ORIGINAL RESEARCH

Research on Crop Ecophysiology
Vol.12/2, Issue 2 (2017), Pages:113 - 128

Agrophysiological Responses of Barley Cultivars to Salt Stress and Zinc Fertilization

MEHRDAD MAHLOOJ[1]*, MOHAMMAD PESSARAKLI[2]
1-* Horticulture Crops Research Department, Isfahan Agricultural and Natural Resources Research and Education Center, AREEO, Isfahan, Iran.
2- School of Plant Sciences, College of Agriculture & Life Sciences, The University of Arizona, Tucson, AZ 85721, USA.

* Corresponding author email: mmahlooji2000@yahoo.com

Received: 25 may 2017 Accepted: 12 Jun 2017

ABSTRACT

In order to study the effects of agrophysiological responses of barley genotypes to salt stress and zinc fertilization, a field experiment was conducted in a strip-split-plot design with three replications. Saline irrigation water in three levels [2 (low), 10 (moderate) and 18 (high) dS m^{-1}] were applied as vertical factors. Three barley genotypes [‘Morocco’ (salt-sensitive), ‘Nosrat’ (semi salt-tolerant) and ‘Khatam’ (salt-tolerant)] were arranged within the vertical factors. The horizontal factors were four zinc fertilizer applications [Nano-ZnO, Zn-EDTA, simultaneous applications of Nano-ZnO + Zn-EDTA, and water (control)]. With increasing salinity, maximal efficiency of PSII (Fv/Fm), chlorophyll content (SPAD), relative water content (RWC), K^+ ion, K^+:Na^+ ratio, number of spike (NS), kernel number per spike (KNS), thousand-kernel weight (TKW), and grain yield (GY) decreased, but electrolyte leakage (EL) and Na^+ ion concentration increased. The tolerant genotype (Khatam) had maximum Fv/Fm, SPAD, RWC, K^+ ion, K^+:Na^+ ratio, KNS and GY. Minus zinc application (check) had minimum Fv/Fm, SPAD, K^+:Na^+ ratio and GY. Nano-ZnO had the highest EL and the lowest KNS, K^+ and Na^+ ions. Zn-EDTA application provided the highest RWC, K^+ ion, K^+:Na^+ ratio, KNS and GY. Overall, it was concluded that Zn-EDTA can be as a proper tool for increasing barely yield under salinity stress conditions.

***KeyWords**:* Barley, Salinity stress, Yield, Zinc applications.

INTRODUCTION

Salinity is a major stress limiting crop production around the world, affecting almost 80 million hectares of agricultural lands (FAO, 2014). Growth of plant species and photosynthetic processes are restricted by salt stress. In this study, we measured major agrophysiological responses, including ion contents (Rahnama *et al.,* 2011), chlorophyll fluorescence, chlorophyll content, relative water content, and electrolyte leakage. These measurements can be used to monitor plant responses to salt stress (Izadi *et al.,* 2014). Therefore, using these measurements to screen for salinity tolerance and reducing expenses are thought to be more reliable than selecting for salt tolerance based on yield (Rahnama *et al.,* 2011).

The chlorophyll content (SPAD) in flag leaves is an important physiological index representing the degree of photosynthesis in plants. Reduction in net photosynthesis under stress has been attributed to reduction in SPAD of plants (Ebrahimi *et al.,* 2014). Decrease in leaf water potential induces stomatal closure and inhibits photosynthetic metabolism with evident changes in the actual quantum efficiency of PSII (Azizpour *et al.,* 2010), while no or little changes and effects are recorded in Fv/Fm (Seckin *et al.,* 2010). Some researchers have demonstrated that it inhibits PSII activity (Hichem *et al.,* 2009), whereas others have indicated that salt stress has no effect on PSII (Demiral and Turkan, 2006). However, some studies have shown changes in chlorophyll (Chl) fluorescence (Fv/Fm ratio after dark-adaptation of the leaf) as a result of salinity stress (Castillo *et al.,* 2005). Chl fluorescence methodology can be conveniently used to screen in a short time many samples for tolerance to abiotic stresses, and also provides useful information about stress tolerance mechanisms (Izadi *et al.,* 2014). Leaf electrolyte leakage is also considered as a good physiological index reflecting the degree of plant injury caused by salt stress. Increasing membrane ion leakage under stress conditions has been reported by Roy *et al.,* (2009). The relative water content of a leaf is a measurement of its relative hydration status to maximum water holding capacity at full turgidity. Reduction in RWC due to water stress has been shown in many sunflower genotypes (Gholinezhad *et al.,* 2009). Also, salinity was shown to decreases relative water content (RWC) of sunflower (Ebrahimian and Bybordi, 2011).

In calcareous soils, zinc precipitates is an unavailable forms to plants (Morshedi and Farahbakhsh, 2012). By reducing the amount of soil moisture in saline soils, Zn and Fe in the soil solution are reduced in mobility. Application of Zn fertilizers is a common practice to correct Zn deficiency. However, soil applications of Zn have not been very successful under furrow irrigation. Most Zn deficiencies can be corrected with foliar zinc application (Christensen and Peacock, 2000). Plant element deficiencies can be compensated for by spraying appropriate foliar solutions to compensate for the deficiency (Cakmak, 2008). Zn is an essential micronutrient, which is deficient in many regions worldwide, such as in calcareous and salt-affected soils of central Iran (Khoshgoftarmanesh *et al.,* 2004). Morshedi and Farahbakhsh (2012) and Keshavarz and Saadat (2015) reported that zinc applications increased yields and had a positive effect on salt tolerance of wheat and barley. Zinc deficiency in plants grown in calcareous soils can be moderately corrected by the application of inorganic zinc salts.

