In *European Journal of Soil Science*, vol. *66*, no. 3, pp. 577–588. DOI: 10.1111/ejss.12242

DEGENS, B.P. 1997. Macro-aggregation of soils by biological bonding and binding mechanisms and the factors affecting these: a review. In *Soil Research*, vol. *35*, no. 3, pp. 431–460. DOI: 10.1071/S96016

DZIADOWIEC, H. – GONET, S.S. 1999. *Przewodnik metodyczny do badań materii organicznej gleb* [Methodical guide-book for soil organic matter studies]. Prace Komisji Naukowych Polskiego Towarzystwa Gleboznawczego, N. 120, Komisja chemii gleb, Zespół Materii Organicznej Gleb, N II/16, 65 p.

EDWARDS, J.H. – WOOD, C.W. – THURLOW, D.L. – RUF, M.E. 1992. Tillage and crop rotation effects on fertility status of a hapludult soil. In *Soil Science Society of American Journal*, vol. *56*, no. 5, pp. 1577–1582. DOI: 10.2136/sssaj1992.03615995005600050040x

FECENKO, J. – LOŽEK, O. 2000. *Výživa a hnojenie poľných plodín* [Nutrition and fertilization of field crops]. Nitra : SPU, pp. 452.

GAIDA, A.M. – PRZEWLOKA, B. – GAWRYJOLEK, K. 2013. Changes in soil quality associated with tillage system applied. In *International Agrophysics*, vol. *27*, no. 2, pp. 133–141. DOI: 10.2478/v10247-012-0078-7

HAYNES, R.J. – NAIDU, R. 1998. Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: a review. In *Nutrient Cycling in Agroecosystems*, vol. *51*, no. 2, pp. 123–137. DOI: 10.1023/A:1009738307837

HUANG, S. – PENG, X. – HUANG, Q. – ZHANG, W. 2010. Soil aggregation and organic carbon fractions affected by long-term fertilization in a red soil of subtropical China. In *Geoderma*, vol. *154*, no. 3–4, pp. 364–369. DOI: 10.1016/j.geoderma.2009.11.009

IUSS Working Group WRB. 2014. *World reference base for soil resources 2014. International soil classification system for naming soils and creating legends for soil maps. Update 2015.* World Soil Resources Reports No. 106, Rome : FAO, pp. 192.

JONCZAK, J. 2014. Effect of land use on the carbon and nitrogen forms in humic horizons of Stagnic Luvisols. In *Journal of Elementology*, vol. *19*, no. 4, pp. 1037–1048. DOI: 10.5601/jelem.2014.19.3.345

KHORRAMDEL, S. – KOOCHEKI, A. – MAHALLATI, M.N. – KHORASANI, R. – GHORBANI, R. 2013. Evaluation of carbon sequestration potential in corn fields with different management systems. In *Soil & Tillage Research,* vol. *133*, pp. 25–31. DOI: 10.1016/S0167-1987(97)00038-X

KUNDU, S. – BHATTACHARYYA, R. – VED-PRAKASH GHOSH, B.N. – GUPTA, H.S. 2007. Carbon sequestration and relationship between carbon addition and storage under rainfed soybean–wheat rotation in a sandy loam soil of the Indian Himalayas. In *Soil & Tillage Research*, vol. *92*, no. 1–2, pp. 87–95. DOI: 10.1016/j.still.2006.01.009

ŁOGINOW, W. – WISNIEWSKI, W. – GONET, S.S. – CIESCINSKA, B. 1987. Fractionation of organic carbon based on susceptibility to oxidation. In *Polish Journal of Soil Science*, vol. *20*, pp. 47–52.

PAUSTIAN, K. – ANDRÉN, O. – JANZEN, H.H. – LAL, R. – SMITH, P. – TIAN, G. – TIESSEN, H. – VAN NOORDWIJK, M. – WOOMER, P.L. 1997. Agricultural soils as a sink to mitigate CO_2 emissions. In *Soil Use Management*, vol. *13*, no. 4, pp. 230–244. DOI: 10.1111/j.1475-2743.1997.tb00594.x

PETH, S. – HORN, R. – BECKMANN, F. – DONATH, T. – FISCHER, J. – SMUCKER, A.J.M. 2008. Three-dimensional quantification of intra-aggregate pore-space features using synchrotron-radiation-based microtomography. In *Soil Science Society of American Journal,* vol. *72*, pp. 897–907. DOI: 10.2136/sssaj2007.0130

PLANTE, A.F. – MCGILL, W.B. 2002. Soil aggregate dynamics and the retention of organic matter in laboratory-incubated soil with differing simulated tillage frequencies. In *Soil & Tillage Research*, vol. *66*, no. 1, pp. 79–92. DOI: 10.1016/S0167-1987(02)00015-6

POLIDORI, A. – TURPIN, B.J. – DAVIDSON, C.I. – RODENBURG, L.A. – MAIMONE, F. 2008. Organic PM2.5: fractionation by polarity, FTIR spectroscopy, and OM/OC ratio for the Pittsburgh aerosol. In *Aerosol Science and Techology*, vol. *42*, no. 3, pp. 233–246. DOI: 10.1080/02786820801958767

PURAKAYASTHA, T.J. – RUDRAPPA, L. – SINGH, D. – SWARUP, A. – BHADRARAY, S. 2008. Long-term impact of fertilisers on soil organic carbon pools and sequestration rates in maize–wheat–cowpea cropping system. In *Geoderma*, vol. *144*, no. 1–2, pp. 370–378. DOI: 10.1016/j.geoderma.2007.12.006

RABBI, S.M.F. – WILSON, B.R. – LOCKWOOD, P.V. – DANIEL, H. – YOUNG I.M. 2015. Aggregate hierarchy and carbon mineralization in two Oxisols of New South Wales, Australia. In *Soil & Tillage Research,* vol. *146*, pp. 193–203. DOI: 10.1016/j.still.2014.10.008

SHEPHERD, T.G. – SAGGAR, S. – NEWMAN, R.H. – ROSS, C.W. – DANDO, J.L. 2001. Tillage-induced changes to soil structure and organic carbon fraction in New Zealand soils. In *Australian Journal of Soil Research*, vol. *39*, pp. 465–489.

SHIMIZU, M.M. – MARUTANI, S. – DESYATKIN, A.R. – JIN, T.J. – HATA, H. – HATANO, R. 2009. The effect of manure application on carbon dynamics and budgets in a managed grassland of Southern Hokkaido, Japan. In *Agriculture, Ecosysems and Environment*, vol. *130*, no. 1–2, pp. 31–40. DOI: 10.1016/j.agee.2008.11.013

ŠIMANSKÝ, V. 2013. Soil organic matter in water-stable aggregates under different soil management practices in a productive vineyard. Archives of Agronomy and Soil Science, vol. *59*, no. *9*, pp. 1207–1214. DOI: 10.1080/03650340.2012.708103

ŠIMANSKÝ, V. – BAJČAN, D. 2014. The stability of soil aggregates and their ability of carbon sequestration. In *Soil & Water Research*, vol. *9*, no. 3, pp. 111–118.

ŠIMANSKÝ, V. – POLLÁKOVÁ, N. 2012. Use of “progressive” soil organic matter parameters for studying of its changes in aggregates in a vineyard. In *Acta fytotechnica et zootechnica*, vol. *15*, no. 4, pp. 109–112.

ŠIMANSKÝ, V. – TOBIAŠOVÁ, E. – CHLPÍK, J. 2008. Soil tillage and fertilization of Orthic Luvisol and their influence on chemical properties, soil structure stability and carbon distribution in water-stable macro-aggregates. In *Soil & Tillage Research*, vol. *100*, no. 1–2, pp. 125–132. DOI: 10.1016/j.still.2008.05.008

SIX, J. – BOSSUYT, H. – DEGRYZE, S. – DENEF, K. 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. In *Soil and Tillage Research,* vol. *79*, pp. 7–31. DOI: 10.1016/j.still.2004.03.008

TONG, X. – XU, M. – WANG, X. – BHATTACHARYYA, R. – ZHANG, W. – CONG, R. 2014. Long-term fertilisation effects on organic carbon fractions in a red soil of China. In *Catena,* vol. *113*, pp. 251–259. DOI: 10.1016/j.catena.2013.08.005

TRIBERTI, L. – NASTRI, A. – GIORDANI, G. – COMELLINI, F. – BALDONI, G. – TODERI, G. 2008. Can mineral and organic fertilisation help sequestrate carbon dioxide in cropland? In *European Journal of Agronomy*, vol. *29*, no. 1, pp. 13–20. DOI: 10.1016/j.eja.2008.01.009

VADJUNINA, A.F. – KORCHAGINA, Z.A. 1986. *Methods of study of soil physical properties.* Moscow : Agropromizdat, 415 p.

WANG, Y. – ZHANG, J.H. – ZHANG, Z.H. 2015. Influences of intensive tillage on water-stable aggregate distribution on a steep hillslope. In *Soil & Tillage Research*, vol. *151*, pp. 82–92. DOI: 10.1016/j.still.2015.03.003

WHALEN, J.K. – CHANG, C. 2002. Macroaggregate characteristics in cultivated soils after 25 annual manure applications. In *Soil Science Society of American Journal*, vol. *66*, no. 5, pp. 1637–1647. DOI: 10.2136/sssaj2002.1637

YANG, X.Y. – LI, P.R. – ZHANG, S.L. – SUN, B.H. – CHEN, X.P. 2011. Long-term-fertilisation effects on soil organic carbon, physical properties, and wheat yield of a loess soil. In *Journal of Plant Nutrition and Soil Science, 174*, pp. 775–784. DOI: 10.1002/jpln.201000134.

ZHANG, B. – PENG, X.H. 2006. Organic matter enrichment and aggregate stabilization in a severely degraded Ultisol after reforestation. In *Pedosphere*, vol. *16*, no. 6, pp. 699–706. DOI: 10.1016/S1002-0160(06)60105-7

Received: August 11, 2017

DE GRUYTER OPEN

Agriculture (Poľnohospodárstvo), 63, 2017 (4): 163−175

Original paper DOI: 10.1515/agri-2017-0016

UPTAKE AND TRANSLOCATION OF SOME HEAVY METALS BY RICE CROP (*ORYZA SATIVA*) IN PADDY SOILS

GHASEM RAHIMI*, ZAHRA KOLAHCHI, AMIN CHARKHABI

College of Agriculture, Bu-Ali Sina University, Hamedan, Iran

RAHIMI, G. – KOLAHCHI, Z. – CHARKHABI, A.: Uptake and translocation of some heavy metals by rice crop (*Oryza sativa*) in paddy soils. Agriculture (Poľnohospodárstvo), vol. *63*, 2017, no. 4, pp. 163–175.

Accumulation of heavy metals in edible crops is amongst major international concerns today. While consuming Lenjan variety of rice is very popular in Iran, limited evidence exists on its safety. Amid increasing public concern about the safety of locally grown and imported rice in the market, a field study was carried out to investigate uptake and translocation of Cd, Pb, Ni, and Zn by a local variety of rice crop (*Oryza sativa*) exposed to contaminated water. At harvest time and in paddy fields, 41 soil and plant samples were collected from four locations of Lenjan, central Iran; irrigated from Zayandeh Rood River. In the laboratory, different parts of the plant were milled, digested via acid digestion method, and then analysed for Cd, Pb, Ni and Zn using atomic absorption spectrophotometry. The results showed that average concentrations of Cd, Pb, Ni and Zn were 1.07, 17.22, 1.73 and 13.75 mg/kg in the plant's stem; and 1.27, 12.32, 1.099 and 19.39 mg/kg in its grain, respectively. In general, both in the plant's stem and grain, the Cd and Pb concentrations were much higher than the FAO/WHO standard and labelled as harmful for consumers. Moreover, among the studied heavy metals, Ni transported very weakly, while Cd and Zn conveyed most easily into the plant's stem and grain. Of course, Pb was the least mobile metal. However, it had highly accumulated in the plant's stem and grain.

Key words: heavy metal, rice plant, translocation factor, paddy soils, paddy crop, *Oryza sativa*

1. INTRODUCTION

Agricultural soil and water contamination has become a severe environmental problem in many developed and developing countries in recent years (Facchinelli *et al*. 2001; Kalavrouziotis *et al*. 2012; Fan *et al*. 2017). Heavy metals, one of such toxic contaminants, are not bio- and thermo-degradable, hence accumulate in the environment up to hazardous levels (Chung *et al*. 2011). Two primary sources of heavy metals in the soil are: (i) the natural background -i.e. metals derived from parent rocks; and (ii) the anthropogenic contamination -i.e. those originated from human activities (Fu *et al*. 2008; Zhao *et al*. 2010). Most of the soil metals today have originated from anthropogenic sources than natural ones (Moura *et al*. 2010). Meanwhile, irrigation of agricultural soil with polluted municipal and industrial wastewater is another vital source of pollution (Mahmoud & Ghoneim 2016).

The soil-to-plant transfer of heavy metals is a process of significant importance (Kalavrouziotis *et al.* 2012). In plants, heavy metals might cause oxidative stress, displace essential metals, disrupt metabolic processes from functioning, and finally reduce yield (Wang-da *et al*. 2006). Furthermore, chronic low-level intake of heavy metals can pose an irreversible detrimental effect on human health

Rahimi Ghasem, Associate Professor (*Corresponding author), Department of Soil Science, College of Agriculture, Bu-Ali Sina University, Hamedan, Iran. E-mail: g.rahimi@basu.ac.ir
Kolahchi Zahra, Assisstant Professor, Department of Soil Science, College of Agriculture, Bu Ali Sina University, Hamedan, Iran. E-mail: zkolahchi@basu.ac.ir
Charkhabi Amin, Postgraduate Student, Department of Soil Science, College of Agriculture, Bu Ali Sina University, Hamedan, Iran. E-mail: amin_charkhabi@yahoo.com

(Rattan *et al*. 2005; Rodriguez Martin *et al*. 2006; Hang *et al*. 2009; Yang *et al.* 2009; Wu & Zhang 2010). Among agricultural products, rice is widely consumed as a staple food worldwide and especially in Asian countries. So, in case of contamination, it could become a significant dietary source of toxic elements compared to other crops (Park *et al*. 2011; Tariq & Rashid 2013). Therefore, its quality can profoundly affect human health. That is why heavy metals contamination in agricultural soils and their transfer into plants have been of increasing concern (Zhao *et al*. 2010).

Rice as a herbal plant contains protein and is ranked as the second highly consumed cereal in the world. Half of the world populations consume rice as their staple food (Rabbani *et al*. 2015). Some researchers showed a gradual increase of some heavy metals such as cadmium in Iranian rice and mentioned the situation as posing a significant threat (Zazoli *et al*. 2006).

The metal transfer factor (TF) is an indicator of heavy metal accumulation in plants. It quantifies the differences in the bioavailability of metal to plant. In other words, TF is an indicator of heavy metal mobility in the soil. Meanwhile, it is considered a critical parameter regarding the accumulation of heavy metals in plants. Of course, factors that contribute to the increase of heavy metal concentration in soil could have an impact on it, namely sludge application or wastewater reuse (Kalavrouziotis *et al*. 2012).

In recent years, researchers have focused their attention on the significance of soil types and genotype, and their impact on the uptake and accumulation of heavy metals in potted experiments (Chung *et al*. 2011; Lai *et al*. 2012). However, the pot experiments may not be able to predict uptake of heavy metals by a crop under actual field conditions. Accordingly, it is crucial to study heavy metals accumulation in plants' natural environment. Multiple metals' pollution of agricultural soils is turned into a common phenomenon, regarding human activities. It is believed that interactions of such different elements create a different toxic effect on an ecosystem compared to that of single pollutant (Liu *et al*. 2007). Therefore, in the present research, we performed a comprehensive study of toxic heavy metals in rice plants and their agricultural soils under natural conditions from renowned Lenjan paddy fields of central Iran.

2. MATERIAL AND METHODS

2.1 *Study area*

The area selected for present study is located in Lenjan, southwest Isfahan Province, central Iran; with a semi-arid climate, and an average annual temperature and rainfall of 15.7ºC and 157.7 mm, respectively (Iran Meteorological Organization 2017). Many active industrial units were placed adjacent to the study area. The growing period of rice plant (*Oryza sativa*) was about 155 days (May– October) in the Lenjan Region. Traditionally, rice is broadly cultivated in the area, with a great yield annually. However, most of the produced rice is consumed by residents. According to the local records, large amounts of chemical fertilisers, pesticides, and manures have been being applied to above-mentioned paddy fields over a long period. Surrounding industrial activities and urbanisation could have also affected the soil environment.

2.2 *Sampling*

Out of the greatest sources of pollution in the region, samples were collected from 41 sites in 2014. Researchers divided them into four districts, namely: 1-*Zarrinshahr*: an industrial and municipal sewage and river irrigation region, 2-*Sede*: a river and municipal sewage irrigation region, 3-*Chamgordan*: an industrial and municipal sewage irrigation region, and 4-*Varnamkhast*: a municipal sewage irrigation region. All collected samples were stored in polyethylene bags and brought to the soil science laboratory of Bu Ali Sina University, Iran for further preparation and treatment.