There is a lack of information on the use of agrophysiological responses as selection markers for barley genotypes under saline stress conditions. Therefore, the purpose of this study was to determine the agrophysiological responses of different barley genotypes to salinity stress and to investigate the role of zinc fertilizer application in reducing the effects of salinity stress.

MATERIALS AND METHODS

Plant material and experimental design

This experiment was conducted in a strip-split-plot design with three replications at Isfahan Rodasht Drainage and Salinity Research Station (32º 30/ N, 52º 9/ E) in 2014-2015. Three irrigation water qualities, including S_1 (2 dS m^{-1}, low salinity as a check), S_2 (10 dS m^{-1}, common salinity in the region), and S_3 (18 dS m^{-1}, high salinity) were evaluated as vertical strips factors. The horizontal factors were four zinc application levels, including Nano zinc-oxide, Zn-EDTA, simultaneous applications of Nano-ZnO and Zn-EDTA and water (check). The application rates of Nano-ZnO and Zn-EDTA were 100 and 1000 g ha^{-1}, respectively. Soil characteristics and chemical analysis of the irrigation water quality are shown in Table 1. Three barley genotypes: 'Morocco' (salt-sensitive), 'Nosrat' (semi-salt tolerant) and 'Khatam' (salt-tolerant) were planted on November 13, 2015. In each plot, there were 6 rows 4 m long. Plant density was 450 seeds m^{-2}.

Table 1. Selected physico-chemical properties of the soil before planting and three levels of water irrigation quality.

Soil characteristics	Amount	Water characteristics	Saline water, (dS m^{-1})		
			S_1=2	S_2=10	S_3=18
pH	7.7	pH	7.7	8.1	7.6
Electrical conductivity (dS m^{-1})	13	Electrical conductivity (dS m^{-1})	1.4	9.7	17.8
Available K^+ (mg kg^{-1})	340	$So_4{}^{2-}$ (meq L^{-1})	0.8	26.9	172.3
Available Zn^{2+} (mg kg^{-1})	0.72	HCo_3^- (meq L^{-1})	2.0	5.7	6.4
Available Fe^{2+} (mg kg^{-1})	5.54	Cl^- (meq L^{-1})	1.4	60	111
Available Na^+ (meq L^{-1})	79.1	Na^+ (meq L^{-1})	1.5	47.8	99.3
Available Ca^{2+}+Mg^{2+} (meq L^{-1})	60	Ca^{2+}+Mg^{2+} (meq L^{-1})	2.6	44	72

Physiological traits

At heading stage, the quantum yield (F_v/F_m) was measured by the uppermost fully-expanded leaf using a fluorometer (chlorophyll fluorometer; Optic Science-OS-30, USA) (Pask *et al.,* 2012). For this purpose, the plants were adapted to darkness for 20 minutes by using one special clamp, then the fluorescence amounts were measured in 1000 (µM photon m^{-2} s^{-1}), and calculation was performed using the following formula (Arnon, 1949):

$$PSII = (F_m - F_0)/F_m = F_v/F_m$$

PSII; quantum yield amount of photosystem II, F_m or maximum fluorescence after a saturated light pulse on plants adapted to darkness and F_0, the minimal fluorescence in the light adapted, which was determined by illumination with far-red light. Chlorophyll meter (SPAD Konica, Minolta) was used to measure chlorophyll content at heading stage. For each treatment, the chlorophyll contents (SPAD) of 10 individual leaves were measured. Relative water contents of the flag leaves were measured as described by Pask *et al.*, (2012), and the electrolyte leakage was measured using the methods of Ahmadizadeh *et al.* (2011).

Nutrients concentration

After harvesting, the concentration of K^+ was determined using an Auto Analyzer (Quikchem IC+FIA 8000 Series) and the concentration of Na^+ was determined with an Atomic Absorption Spectrometer (Perkin Elmer Model 3110, USA) (Bauder *et al.*, 2014).

Grain yield measurement and statistical analysis

Grain yield was measured in 0.4×4 m^2 plots. Analyses of variances were conducted on the data to determine the significant of differences among the treatments using the general linear model (GLM) in SAS software (Version 9.1, SAS Institute, Cary, NC). Mean comparisons were conducted using Fisher's least significant differences (LSD) test at ($p \leq 0.05$). Relationships between agrophysiological traits were examined using simple linear correlations.

RESULTS AND DISCUSSION

The results of the analysis of variance of the data indicated that the effects of saline irrigation water were significant on chlorophyll content, relative water content, K^+ and Na^+ ions, K^+:Na^+ ratio, number of spike (NS); kernel number per spike; thousand-kernel weight, and grain yield. The effects of zinc applications were significant on SPAD, electrolyte leakage, K^+ and Na^+ ions, and K^+:Na^+ ratio. The genotype had significant effects on all of the agrophysiological responses (Fv/Fm, SPAD, RWC, EL, K^+, Na^+, K^+:Na^+, NS, KNS, TKW, and GY) (Table 2).