2.2.1 Soil sampling

Soil samples were collected from the fields' 0–20 cm soil layer. At first, they were air-dried at room temperature, then finely powdered, homogenised, and grinded to pass through a 2-mm nylon sieve. Later the samples were analysed for some of their physical and chemical properties. The pH (at the ratio of 1:5 soil-distilled water), EC (at the ratio of 1:5 soil-distilled water), calcium carbonate equivalent, soil texture, and soil organic matter plus cation exchange capacity were identified according to Thomas (1996), Sims (1996), Bauycos (1962), and Rowell (1994) guidelines; respectively. Total and available concentrations of heavy metals were measured by the method of Sposito *et al*. (1982) and Lindsay and Norvell (1978).

2.2.2 Water sampling

Water samples were collected from 2 sites (The Zayandeh Rood river and municipal wastewater). They were preserved in 1-L polypropylene sampling bottles at 4°C in darkness and analysed within 48 h (Hai *et al*. 2009).

2.2.3 Plant sampling

The plants' samples were taken in their maturity from approximately very same locations where the soils were sampled and simultaneously for further studies on the heavy metals mobility and bioavailability. In the laboratory, sampled rice plants were separated into their different parts such as roots, stems and grains; Then, they were washed three times with distilled water, rinsed with deionised water, and finally dried in an oven at 65°C. Later, the samples' dry weights were determined, and afterwards, the plant parts grinded with a tissue grinder (Liu *et al*. 2007). Samples digested with 8 mL of 70% HNO_3, then cooling to room temperature, were filtered through a 0.45-μm membrane filter, and adjusted to a final volume of 25 ml (Park *et al*. 2011). At last, some metal concentrations including (Cd, Pb, Ni and Zn), in three parts of the plant comprising (roots, stems and seeds) were determined using atomic absorption spectrophotometry.

2.3 *Bio-accumulation factor*

The bio-accumulation factor (BAF) is defined as the ratio of an element's concentration in plant's grain to that element's concentration in the corresponding soil. BAF was calculated for each plant sample to quantify the plant's bio-accumulation effect, up-taking heavy metals from the soils (Hang *et al*. 2009). The BAF was computed with the following formulae (eq. 1):

$$BAF = \frac{Cr}{Cs} \qquad \text{eq. 1,}$$

where Cr and Cs represented the heavy metals'

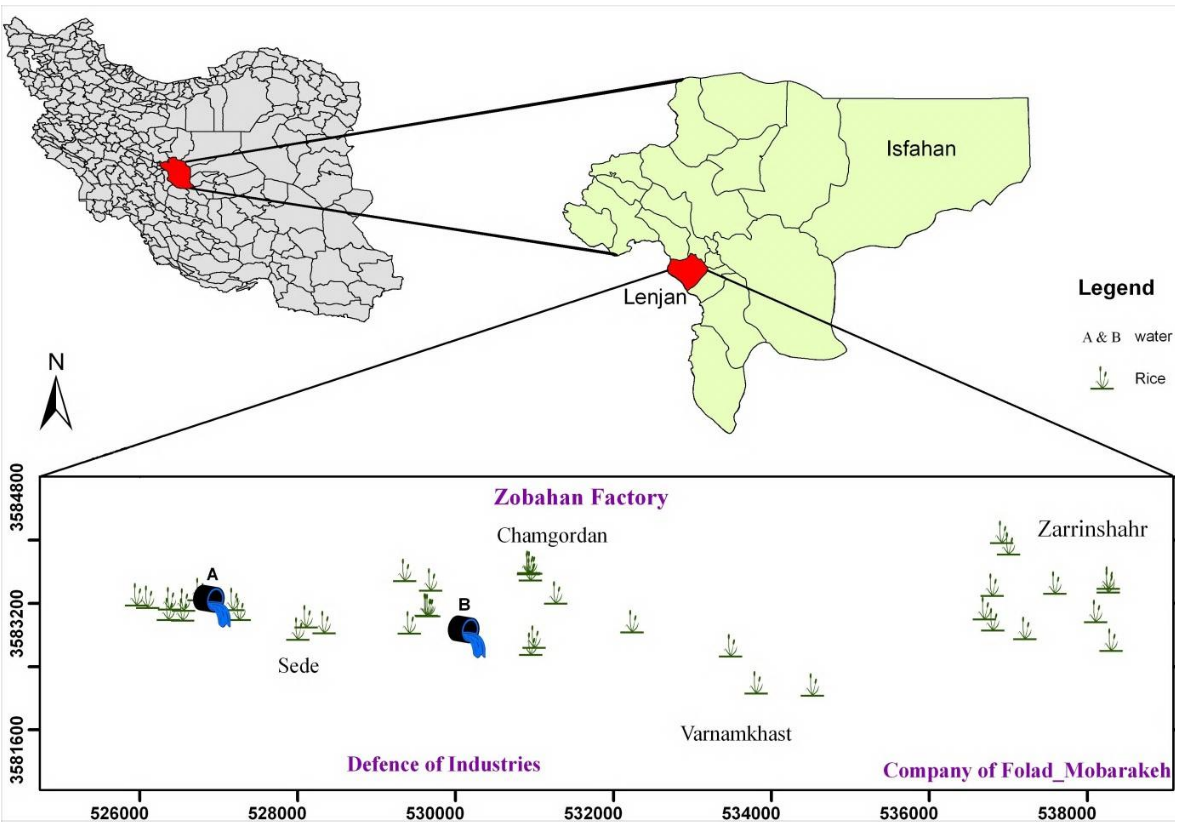


Figure 1. Map of the study area and four sampling locations

concentrations in the grain and soils extracts; respectively, on a dry weight basis (Hang *et al*. 2009; Singh *et al*. 2011).

2.4 *Transfer of heavy metals from the soils to the rice plants*

Concentrations of heavy metals in rice plant vary depending on total metal concentrations in their paddy soils (Jung & Thornton 1997). Therefore, the transfer factor (TF) of the heavy metals were calculated by dividing the concentration of every metal in the plant over its total concentration in the soil. Higher TFs reflect relatively poor retention in soil or greater efficiency of the plant to absorb metal; while lower TFs indicate strong sorption of metal to the soil colloid (Zhen *et al*. 2009).

Moreover, the translocation factor is calculated as the ratio of metal concentration in aerial parts of any plant over that metal's concentration in the plant root. In other words, TF = (C_{aerial} / C_{root}), where, C_{aerial} is the metal concentration in plant's aerial part, and C_{root} is that metal concentration in the plant's root (Tiwari *et al*. 2011; Singh *et al*. 2011).

3. RESULTS AND DISCUSSION

3.1 *Physico-chemical parameters of the soil*

As shown in Table 1, the main physicochemical parameters determined for topsoil's from the study area were as the followings: (i) The OM contents were within the range of 0.76–4.14%, (ii) values of pH fell in a narrow range (7.02–8.24), indicating sub-alkaline conditions for all the sampled topsoils; (iii) values of CEC showed high variation (10.1–47.56 cmol/kg) with a mean value of 20.81 cmol/kg. (IV) The $CaCO_3$ content ranged from 17.91 to 36.83; (V) the electrical conductivity ranged from 0.152–3.266 dS/m. Also, the contents of clay, silt and sand varied between 16–31, 16–48 and 27–57 percentages, respectively.

3.2 *Soil contamination*

Total and available heavy metal concentrations in the soils are presented in Table 2. Total and available concentrations of Cd and Pb, measured in the soils exceeded those in arable soils not subject to gross anthropogenic pollution, reported by Bi *et al*. (2010) that ranged between 0.1–2 mg/kg and 20–50 mg/kg; respectively.

The maximum allowable concentration of Ni in agricultural soils was proposed to be 50 mg/kg (Kabata Pendias 2010). However, in the present study, the mean concentration of Ni, obtained from all the sampled regions, was higher than what considered safe for agricultural soils.

The threshold of Zn is defined at 60 mg/kg in the topsoil (Sposito 1989; Manata & Angelone 2002; Kabata Pendias 2010). In comparison with the above standard, in approximately 50% of the samples, the Zn concentrations were higher than the standard, emphasising on anthropogenic sources of the contaminant which might be due to irrigating the rice crops with industrial and municipal wastewater. Tiwari *et al*. (2011) found that agricultural soils which repeatedly irrigated with industrial effluent were contaminated with Pb, Cd, Cu, Fe, Mn, Ni and Zn.

The DTPA extracted-metal contents, which are commonly recognised as available for uptake by plants, also differed among the soils. Among the four heavy metals in the study, Zn showed the most substantial relative difference, presented as the ratio of the maximum/minimum concentration, which was

T a b l e 1

Some soil properties in the study area

	$CaCO_3$ [%]	pH	EC [dS/m]	CEC [cmol/kg]	OM [%]	Clay [%]	Silt [%]	Sand [%]
Min	17.91	7.02	0.152	10.10	0.76	16.00	16.00	27.00
Max	36.83	8.24	3.266	47.56	4.14	31.00	48.00	57.00
Mean	24.80	7.68	0.487	20.81	2.49	23.28	33.43	43.27

1.51 Pb ranked the second with a relative difference of 1.28 among the locations. Meanwhile, the other two elements had smaller differences, -i.e. 1.21 and 1.14 for the Cd and Ni, respectively. It could be due to different levels of contamination.

3.3 *Chemical properties and metal concentrations of the water samples*

The mean values for chemical properties and heavy metal concentrations -i.e. Cd, Pb, Ni and Zn, in the sampled water from a local river and municipal wastewater are shown in Table 3. The pH of river water samples ranged from 8.04 to 8.31; while those of sampled municipal wastewater varied between 7.27 to 7.38 in the study area. The electrical conductivities of the wastewater samples were higher perhaps due to concentrated salts previously reported in municipal wastewater (Kiziloglu *et al.* 2008). According to the Table 3, Cd, Pb and Zn concentrations in river water and municipal wastewater, commonly utilised for irrigation, were below the standard. Nevertheless, Ni concentrations in both groups of sampled water exceeded the pollution standards. The higher concentration of Cd, Pb and Zn in the river, compared to municipal wastewater, can be attributed to the later discharge of industrial pollutants into the river. Of course, depending on the type of industrial activity, discharged waste into the water resources may contain different heavy metals. Continued use of such polluted water would lead to accumulation of heavy metals in soil and plant (Singh *et al*. 2010; Mahmoud & Ghoneim 2016).

3.4 *Tracing the elements' concentrations in rice plant's parts*

3.4.1 Cd

The range and mean concentrations of Cd, Pb, Ni and Zn in various parts of the plants cultivated

T a b l e 2

Concentrations [mg/kg] of total and available heavy metal in the soils

Elements	*Zarrinshahr*	*Sede*	*Varnamkhast*	*Chamgordan*	aggregate	MAC*
Cd-Total	1.320	2.360	0.628	1.051	1.700	0.1–2[a]
Cd-DTPA-extractable	0.067	0.078	0.076	0.064	0.073	–
Pb-Total	48.700	44.600	80.230	71.010	53.540	20–50[a]
Pb-DTPA-extractable	3.140	3.126	2.520	2.440	2.956	–
Ni-Total	63.920	54.710	55.410	52.920	52.460	50[b]
Ni-DTPA-extractable	2.270	2.195	2.170	1.980	2.177	–
Zn-Total	95.436	63.032	51.755	50.352	66.648	60[c]
Zn-DTPA-extractable	8.065	7.061	5.410	7.317	7.129	–

*: maximum allowable concentrations (MAC)
a: Bi *et al*. (2010)
b: Kabata Pendias (2010)
c: Sposito (1989); Kabata Pendias (2010); Manata and Angelone (2002)

T a b l e 3

Some chemical properties and heavy metals' concentration in the sampled river and municipal wastewater

	pH	EC	Cd	Pb	Ni	Zn
Zayandeh Rood River	8.3	0.276	0.005	0.14	0.481	0.150
Municipal Wastewater	7.3	0.820	0.002	0.05	0.507	0.033
MAC[a]	–	–	0.020	5.00	0.200	2.000
MAC[b]	–	–	0.010	–	–	5.000

MAC[a]: maximum allowable concentrations for irrigation purposes, Ayers and Westcott (1985)
MAC[b]: maximum allowable concentrations for irrigation purposes, Montgomery (1985)

on paddy soils from four different areas of Lenjan, Iran are presented in Table 4. The mean value of Cd concentration in roots, stems and seeds of rice from different regions were 1.57, 1.07 and 1.27 mg/kg on dry weight basis, respectively. Fakoor Janati *et al*. (2011) studying 100 samples of rice purchased from some supermarkets in Iran, discovered 21 ng/g as the maximum content of Cd in rice. Zazoli *et al*. (2006) found that mean Cd concentration in Tarom rice (another variety of Iranian rice) was 0.41±0.17 mg/kg on dry weight basis. The allowable amount of Cd in plants (as shown in Table 5) has been reported to be 0.01–0.3 mg/kg dry weight (Alloway 1968). Moreover, the healthy food standard of Cd in rice, set by FAO/WHO codex (1984), was 0.3 mg/kg dry weight. Therefore, the average content of Cd in stems and grains of the sampled rice were 3.5 and 4.2 times more than the permissible limit, respectively. Findings revealed that amounts of Cd in all samples were above 0.3 mg/kg level. Also, Table 4 shows that concentrations of Cd in the plant's grains were higher than its stems. It was consistent with evidence that proved Cd possessed high mobility and easy absorption properties in plants, and could be easily absorbed by plants' root and skin, and then enter its tissues (Kabata Pendias 2010). With no proven beneficial effects on plants and animals; Cd proved to be toxic to plants, by reducing their photosynthesis, and water/nutrients uptake (Singh *et al*. 2011). It confirmed findings by Casado *et al*. (2008) that showed high Cd concentration in grasses induced by polluted soils.

The highest amount of Cd, in different parts of the plant, was seen in the *Sede* region. However, ANOVA analysis revealed that there wasn't a significant difference ($P < 0.05$) in Cd contents of the rice plant from the four different regions (Table 4).

Results from previous studies (Khan *et al*. 2008) demonstrated that plants grown on wastewater-irrigated soils contaminated with heavy metals could pose a significant health risk to humans. Opposite to current study, Liu *et al*. (2007) stated that amounts of Cd in rice grains irrigated with municipal wastewater, river, and ground-water were 0.016, 0.038 and 0.0079 mg/kg; respectively.

3.4.2 Pb

Among the four regions, the highest, but not

T a b l e 4

Concentrations of heavy metals in various parts of the rice plants [mg/kg dw]

	Zarrinshahr (n= 11)		*Sede* (n= 21)		*Chamgordan* (n= 5)		*Varnamkhast* (n= 4)		Total (n= 41)
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Mean
Cd									
Root	1.20–1.75	1.58	1.05–2.15	1.60	1.20–1.75	1.41	1.35–1.80	1.56	1.57
Stem	0.75–1.15	0.92	0.50–5.45	1.20	0.80–1.10	0.92	0.80–1.50	1.05	1.07
Grain	1.02–1.60	1.27	1.05–2.05	1.28	1.25–1.40	1.32	1.10–1.25	1.18	1.27
Pb									
Root	22.5–42.5	29.34	24.0–34.0	27.82	25.50–28.00	26.30	23.0–30.0	26.37	27.90
Stem	11.0–28.0	18.72	9.5–24.0	16.35	11.33–21.50	18.23	11.5–23.5	16.37	17.22
Grain	11.0–15.3	12.65	10.2–14.2	12.36	11.00–12.75	11.70	24.5–28.5	11.94	12.32
Ni									
Root	5.85–18.7	11.80	2.7–17.05	9.98	5.5–16.2	11.35	7.15–15.20	11.20	10.75
Stem	1.52–2.85	2.13	0.5–2.60	1.50	1.4–2.1	1.78	1.13–3.06	1.75	1.73
Grain	0.10–2.50	1.19	0.2–3.00	1.07	0.1–2.3	1.10	0.45–1.50	0.95	1.099
Zn									
Root	19.60–56.3	29.27	12.6–50.3	23.83	9.24–33.70	23.48	13.60–37.40	28.57	25.71
Stem	6.13–36.2	16.20	0.23–37.9	12.70	4.00–19.31	11.77	6.30–25.39	14.96	13.75
Grain	16.20–33.6	24.26	5.08–35.0	18.75	8.25–17.30	14.17	8.02–24.50	15.84	19.39

significant, concentration of Pb in the roots, stems and seeds of the plant were seen in *Zarrinshahr* as shown in Table 4. While FAO (1984) recommended 5 mg/kg as the safe limit of Pb uptake concentration for plants, Alloway (1968) and Kabata Pendias (2010) reported 3 mg/kg as the tolerable limit of Pb for upper parts of the plants. Meanwhile, Hang *et al*. (2009) introduced the 0.2 mg/kg as the Pb limit. Comparatively studying, Pb concentrations in the studied rice plants were much higher than the standard limit. Jahed Khaniki and Zazoli (2005) also found higher than the FAO/WHO guidelines' Pb content in the rice plants from northern Iran.