Photosynthetic parameters

Chlorophyll fluorescence

The maximal quantum yield (F_v/F_m), which characterizes maximum efficiency of PSII photochemistry, can be used as a good estimation of photosynthetic performance. The effects of salinity levels and zinc applications on Fv/Fm were not significant, but genotypic differences in Fv/Fm were significant (Table 2). In comparison with S_1, Fv/Fm declined about 0.5 and 1% in S_2 and S_3, respectively. It has been reported that mild-salinity levels do not induce sustained photodamage to PSII as revealed by unvaried Fv/Fm ratio in plants (Naumann *et al.*, 2007) even in reduction of leaf gas exchanges. Salt-tolerant genotype (Khatam) had higher Fv/Fm than the salt-sensitive genotype (Morocco). The results show that the leaf Fv/Fm gradually decreased with increasing salinity in barley (Table 2). This could result from damaged leaf cell membranes and irreversible photoinhibition resulting from salt stress. These results are in agreement with those reported by James *et al.,* (2002).

Chlorophyll content (SPAD)

Salinity levels had a significant ($p \leq 0.01$) effect on SPAD (Table 2). SPAD was reduced due to high salinity stress. With increasing salinity up to 10 dS m^{-1} (S_2), SPAD increased approximately 5%, but in S_3 (18 dS m^{-1}) SPAD reduced about 2.6%, in comparison with S_1 (2 dS m^{-1}). Maximum SPAD was at a medium level of salt and the greatest reduction was observed in S_3 treated with high saline water. However, under lower salt stress (S_2), the SPAD was higher than control (S_1). Conditions of medium salinity (S_2) may stimulate photosynthesis due to tolerance mechanisms such as leaf area reduction and leaf thickness increasing the concentration of chlorophyll in the leaf surface. In the studies of Mohammadkhani and Heidari (2007), SPAD increased at moderate stress level. Increasing the salinity level up to 18 dS m^{-1} lowered SPAD values. Reducing the SPAD could be due to destroying the chloroplasts and a reduction in the amount of chlorophyll. Similar results have been reported by Azizpour *et al.,* 2010.

The effects of zinc application treatments on SPAD were significant ($P \leq 0.01$). Simultaneous application treatments (F_3=Nano-ZnO and Zn-EDTA) increased SPAD to about 10%, in comparison with F_4 (check=only water). SPAD of F_4 was the lowest and the differences between F_1 (Nano-ZnO), F_2 (Zn-EDTA) and F_4 were not statistically significant (Table 2). SPAD increased by zinc applications (F_1, F_2 and F_3). Higher chlorophyll accumulation may be due to complementary effects of nutrients like zinc, magnesium, iron and sulfur. The positive effects of zinc applications under salt stress were: protecting chlorophyll against free radicals, removing the reactive oxygen species, preventing the degradation of chlorophyll, increasing potassium concentration in the leaves, reducing sodium in the plasma membrane and maintaining cell integrity (Pask *et al.,* 2012). This is consistent with our study and others (Cakmak 2008) in which zinc applications improved SPAD.

SPAD was significantly affected by genotype ($p \leq 0.01$). SPAD of the tolerant genotype (G_3=Khatam) was the highest. SPAD decreased in Morocco (G_1=sensitive) and Nosrat

(G_2=semi tolerant) about 14 and 12%, respectively as compared to G_3 (Table 2) because of less degradation of chlorophyll in the salt tolerant barley genotype (Khatam). Other studies have shown a similar results (Kumar Parida and Bandhu Das, 2005).

Relative water content (RWC)

With increasing salinity levels, RWC declined significantly ($p \leq 0.05$). There was about 4 and 7% reduction in RWC in S_2 and S_3, respectively in comparison with S_1. The RWC differences were small and not significant between S_1 and S_2 or S_2 and S_3, but it was significant between S_1 and S_3 (Table 2). High sodium ion absorption under saline conditions may have impaired water absorption and reduced RWC. Reduction in RWC may be due to reduced water, high concentrations of sodium and chloride ion, and reduced leaf area (Munns and Tester, 2008; Ebrahimian and Bybordi, 2011).

RWC was not affected significantly by zinc applications (Table 2). However, application of Zn-EDTA slightly increased RWC, but Nano-ZnO application reduced it, as compared to F_4. Although zinc is considered to protect vital cell components under stress, it is not known to increase the water absorption potential of plants or affect RWC. Consequently, lack of a significant effect of zinc applications on RWC in the present experiment may be acceptable. These results are in accordance to those reported by Cakmak, 2008.

RWC was significantly ($p \leq 0.01$) affected by genotypes. Tolerant (G_3) and sensitive (G_1) genotypes had maximum and minimum RWC, respectively. There was about 12 and 7% reduction in RWC in G_1 and G_2, respectively, in comparison with G_3 (Table 2). Although G_2 has been shown to be more saline tolerant than genotype G_1, the significant decreases in RWC due to salinity stress implies that G_2 is also slightly sensitive to saline water stress. Ganji Arjenaki *et al.*, (2012) showed similar results in wheat and reported that tolerant genotypes maintained higher RWC under stress than the sensitive ones.

It was also found that higher RWC indicates a better plant water status. Thus, it can be assumed that increase in RWC has increased the chlorophyll content and Fv/Fm (Table 2). The ability of plants to maintain their RWC under stress conditions has been suggested as a tolerance mechanism (Kadkhodaei *et al.*, 2014; Maghsoodi and Razmjoo, 2014). In our study, RWC decreased in all genotypes under salt stress, but the decline was genotype-salinity-level specific and tolerant genotpes had higher RWC than the sensitive ones (Table 2). RWC was positively correlated (r= 0.45**) with salinity tolerance of genotypes (Table 3). Similar results have been reported by Kadkhodaei *et al.* (2014).

Electrolyte leakage (EL)

The effects of salinity levels on EL were not significant. Despite the lack of significant effects of salinity levels on EL, there was an increase of about 7 and 10% in EL in S_2 and S_3, respectively, compari to S_1. EL in the mild salinity treatment (S_2) increased with the salinity gradient, but no difference was observed between S_1 and S_2. However, at the 18 dS m^{-1} salinity level (S_3), EL was significantly higher than at S_1. This implies that the high levels of salinity exhibited more EL effects. The results show that EL gradually increased with increasing salinity. This has been attributed to leaf cell membranes being damaged by salt stress (Kaya *et al.,* 2001).

The effects of zinc application treatments on EL of the flag leaf were statistically significant ($p \leq 0.01$). Nano-ZnO (F_1) and simultaneous applications of (Nano-ZnO + Zn-EDTA) (F3) increased EL. The highest EL were in treatments with Nano particle contents (F_1 and F_3). Under environmental stresses, plant membranes are subject to changes often associated with the increases in permeability and loss of integrity (Bilal *et al.,* 2015). Commonly, changes in EL have been measured to detect stress injury of the cell membrane. Bilal *et al.,* 2015 reported that salinity increased electrolyte leakage in the leaves of *Vigna radiata* (L.). Varietal differences in EL were significant ($p \leq 0.01$). There were about 10 and 15% increases in EL in G_2 and G_1, respectively, in comparison with G_3. No significant differences between G_1 and G_2 were obtained, but G_2 had a lower EL than G_1. However, the results showed that EL in G_3 (salt tolerant) and G_2 (semi salt tolerant) were lower in comparison with the G_1 (salt sensitive). These results are in agreement with that of Roy *et al.,* 2009.

EL was used to assess membrane permeability. The leaf EL is considered a good physiological marker reflecting the amount of plant membrane damage caused by salt stress (Kaya *et al.,* 2001)

Table 2. Effects of water quality and fertilizer application on photosynthetic parameters of barley genotypes

Treatments	Fv/Fm	SPAD value	Relative water content (RWC%)	Electrolyte leakage (EL%)	K^+ ,(mg kg^{-1})	Na^+ ,(mg kg^{-1})	K^+**:**Na^+ ratio	Number of spike (NS)	Kernel number per spike (KNS)	Thousand-kernel weight (gr) (TKW)	Grain yield (GY) ,(kg ha^{-1})
Quality(dS m^{-1}):											
S_1=2	0.799a	44.97b	87.27a	35.42b	1.57a	0.61b	2.64a	517.92a	32.57a	37.86a	6006.30a
S_2=10	0.795a	47.36a	83.99ab	37.80ab	1.54b	0.63b	2.54b	457.81b	29.30b	34.98b	4592.20b
S_3=18	0.792a	43.83c	81.34b	38.98a	1.47c	0.70a	2.22c	389.19c	25.50c	26.94c	2054.40c
LSD 5%	0.015	0.98	4.22	3.14	0.03	0.02	0.10	30.36	1.65	1.28	361.04
Fertilizer:											
F_1=Nano-ZnO	0.795ab	44.06b	83.06 a	41.38a	1.49c	0.62b	2.44b	454.63a	28.09b	32.90a	4163.30a
F_2=Zn-EDTA	0.794ab	45.48b	84.92 a	34.09b	1.57a	0.65a	2.53a	457.07a	29.06ab	33.98a	4365.10a
F_3=Mix	0.801a	48.15a	84.89 a	39.53a	1.52b	0.66a	2.39b	448.41a	29.58ab	33.81a	4209.80a
F_4=Check	0.790b	43.86b	83.93 a	34.60b	1.54b	0.64a	2.38b	459.78a	29.77a	32.35a	4132.40a
LSD 5%	0.015	1.18	3.80	1.95	0.02	0.02	0.07	28.01	1.52	1.85	386.9
Genotype:											
G_1=Moroco	0.795ab	42.57c	79.34c	39.67a	1.34c	0.74a	1.82c	567.56a	18.02c	34.43a	3843.59b
G_2=Nosrat	0.789b	43.89b	83.68b	38.06a	1.52b	0.67b	2.30b	427.03b	33.27b	31.96c	4402.67a
G_3=Khatam	0.801a	49.70a	89.59a	34.46b	1.73a	0.55c	3.18a	370.32c	36.08a	33.39b	4406.68a
LSD 5%	0.015	2.03	1.98	1.92	0.02	0.01	0.06	15.14	1.52	0.82	176.41
S (water quality)	ns	**	*	ns	**	**	**	**	**	**	**
F (zinc fertilizer)	ns	**	ns	**	**	**	**	ns	ns	ns	ns
S*F	**	**	ns	*	**	**	*	ns	ns	**	*
G (genotype)	*	**	**	**	**	**	**	**	**	**	**
G*S	ns	**	ns	ns	**	ns	**	**	**	**	**
G*F	**	**	ns	*	**	**	**	**	*	*	ns
G*S*F	ns	ns	ns	**	**	**	**	**	ns	**	*
CV%	2.23	5.09	4.97	10.81	3.09	4.63	5.52	7.02	10.97	5.20	8.82

Ns,* and **: non significant and significant at 5% and 1% probability levels,respectively.