While Pb may easily be absorbed by plant roots and stems, only a small proportion of it moves into the aerial parts of the plants (Pais & Jones 1997; Agarval 2002). In general, it is less mobile in the rice plants (Liu *et al*. 2007). Moreover, no significant correlations were observed between Pb concentrations in the sampled soils and aerial parts of the plant in the study (Table 6). Fu *et al*. (2008), in their study regarding heavy metal pollution in the rice crop, obtained a low correlation between soil and high levels of heavy metals in rice grains representing atmospheric deposition as a significant potential source of metals contamination of rice grains. Therefore, it seems to us that in the present study, an external factor such as atmospheric deposition could be blamed for Pb accumulation in aerial parts of the studied rice plants.

Anthropogenic entry and accumulation of Pb in the human food chain initiated from cultivated crops (Sillanppa & Jansson 1992; Pais & Jones 1997). Hang *et al*. (2009) in a risk assessment of contaminants in soil and rice from Changshu City, China discovered that concentrations of some heavy metals such as Pb in the plant could be related to substantial industrial activities in the region.

3.4.3 Ni

Ni concentrations in the rice grains were less than other parts of studied plans in paddy fields from various regions. The maximum concentrations of Ni in the roots, stems and seeds of the plant were found in *Zarrinshahr*, again. Not a substantial difference was revealed among the regions (Table 4). The Ni content in the plant roots was higher than other plant parts, and the Standard of China (2005). In the present study, Ni concentrations in sampled waters from the Zayandeh Rood river and municipal wastewater had reached the critical level (Table 3). It could play

T a b l e 5

Maximum levels of contaminants in plant tissues quoted from various sources

Reference	Cd	Ni	Zn	Pb
FAO and WHO (1984)	0.3	20	60	5
SEPA (2005)*	0.2	10	100	9
Doberman and Fairhurst (2000)	–	–	20[a], 40[b]	–
Alloway (1968)	0.3	–	100	3
Hang *et al*. (2009)**	0.2		50	0.2

*: State Environmental Protection Administration, China
**: Maximum levels of contaminants in foods
a and b: Maximum levels of Zn in grain and stem, respectively, Doberman and Fairhurst (2000)

T a b l e 6

Pearson correlation coefficients between Cd, Pb, Ni and Zn concentrations in soils and the plant parts

Parts of the plant	Cd	Pb	Zn	Ni
Root	0.322*	–0.134	0.316*	0.353*
Stem	0.080	0.020	0.199	0.190
Grain	0.030	–0.220	0.080	0.240

*significant difference at $p < 0.05$ by the Duncan test a significant role in the consequent contamination of local soil and plants. Rattan *et al*. (2005) by investigating the impact of municipal effluent on the amount of Ni in cereals, illustrated that Ni uptake was dependent on its concentration in irrigation water.

3.4.4 Zn

The average Zn concentration in the stems was less than that of the roots and grains of the paddy crop across various regions. The maximum allowable concentration of zinc in plants varies in different sources. FAO and WHO (1984) reported the normal concentration of Zn in plants to be 60 mg/kg dry weight; whereas, Hang *et al*. (2009), SEPA (2005) and Alloway (1968) proposed 100 mg/kg as the permissible limit (Table 5). According to the proposed limits, the Zn contents in the stems and grains of the plant stood within the normal range. Of course, the Zn concentration in the rice roots cultivated in *Zarrinshahr* region was higher than expected, and exceeded the FAO (1984) standards.

Zarrinshahr had the highest total and available concentration of Zn in soils, as well (Table 2). Releasing industrial and municipal wastewater into water resources could have led to increasing concentrations of Zn in the soil and plants of the study area. Abbas *et al*. (2007) in a study on the effects of contaminated water of Nullah Dek on rice paddy saw elements accumulation in different parts of rice plant and soil. Moreover, they delineated that concentrations of all trace elements including (Zn, Cu, Fe and Mn) were increased by applying the Nullah Dek water which was already contaminated with industrial effluents carrying different micronutrients.

3.5 *Translocation of metals from soil into the rice plants*

3.5.1 Bio-accumulation factor

Figure 2 shows the bio-accumulation factors (BAF) calculated for heavy metal transfer from soils to the rice grain. The BAFs for heavy metals across studied regions in a descending order were as the following: Cd (*Varnamkhast* > *Zarrinshahr* > *Chamgordan* > *Sede*, with a significant difference in TF values among different regions), Ni (*Chamgordan* > *Zarrinshahr* > *Sede* > *Varnamkhast*, with no significant difference in TF values among different regions), Pb (*Sede* > *Zarrinshahr* > *Varnamkhast* > *Chamgordan*, with a significant difference in TF values among different regions), and Zn (*Varnam-*

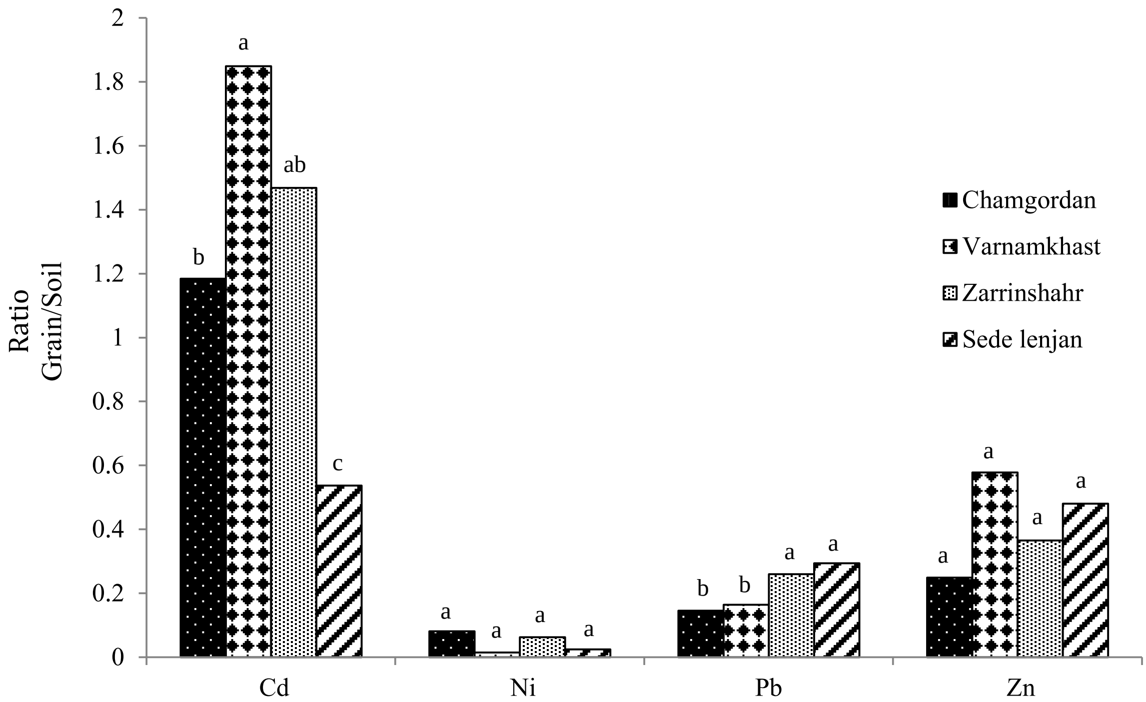


Figure 2. BAFs of the metals in the study area

khast > *Sede* > *Zarrinshahr* > *Chamgordan*, with no significant difference in TF values among different regions).

Principally, the food chain (soil–plant–human) is recognised as the major pathway for human exposure to soil contamination. The soil-to-plant transfer is one of the key components of human exposure to metals through the food chain. When BCF < 1 or BAF = 1, this denotes that the plant only absorbs but do not accumulate heavy metals; when BCF > 1,