In each column and for each experimental factor means with a common letter are not statisticlas different

Table 3. Coefficient correlations between traits of three barley- genotypes grown under different salinity levels

Traits	Grain yield (GY)	Fv/Fm	SPAD value	Relative water content (RWC)	Electrolyte leakage (EL)	K^+ ion	Na^+ ion	K^+**:**Na^+ ratio	Number of spike (NS)	Kernel number per spike (KNS)	Thousand-kernel weight (TKW)
GY	1										
Fv/Fm	0.04 ns	1									
SPAD	0.21 *	0.05 ns									
RWC	0.45 **	0.16 ns	0.51 **	1							
EL	-0.21 *	-0.06 ns	-0.24 *	-0.26 **	1						
K+	0.22 *	0.18 ns	-0.43 **	-0.50 **	-0.26 **	1					
Na+	-0.46 **	-0.18 ns	-0.47 **	-0.60 **	0.30 **	-0.63 **	1				
K^+**:**Na^+	0.33 **	0.20 *	0.53 **	0.62 **	-0.33 **	-0.89 **	-0.90 **	1			
NS	0.36 **	-0.03 ns	-0.36 **	-0.32 **	0.07 ns	-0.40 **	0.39 **	-0.46 **	1		
KNS	0.46 **	0.02 ns	0.45 **	0.62 **	-0.31 ns	0.59 **	-0.71 **	0.70 **	1	1	
TKW	0.89 **	0.06 ns	0.20 *	0.32 **	-0.11 ns	0.11 ns	-0.28 **	0.20 *	0.45 **	0.16 ns	1

ns, non significant. ** and *, significant at 0.01 and 0.05 probability levels, respectively

.

In our study, EL decreased in genotypes under salt stress, but the decrease was genotype-salinity-level specific and the tolerant genotpes had lower EL than the sensitive ones (Table 2). EL was negatively correlated (r= -0.21**) with the salinity tolerance of the genotypes (Table 3) which is in agreement with the results of Peng *et al.,* 2008.

Shoot nutrient element contents

The effects of salinity levels, zinc applications and genotypic differences in K^+, Na^+ and K^+:Na^+ were significant ($p \leq 0.01$). With increasing salinity, K^+ and K^+:Na^+ were reduced, but Na^+ contents increased in the shoots. K^+ and K^+:Na^+ contents in the shoots increased by Zn-EDTA applications, but decreased in Nano-ZnO applications in comparison to the check treatment (fertilizer without Zn). K^+ content and K^+:Na^+ ratio were greater in the salt tolerant genotype (Khatam) than the semi salt tolerant genotype Nosrat and the salt sensitive genotype Morocco. There was a positive correlation between grain yield and K^+ and K^+:Na^+ ratio and a negative correlation between Na^+ concentration in shoots and grain yield. Similar results have been reported by Khorshidi *et al.,* (2009). With increasing salinity, the Na+ ion concentration increased in shoot tissues causing an imbalance of nutrient elements and toxic effects on plant growth. Some reports have indicated that an antagonism between absorption of K^+ and Na^+ occurs at the root surface under salinity stress (Ahmadi *et al.,* 2009). Low K^+:Na^+ ratio may indicate increasing K^+ and Ca^{2+} concentrations. The effect of potassium is considered to its ease in the penetration into the plant cell which results in higher protoplasmic change to increase water retaining capacity and resistance to water stress (Tammam *et al.,* 2008). Bartels and Sunkar (2005) have also reported the role of potassium in raising salt tolerance of rice, cotton, wheat, and barley plants, respectively.

Investigations dealing with the development of salt-tolerant varieties have concentrated on the uptake, transport, and accumulation of K^+, Na^+, and Ca^{2+} in plants (Morshedi and Farahbakhash, 2012; Munns and Tester, 2008; James *et al.,* 2008). The concentrations of these nutrients and their ratios (e.g., K^+:Na^+ and Ca^{2+}:Na^+) are reliable, useful and widely used as screening parameters in ranking varieties for their tolerance to salt toxicity. Tavakoli *et al.,* (2010) reported that the salt tolerant barley genotype `Afzal' produced higher dry mass compared to the salt sensitive genotype under salt stress conditions (200 mM NaCl) and that the higher tolerance in the genotype 'Afzal' was associated with higher K^+:Na^+ ratio in the shoots.

Sodium is toxic to cell metabolism and its high concentrations causes reduction in photosynthetic rate and growth (Ehsanzadeh *et al.,* 2009). Increase of Na^+ concentrations in the shoot is one of the plant responses to salinity stress and high Na^+ ion concentrations can change the absorption and accumulation of K^+ ions, which are actively involved in turgor adjustment. A strong negative correlation was found between grain yield and Na^+ concentration (r= -0.46**), and an inverse relationship between shoot Na^+ and K^+ contents (r= -0.63**), and also Na^+ and K^+:Na^+ (r= -0.90**) of barley genotypes grown under different salinity levels (Table 3). Houshmand *et al.,* (2005) reported that the K^+ contents significantly decreased with increasing salinity levels.

Agronomical parameters

Grain yield (GY)

GY was significantly affected by irrigation water quality and genotype ($p \leq 0.01$). GY reduced with increasing salinity levels. GY was reduced in S_2 and S_3 by 24 and 66%, respectively, in comparison with S_1. GY was not affected by zinc application treatments. No significant differences in GY were found among the zinc applications (Table 2). Although zinc fertilizer effects on GY were not significant, F_2 and F_3 had 6 and 2%, higher GY respectively, in comparison to F_4. The highest GY was produced in Zn-EDTA application treatments (Table 2). GY was significantly ($p \leq 0.01$) affected by genotype. GY of the tolerant genotype (G_3=Khatam) was the highest and reduced the most in Morocco (G_1). Despite of non-significant effect of genotypes (G_2 and G_3) on grain yield (GY), there was about 14% increase in GY in comparison with G_1. GY was reduced by both salinity treatments, but genotypic differences were highly significant at high salinity level (S_3). At low (S_1) to moderate (S_2) salinity levels, osmotic stress impacts growth and ionic stress (Na^+-specific effect) at high salinity level (S_3) negatively influences reproductive growth and grain yield (Munns and Tester, 2008). High concentrations of Na^+, which accumulate in the chloroplasts under salinity stress, are known to damage thylakoid membranes and inactivate electron transport and photophosphorylation of isolated thylakoid membranes causing a reduction in photosynthetic capacity (Ashraf and Harris, 2013). Moreover, reduction in yield could be due to decrease in water absorption by plant tissues along with reduction in cellular growth and development as well as the decrease in growth of the plants under salt stress as suggested by pirasteh *et al.* (2016). In agreement with our results, Ashrafi *et al.,* (2014) found that salinity reduced plants' dry weights, but the reduction was cultivar-salt-level-specific due to genetic differences as well as interactions.

GY was positively correlated with shoot K^+:Na^+ ratio (r=0.33**). Negative effects of a low K^+:Na^+ ratio results in lower GY. Khatam with the lowest Na^+ and highest K^+, and Nosrat with low Na^+ and high K^+, and therefore relatively high shoot K^+:Na^+ ratio should be suitable barley genotypes to tolerate the adverse effects of salinity stress. The significant negative correlation between Na^+ concentration and GY (r=-0.46) (Table 3) indicates that the reduction in source activity may affect the accumulation of photo-assimilates within the grains, and the salt-sensitive genotypes (Morocco and Nosrat) with higher Na^+ concentrations may affected more by salinity stress than the tolerant genotype (Khatam) (Mahmood, 2011; Shafaqat, 2012). Poustini and Siosemardeh (2004) and Rahnama *et al.* (2011) have reported that salt tolerant wheat genotypes with the low leaf Na^+ concentration produced grain yields higher than that of the sensitive ones.

Yield components

The effects of salinity levels and genotypes on yield components were highly significant ($p \leq 0.01$), but there were no significant effects of fertilizer applications. Reduction of number of spike (NS) (12 and 25%), kernel number per spike (KNS) (11 and 22%) and thousand-kernel weight (TKW) (8 and 29%) were observed in S_2 and S_3, respectively, in comparison to S_1. Genotypic differences in NS, KNS, and TKW were significant. Khatam (G_3=tolerant) had the highest KNS and lowest NS, but Morocco (G_1=sensitive) had the lowest KNS and the highest NS and TKW (Table 2).

Zn-EDTA application and the Khatam salt tolerant genotype provided higher grain yield, 1000-kernel weight, and K^+ and K^+:Na^+ ratios. Moreover, a significant positive correlation between grain yield with these traits (1000-kernel weight, and K^+ and K^+:Na^+ ratios) showed that the mechanism which caused to maintain high K^+:Na^+ ratio, exclusion of Na^+ or retention of K^+ in shoots, is a key marker for salt avoidance in terms of grain yield.

The Khatam (salt-tolerant) and Nosrat (semi salt-tolerant) are comparatively higher in kernel number per spike, relative water content, Fv/Fm, K^+, K^+:Na^+, and lower Na^+ ion than the salt sensitive genotype Morocco. These findings show that these agrophysiological traits could be the key factors involved in salt tolerance. They could also be used to screen many genotypes in a short time and provide useful information about stress tolerance mechanisms.

The decrease in GY of salt-tolerant genotypes (Khatam and Nosrat) was mainly attributed to a decline in NS, but in the salt-sensitive genotype (Morocco) it was due to a reduction in KNS. Decrease in KNS of Morocco could be due to the lack or availability of photoassimilates accumulation before anthesis that may have reduced the KNS per plant. On the other hand, in Khatam and Nosrat cultivars, there may have been no limitation in photoassimilates accumulation before anthesis resulting in more KNS. Table 3 shows that a significant positive correlation exists between GY and NS (r = 0.33**). Significant correlations also exist between GY and KNS (r = 0.46 **) and TKW (r = 0.89**).