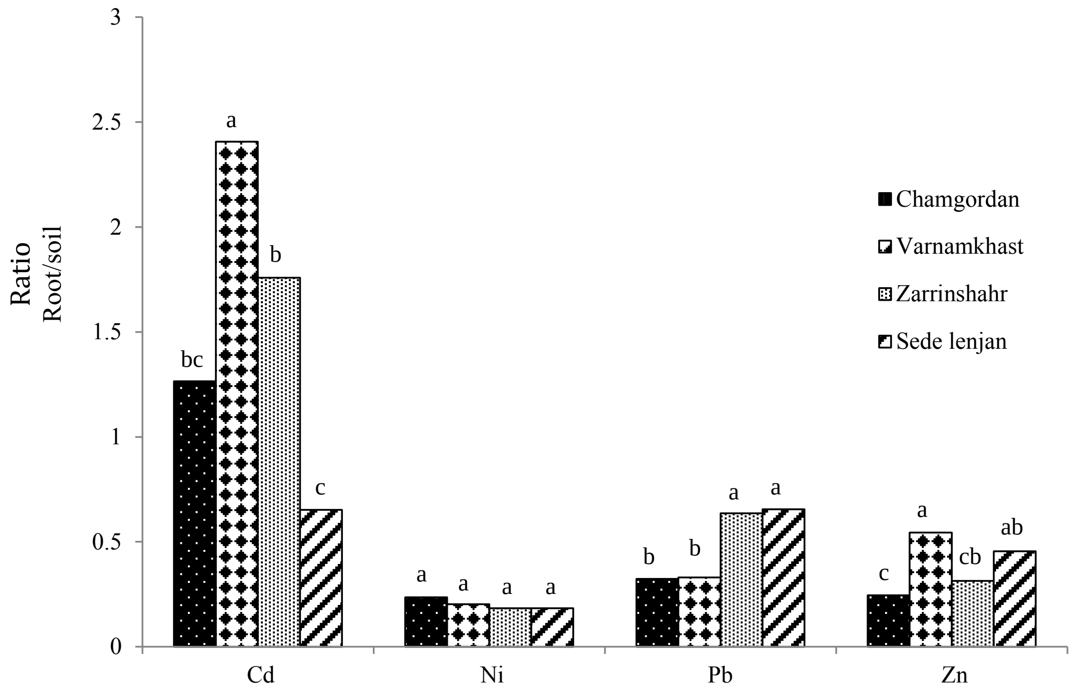


Figure 3. Metal transfer factor from soil to roots of rice (same letters indicate no significant difference)

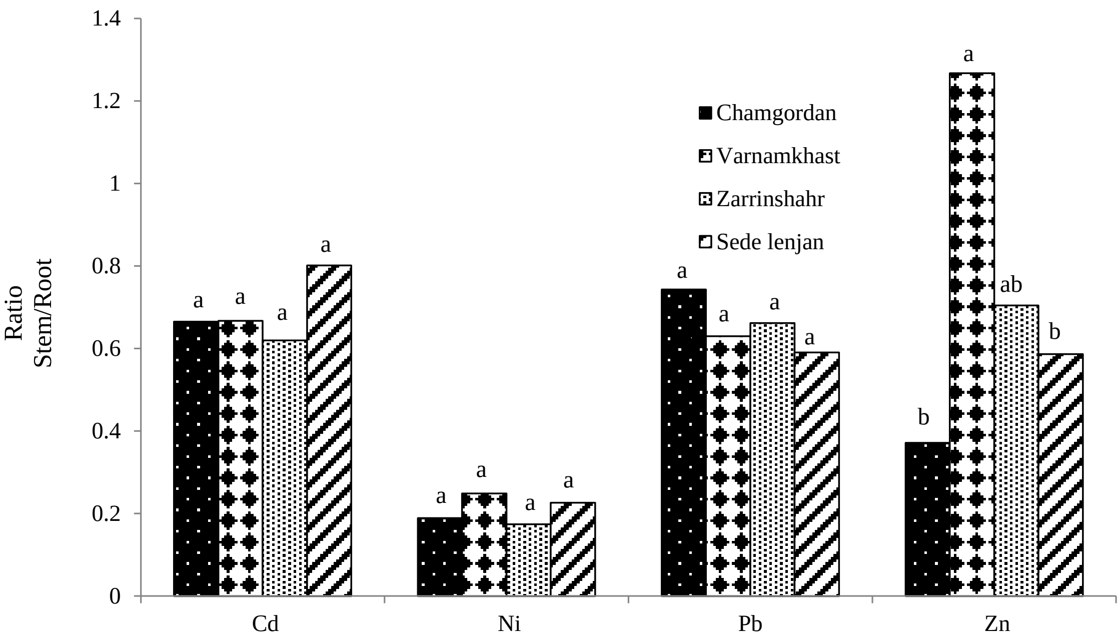


Figure 4. Metals transfer factor from root to stem of rice (same letters indicate no significant difference)

this indicates that plant accumulates metals (Singh *et al*. 2011). BAF values of Pb, Ni and Zn, were less than 1 in the rice grain. The results indicated that metal bioavailability was low in the study area.

3.5.2 Translocation of metals from roots into the upper parts of the rice plant

The translocation factor (TF) is an indication of the degree of metal translocation from soil to plant. It expresses a plant's capacity to store heavy metals in its upper part. The TF is described as the ratio of metal concentration in the upper part to that in the roots (Boularbah *et al*. 2006). Figures 3, 4 and 5 show the translocation factors (TF) of different heavy metals from soil to the rice plant. The TF is regarded as one of the key components of human exposure to heavy metals through the food chain (Singh *et al*. 2011). The TFs of metals from soil to root (TF_{Soil}), root to stem (TF_{Root}) and stem to grain (TF_{stem}) were calculated in the study. The average soil-to-root translocation (TF_{Soil}) were found to be in order of Cd (1.52) > Pb (0.486) > Zn (0.389) > Ni (0.201). It is illustrated that rice root accumulated high quantities of Cd^{+2} when grown in non-polluted areas, hence Cd^{+2} was proved to be more bioavailable to plants than other heavy metals, resulting in a higher biological absorption coefficient for Cd (Singh *et al*. 2011). The TF_{Root} values were found in the following order: Zn (0.732) > Cd (0.688) > Pb (0.656) > Ni (0.209).

The root-to-straw translocation values in the study area were less than 1 (except Zn), with no significant difference in TF values among different regions. The translocation values for the straw to grain (TF_{Straw}) in the study area were found in the following order: Zn (1.228) > Cd (0.854) > Pb (0.456) > Ni (0.24). For most metals (except Zn), the straw-to-grain TF values were less than 1, with no significant difference in TF values among different regions. It is because most of the heavy metals are often confined in the roots after paddy plant uptake. Also, for all the heavy metals, the TF values at stem were less than the grain, while at the grains, the values come close to the TF at the root. It appears due to atmospheric deposition and the plants' exposure to factory chimneys all over the years.

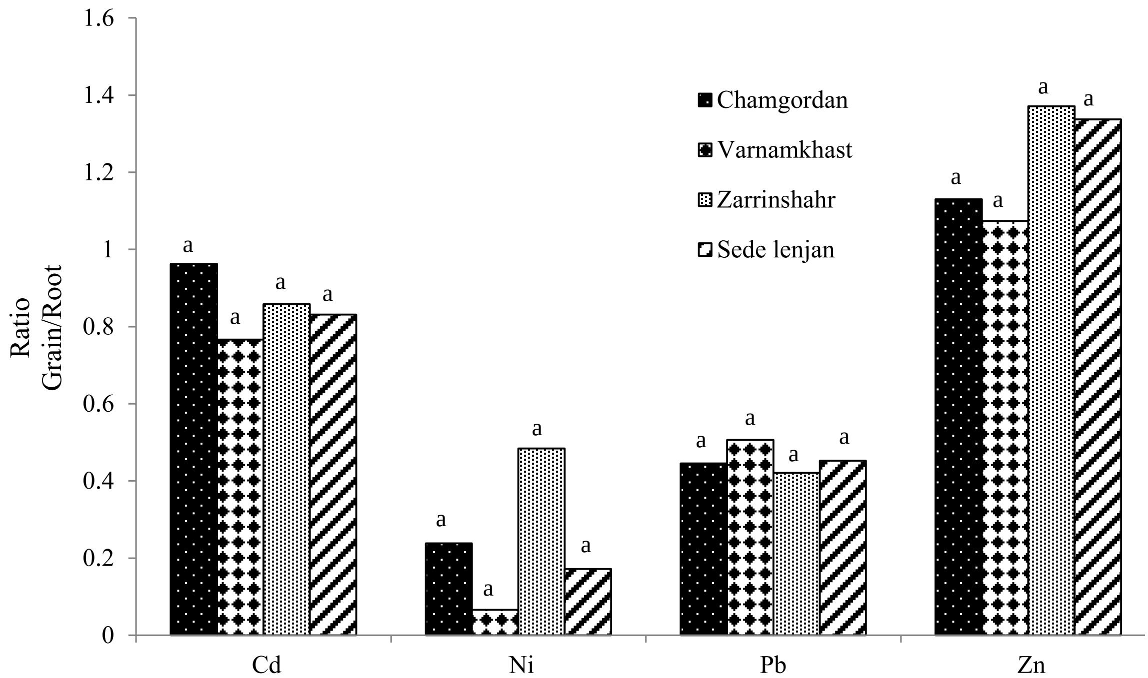


Figure 5. Metal transfer factor from root to grain of rice (same letters indicate no significant difference)

3.5.3 Coefficients of correlation between metals in the soils and the rice plants

Variability in soil properties and plant growth conditions often lead to not a direct relationship between total metal concentrations in soils and plants (Obrador *et al*. 2007). However, one of the reasons for the lack of a significant relationship between metal concentrations in soils and plants can be attributed to sources of contamination in the soil. In the present study, the concentrations of Cd, Zn and Ni in soil with their concentrations in the rice plant's roots showed a significant positive correlation (Table 6). The correlations between the stem and seed were positive but not statistically significant. Singh *et al*. (2010) in a study, obtained a positive correlation between the concentration of Cd in soil and its concentration in rice, but same correlations were negative for Ni. Also, Hang *et al*. (2009) found out a significant positive correlation (r = 0.353, $p < 0.05$) between Cd, Zn and Ni concentrations in soils and rice. In the present study, the concentration of Pb in the soil did not correlate with the amount absorbed by the rice, which was consistent with the findings of Zhao *et al*. (2010).

Pb is taken up by plants to some extent. Plants took up only 0.003–0.005% of the total soil Pb (Kalavrouziotis *et al*. 2012). However, in the present study, relatively high accumulation of Pb was observed in stems and grains of the plant (Figures 4 and 5). Data revealed that Pb concentrations in all examined parts of the rice plant were much higher than the preset normal standard -i.e. (1–5 mg/kg DM. It was consistent with the results of Nayek *et al*. (2010). It seems to us that adjacent factory chimneys could be blamed for such high Pb concentrations.

CONCLUSIONS

This study determined the accumulation of heavy metals in some paddy soils and rice plants which were collected simultaneously at harvest period. Meanwhile, the farms had been irrigated with contaminated water amid rising public and consumer concerns about the safety and health of local and imported rice in the market. It was found out that contrary to residents' expectations, anthropogenic industrial, municipal and agricultural activities have been changing the famous image of Lenjan aromatic rice for long, exceeding many safety standards in some cases. Based on the data obtained in the study, the agricultural soils, collected from four different locations in Lenjan region, central Iran were severely contaminated by Cd, followed by Ni, Pb and Zn. Assessed levels of Cd, Pb, Ni and Zn were above the standard limits in this area. However, concentrations of metals in the studied water samples were found to be within the permissible limit set by international authorities, except for Ni. High concentrations of Ni in the Zayandeh Rood river could be attributed to the aggregating discharge of municipal and industrial wastewater. The BAF for Cd was at maximum compared to other metals among the four locations. Most of the studied heavy metals were found accumulated mostly in the roots of the paddy plant. All of them had concentrations higher than the standard levels. Meanwhile, some plant parts, including the stems and grains contained, relatively high concentrations of Cd and Pb in comparison with the standards in all studied locations. They were much higher than the standards of FAO/WHO, meaning that consumption of such rice could be harmful to consumers (both humans and animals). In some cases, they had far exceeded the critical level. Low correlation discovered between the concentration of Pb in different parts of the plant and the soil on one, hand, and the relatively high metal contents detected in the rice grains on the other, suggested that aerial deposition was a potential source of metal contamination in rice. In regards to the national food safety criteria, Pb content in all rice samples exceeded the national MAC. In general, factors such as municipal and industrial wastewater increased the traced metal concentrations. Stricter monitoring of soil, plant, and water quality, together with tougher implementation of governmental regulations are among prerequisites to decline potential health hazards caused by irrigating with metal-polluted river water.

REFERENCES

ABBAS, S.T. – SARFRAZ, M. – MEHDI, S.M. – HASSAN, G. – UR-REHMAN, O. 2007. Trace elements accumulation in soil and rice plants irrigated with the contaminated water. In *Soil & Tillage Research*, vol. *94*, pp. 503–509.

AGARVAL, S.K. 2002. *Pollution management: water pollution*. New Delhi : A. P. H. Publ. 384 pages.

ALLOWAY, W.H. 1968. Environmental cycling of trace elements. In *Advanced Agronomy,* vol. *20*, pp. 255–265.
AYERS, R.S. – WESTCOTT, D.W. 1985. *Water quality for agriculture*. Irrigation and Drainage, Paper 29, Rome, Italy : FAO, 97 pages.
BAUYCOS, G.J. 1962. Hydrometer methods improved for making particle size of soils. In *Agronomy Journal,* vol. *56*, pp. 464–465.
BI, X. – REN, L. – GONG, M. – HE, Y. – WANG, L. – MA, Z.H. 2010. Transfer of cadmium and lead from soil to mangoes in an uncontaminated area, Hainan Island, China. In *Geoderma*, vol. *155*, pp. 115–120.
BOULARBAH, A. – BOULARBAH, CH. – BITTON, G. – ABOUDRAR, W. – OUHAMMOU, A. – MOREL, J.L. 2006. Heavy metal contamination from mining sites in South Morocco: assessment of metal accumulation and toxicity in plants. In *Chemosphere*, vol. *63*, pp. 811–817.
CASADO, M. – ANAWAR, H.M. – GARCIA-SANCHEZ, A. – REGINA, I.S. 2008. Cadmium and zinc in polluted mining soils and uptake by plants (El Losar mine, Spain). In *International Journal of Environment and Pollution*, vol. *33*, no. 2–3, pp. 146–159.
CHUNG, B.Y. – SONG, C.H. – PARK, B.J. – CHO, J.Y. 2011. Heavy metals in brown rice (Oryza sativa L.) and soil after long-term irrigation of wastewater discharged from domestic sewage treatment plants. In *Pedosphere*, vol. *21*, no. 5, pp. 621–627.
DOBERMAN, A. – FAIRHURST, T.H. 2000. *Rice*: *Nutrient disorders and nutrient management*. Handbook series. International Rice Research Institute, Manila, p. 191. ISBN 981-04-2742-5
FACCHINELLI, A. – SACCHI, E. – MALLEN, L. 2001. Multivariate statistical and GIS-based approach to identify heavy metal sources in soils. In *Environmental Pollution*, vol. *114*, pp. 313–324.
FAKOOR JANATI, S.S. – BEHESHTI, H.R. – FEIZY, J. – KHOSHBAKHT FAHIM, N. 2011. Determinations of cadmium, lead, arsenic and mercury in rice from Iran. In *International Journal of Industrial Chemistry*, vol. *2*, no. 4, pp. 196–200.
FAN, Y. – ZHU, T. – LI, M. – HE, J. – HUANG, R. 2017. Heavy metal contamination in soil and brown rice and human health risk assessment near three mining areas in central China. In *Journal of Healthcare Engineering*, vol. *2017*, Article ID 4124302, 9 pages. DOI:10.1155/2017/4124302
FAO and WHO. 1984. Contaminants: joint FAO/WHO food standards program. Codex Alimentarius XVII, First edition, pp. 1–33.
FU, J. – ZHOU, Q. – LIU, J. – LIU, W. – WANG, TH. – ZHANG, Q. – JIANG, G. 2008. High levels of heavy metals in rice (Oryza sativa L.) from a typical E-waste recycling area in southeast China and its potential risk to human health. In *Chemosphere,* vol. *71*, pp. 1269–1275.
HAI, X. – ZHANG, Y.L. – GENG-MAO, ZH. – JIA-GUE, J. – SHI-XUE, Y. – ZHAO-PU, L. 2009. Anthropogenic impact on surface water quality in Taihu Lake Region, China. In *Pedosphere*, vol. *19*, no. 6, pp. 765–778.
HANG, X. – WANG, H. – ZHOU, J. – MA, CH. – DU, CH. – CHEN, X. 2009. Risk assessment of potentially toxic element pollution in soils and rice (Oryza sativa) in a typical area of the Yangtze River Delta. In *Environmental Pollution*, vol. *157*, pp. 2542–2549.
Iran Meteorological Organization 2017. Retrieved from http://www.irimo.ir/far/wd/701-Weather-Forcast-Esfahan.html?id=17556.
JAHED KHANIKI, GH.R. – ZAZOLI, M.A. 2005. Cadmium and Lead Contents in Rice (Oryza sativa) in the North of Iran. In *International Journal of Agriculture & Biology,* vol. *7*, no. 6, pp. 1026–1029.
JUNG, M.CH. – THORNTON, I. 1997. Environmental contamination and seasonal variation of metals in soils, plants and waters in the paddy fields around a Pb-Zn mine in Korea. In *Science of the Total Environment*, vol. *198*, pp. 105–121.
KABATA PENDIAS, A. 2010. *Trace elements in soils and pl*ants. Boca Raton, London, New York : CRC Press.
KALAVROUZIOTIS, I.K. – KOUKOULAKIS, P. – KOSTAKIOTI, E. 2012. Assessment of metal transfer factor under irrigation with treated municipal wastewater. In *Agricultural Water Management*, vol. *103*, pp. 114–119.
KHAN, S. – CAO, Q. – ZHENG, Y.M. – HUANG, Y.Z. – ZHU, Y.G. 2008. Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. In *Environmental Pollution*, vol. *152*, pp. 686–692.
KHAN, S. – REHMAN, S. – CAO, Q. – JEHAN, N. – SHAH, M.T. 2011. Uptake and translocation of lead and pyrene by ryegrass cultivated in aged spiked soil. In *International Journal of Environment and Pollution*, vol. *45*, no. 1–3, pp. 110–122.
KIZILOGLU, F.M. – TURAN, M. – SAHIN, U. – KUSLU, Y. – DURSUN, A. 2008. Effects of untreated and treated wastewater irrigation on some chemical properties of cauliflower (Brassica olerecea L. var. botrytis) and red cabbage (Brassica olerecea L. var. rubra) grown on calcareous soil in Turkey. In *Agricultural Water Management*, vol. *95*, pp. 716–724.
LAI, Y. – XU, B. – HE, L. – LIN, M. – CAO, L. – WU, Y. – MOU, SH. – HE, SH. 2012. Cadmium uptake by and translocation within Rice (Oryza Sativa L.) seedlings as affected by iron plaque and Fe2o3. In *Pakistan Journal of Botany*, vol. *44*, no. 5, pp. 1557–1561.
LINDSAY, W.L. – NORVELL, W.A. 1978. Development of a DTPA test for zinc, iron, manganese, and copper. In *Soil Science Society American Journal*, vol. *42*, pp. 421–428.
LIU, W.X. – SHEN, L.F. – LIU, J.W. – WANG, Y.W. – LI, S.R. 2007. Uptake of Toxic Heavy Metals by Rice (Oryza sativa L.) Cultivated in the Agricultural Soil near Zhengzhou City, People's Republic of China. In *Bulletin of Environmental Contamination Toxicology*, vol. *79*, no. 2, pp. 209–213.
MAHMOUD, E.K. – GHONEIM, A.M. 2016. Effect of polluted water on soil and plant contamination by heavy metals in El-Mahla El-Kobra, Egypt. In *Solid Earth,* vol. *7*, pp. 703–711.
MANATA, D.S. – ANGELONE, M. 2002. Heavy metals in urban soils. A case study from the city of Palermo. In *Science of the Total Environment*, vol. *300*, no. 1, pp. 229–243.
MONTGOMERY, J.M. 1985. *Water treatment, principle and design*. New York : John Wiley & Sons, 696 pages.
MOURA, M.C.S. – MOITA, G.C. – NETO, J.M.M. 2010. Analysis and assessment of heavy metals in urban surface soils of Teresina, Piauí State, Brazil: a study based on multivariate analysis. In *Comunicata Scientiae*, vol. *1*, no. 2, pp. 120–127.
NAYEK, S. – GUPTA, S. – SAHA, R.N. 2010. Metal accumulation and its effects in relation to biochemical response of vegetables irrigated with metal contaminated water and wastewater. In *Journal of Hazardous Materials,* vol. *178*, pp. 588–595.
OBRADOR, A. – ALVAREZ, J.M. – LOPEZ-VALDIVIA, L.M. – GONZALEZ, D. – NOVILLO, J. – RICO, M.I. 2007. Relationships of soil properties with Mn and Zn distribution in acidic soils and their uptake by a barley crop. In *Geoderma,* vol. *137*, pp. 432–443.
PAIS, I. – JONES, J.B. 1997. *The Handbook of Trace Elements*. Boca Raton, Florida : St. Lucie Press, 240 pages.