It can be concluded that in the present investigation high salinity decreased agrophysiological parameters (chlorophyll fluorescence, chlorophyll content, relative water content, K^+ ion, K^+:Na^+ ratio, number of spike, kernel number per spike, thousand-kernel weight, grain yield), but increased leaf electrolyte leakage of the flag leaf and Na^+ ion concentration of shoots. This study highlighted the close relationships that exist between grain yield with 1000- kernel weight (R= 0.89**), Na^+ (R= -0.46**), relative water content (0.45**), K^+:Na^+ ratio (R= 0.33**), K^+ ion (R=0.22*), chlorophyll content (r= 0.21**), and electrolyte leakage (R= -0.21**).

REFERENCES

Ahmadi A, Emam Y, Pessarakli M. 2009. Response of various cultivars of wheat and maize to salinity stress. Journal of Food, Agriculture & Environment, 7 (1):123-128.

Ahmadizadeh M, Valizadeh M, Zaefizadeh M, Shahbazi H. 2011. Antioxidative Protection and Electrolyte Leakage in Durum Wheat under Drought Stress Condition. Journal of Applied Sciences Research, 7(3):236-246.

Arnon DI. 1949. Copper enzymes in isolated chloroplast polyphenol oxidase in *Beta vulgaris*. Plant Physiology, 24 (1):1-15.

Ashraf M, Harris PJC. 2013. Photosynthesis under stressful environments: An overview. Photosynthetica, 51(2):163-190.

Ashrafi E, Razmjoo J, Zahedi M, Pessarakli M. 2014. Selecting alfalfa cultivars for salt tolerance based on some physiochemical traits. Agronomy Journal, 106(5):1758-1764.

Azizpour K, Shakiba MR, Khosh Kholg Sima NA, Alyari H, Mogaddam M, Esfandiari E, Pessarakli M. 2010. Physiological response of spring durum wheat genotypes to salinity. Journal of Plant Nutrition, 33:859-873.

Bauder TA, Waskom RM, Sutherland PL, Davis JG. 2014. Irrigation water quality criteria. Colorado state university extension. Fact Sheet No. 0.506, Revised 10/14.

Bartels D, Sunkar R. 2005. Drought and salt tolerance in plants. Critical Reviews in Plant Sciences, 24:23-58.

Bilal AM, Tanveer AK, Qazi F. 2015. 24-epibrassinolide and spermidine modulate photosynthesis and antioxidant systems in Vigna radiata under salt and zinc stress. International Journal of Advanced Research, 3(5):592-608.

Cakmak I. 2008. Enrichment of cereal grains with zinc: Agronomic or genetic biofortification. Plant and Soil, 302:1-17.

Castillo JM, Rubio-Casal AE, Redondo S, Alvarez- López AA, Luque T, Luque C, Nieva FJ, Castellanos EM, Figueroa ME. 2005. Short term responses to salinity of an invasive cordgrass. Biological Invasions, 7:29-35.

Demiral T, Turkan I. 2006. Exogenous glycinebetaine affects growth and proline accumulation and retards senescence in two rice cultivars under NaCl stress. Environmental and Experimental Botany, 56:72-79.

Christensen LP, Peacock WL. 2000. Mineral Nutrition and Fertilization. In: Blank S, Christensen P, Fidelibus M, Hirschfelt D, Klonsky K, Peacock W, Williams L (Eds). Producing the crop, pp. 102-114. University of California, Agricultural and Natural Resources Publication 3393, Oakland, CA.

Ebrahimian E, Bybordi A. 2011. Exogenous silicium and zinc increase antioxidant enzyme activity and alleviate salt stress in leaves of sunflower. Journal of Food, Agriculture and Environment, 9(1):422-427.

Ebrahimi M, Khajehpour MR, Naderi A, Majde Nassiri B (2014). Physiological responses of sunflower to water stress under different levels of zinc fertilizer. International Journal of Plant Production, 8(4):483-504.

Ehsanzadeh P, Sabagh Nekoonam M, Nouri Azhar JH, Pourhadian H, Shaydaee S (2009). Growth, chlorophyll, and cation concentration of tetraploid wheat on a solution high in sodium chloride salt: hulled Versus free-threshing genotypes. Journal of Plant Nutrition, 32: 8-70.

FAO .2014. Global network on integrated soil management for sustainable use of salt-affected soils.

Ganji Arjenaki F, Jabbari R, Morshedi A. 2012. Evaluation of drought stress on relative water content, chlorophyll content and mineral elements of wheat *(Triticum aestivum* L.*)* varieties. International Journal of Agriculture and Crop Sciences, 4:726-729.

Gholinezhad E, Aynaband A, Hassanzade A, Noormohamadi G, Bernousi I. 2009. Study of the effect of drought stress on yield, yield components and harvest index of sunflower hybrid Iroflor at different levels of nitrogen and plant population. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 37(2):85-94.

Hichem H, Ea N, Mounir D. 2009. Effects of salt stress on photosynthesis, PSII photochemistry and thermal energy dissipation in leaves of two corn (*Zea mays* L.) varieties. Photosynthetica, 47:517-526.