PARK, B.J. – LEE, J.H. – KIM, W.I. 2011. Influence of soil characteristics and arsenic, cadmium, and lead contamination on their accumulation levels in rice and human health risk through intake of rice grown nearby abandoned mines. In *Journal of Korean Society Applied Biology Chemical*, vol. *54*, no. 4, pp. 575–582.

RABBANI, D. – MOSTAFAII, GH.R. – DEHGHANI, R. – GILASI, H. – HOSEIN ABADI, Z. 2015. Evaluation of heavy metals in Iranian and Non-Iranian rice supplied by shopping centers of Kashan, Iran. In *Health Sciences*, vol. *2*, no. 1, pp. 25–29.

RATTAN, R.K. – DATTA, S.P. – CHHONKAR, P.K. – SURIBABU, K. – SINGH, A.K. 2005. Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater-a case study. In *Agriculture, Ecosystems and Environment*, vol. *109*, pp. 310–322.

RODRIGUEZ MARTIN, J.A. – LOPEZ ARIAS, M. – GRAUCORBI, J.M. 2006. Heavy metals contents in agricultural topsoils in the Ebro basin (Spain). Application of the multivariate geostatistical methods to study spatial variations. In *Environment Pollution*, vol. *144*, pp. 1001–1012.

ROWELL, D.L. 1994. *Soil Science: Method and Application*. Harlow : Longman Group.

SEPA 2005. *The limits of pollutants in food*. China: State Environmental Protection Administration. GB2762-2005.

SILLANPPA, M. – JANSSON, H. 1992. *Status of cadmium, lead, cobalt*. FAO Soils Bulletin 65. Rome, Italy.

SIMS, J.T. 1996. *Lime requirement method of soil analysis, parts: chemical methods.* Madison, Wisconsin. USA.

SINGH, A. – SHARMA, R.K. – AGRAWAL, M. – MARSHALL, F.M. 2010. Health risk assessment of heavy metals via dietary intake of foodstuffs from the wastewater irrigated site of a dry tropical area of India. In *Food and Chemical Toxicology*, vol. *48*, pp. 611–619.

SINGH, J. – UPADHYAY, S.K. – PATHAK, R.K. – GUPTA, V. 2011. Accumulation of heavy metals in soil and paddy crop (Oryza sativa), irrigated with water of Ramgarh Lake, Gorakhpur, UP, India. In *Toxicological & Environmental Chemistry*, vol. *93*, no. 3, pp. 462–473.

SPOSITO, G. 1989. *The chemistry of soils*. New York : Oxford University Press, 277 pages.

SPOSITO, G. – LUND, J. – CHANG, A.C. 1982. Trace metal chemistry in arid-zone field soils amended with sewage sludge: I. Fractionation of Ni, Cu, Zn, Cd, and Pb in solid phases. In *Soil Science Society American Journal*, vol. *46*, pp. 260–264.

TARIQ, S.R. – RASHID, N. 2013. Multivariate analysis of metal levels in paddy soil, rice plants, and rice grains: A case study from Shakargarh. In *Pakistan Journal of Chemistry,* vol. *2013*, Article ID 539251, 10 p. http://dx.doi.org/10.1155/2013/539251

THOMAS, G.W. 1996. Soil pH and soil acidity in methods of soil analysis. In KLUTE, A. (Ed.). Part 3. *Chemical methods*. Madison, Wisconsin, USA, pp. 475–490.

TIWARI, K.K. – SINGH, N.K. – PATEL, M.P. – TIWARI, M.R. – RAI, U.N. 2011. Metal contamination of soil and translocation in vegetables growing under industrial wastewater irrigated agricultural field of Vadodara, Gujarat, India. In *Ecotoxicology and Environmental Safety*, vol. *74*, pp. 1670–1677.

WANG-DA, CH. – GUO-PING, ZH. – HAI-GEN, Y. – WEI, W. – MIN, X. 2006. Genotypic and environmental variation in cadmium, chromium, arsenic, nickel, and lead concentrations in rice grains. In *Journal of Zhejiang University Science B*., vol. *7*, no. 7, pp. 565–571.

WU, CH. – ZHANG, L. 2010. Heavy metal concentrations and their possible sources in paddy soils of a modern agricultural zone, southeastern China. In *Environmental Earth Science*, vol. *60*, pp. 45–56.

YANG, P. – MAO, R. – SHAO, H. – GAO, Y. 2009. An investigation on the distribution of eight hazardous heavy metals in the suburban farmland of China. In *Journal of Hazardous Materials,* vol. *167*, pp. 1246–1251.

ZAZOLI, M. – BAZRAFSHAN, E. – HAZRATI, A. – TAVAKKOLI, A. 2006. Determination and estimation of Cadmium intake from Tarom rice. In *Journal of Applied Science Environment*, vol. *10*, no. 3, pp. 147–150.

ZHAO, K. – LIU, X. – XU, J. – SELIM, H.M. 2010. Heavy metal contaminations in a soil-rice system: Identification of spatial dependence in relation to soil properties of paddy fields*.* In *Journal Hazardous Material*, vol. *181*, pp. 778–787.

ZHEN, H.X. – MEI, ZH.D. – QIAN, H.D. – LONG, C. – LIN, ZH.H. – HUI, W. 2009. Heavy metal transfer from soil to vegetable in southern Jiangsu Province, China. In *Pedosphere*, vol. *19*, no. 3, pp. 305–311.

Received: July 31, 2017