Houshmand S, Arzani A, Meaibody AM, Feizi M. 2005. Evaluation of salt-tolerant genotypes of durum wheat derived from in vitro and field experiments. Field Crops Research, 91:345-354.

Izadi MH, Rabbani J, Emam Y, Pessarakli M, Tahmasebi A. 2014. Effects of salinity stress on physiological performance of various wheat and barley cultivars. Journal of Plant Nutrition, 37(4):520-531.

James RA, Rivelli AR, Munns R, Von Caemmerer S. 2002. Factors affecting CO_2 assimilation, leaf injury and growth in salt-stressed durum wheat. Functional Plant Biology, 29:1393-1403.

James RA, Caemmerer SV, Condon AGT, Zwart AB, Munns R. 2008. Genetic variation in tolerance to the osmotic stress component of salinity stress in durum wheat. Functional Plant Biology, 35:111-123.

Kadkhodaei A, Razmjoo J, Zahedi M, Pessarakli M. 2014. Selecting sesame genotypes for drought tolerance based on some physiochemical traits. Agronomy Journal, 106(1):111-118.

Kaya C, Kirnak H, Higgs D. 2001. Enhancement of growth and normal growth parameters by foliar application of potassium and phosphorus on tomato cultivars grown at high (NaCl) salinity. Journal of Plant Nutrition, 24(2):357-367.

Keshavarz P, Saadat S. 2016. Assessment of salinity tolerance threshold for two wheat genotypes in response to zinc. Archives of Agronomy and Soil Science, 62(2):247-259.

Khorshidi MB, Yarnia M, Hassanpanah D. 2009. Salinity effect on nutrients accumulation in alfalfa shoots in hydroponic condition. Journal of Food, Agriculture and Environment, 7 (3&4):787-790.

Khoshgoftarmanesh AH, Shariatmadari H, Karimian N, Kalbasi M, Khajehpour MR. 2004. Zinc efficiency of wheat cultivars grown on a saline calcareous soil. Journal of Plant Nutrition, 27:1953-1962.

Kumar Parida A, Bandhu Das A. 2005. Salt tolerance and salinity effects on plants: a review. Ecotoxicology and Environmental Safety, 60:324-349.

Maghsoodi M, Razmjoo R. 2014. Exploration of root and shoot physiological traits for selecting drought tolerance in alfalfa (*Medicago sativa* L.*)*. Advances in Applied Agricultural Science, 12(2):32-55.

Mohammadkhani N, Heidari R. 2007. Effects of drought stress on soluble proteins in two maize varieties. Turkish Journal of Biology, 32:23-30.

Mahmood K. 2011. Salinity tolerance in barley (*Hordeum vulgare* L.): Effects of varying NaCl, K+/Na+ and NaHCO3 levels on cultivars different in tolerance. Pakistan Journal of Botany, 43(3):1651-1654.

Morshedi A, Farahbakhash H. 2012. The role of potassium and zinc in reducing salinity and alkalinity stress conditions in two wheat genotypes. Archives of Agronomy and Soil Science, 58(4):371-384.

Munns R, Tester M. 2008. Mechanisms of salinity tolerance. Annual Review of Plant Biology, 59:651-681.

Naumann JC, Young DR, Anderson JE. 2007. Linking leaf chlorophyll fluorescence properties physiological responses for detection of salt and drought stress in costal plant species. Physiology Plant, 131:422-433.

Pask AJD, Pietragalla J, Mullan DM, Reynolds MP. 2012. Physiological breeding II: a field guide to wheat phenotyping, CIMMYT.

Peng YL, Gao ZW, Gao Y, Liu GF, Sheng LX, Wang DL. 2008. Eco-physiological Characteristics of Alfalfa Seedlings in Response to Various Mixed Salt-alkaline Stresses. Journal of Integrative Plant Biology, 50(1):29-39.

Roy R, Agrawal V, Gupta SC. 2009. Comparison of drought-induced polypeptides and ion leakage in three tomato cultivars. Biology Plantarum, 53:685-690.

Pirasteh-Anosheh H, Ranjbar G, Pakniyat H, Emam Y. 2016. Physiological mechanisms of salt stress tolerance in plants: An overview. In: Mahgoub Azooz M, Ahmad P (eds), Plant-

Environment Interaction: Responses and Approaches to Mitigate Stress. pp. 141-160. John Wiley & Sons.

Poustini K, Siosemardeh A. 2004. Ion distribution in wheat cultivars in response to salinity stress. Field crops Research, 85:125-133.

Rahnama A, Poustini K, Tavakkol-Afshari R, Ahmadi A, Alizadeh H. 2011. Growth properties and ion distribution in different tissues of bread wheat genotypes *(Triticum aestivum* L.*)* differing in salt tolerance. Journal of Agronomy and Crop Science, 197:21-30.

Statisticat Analysis System Institute Inc. 2007. SAS User's guide: Statistics. Version 9.1.3 Edition. Cary (NC): SAS Institute.

Seckin B, Turkan I, Hediye Sekmen A, Ozfidan C. 2010. The role of antioxidant defense systems at differential salt tolerance of *Hordeum marinum* Huds. (sea barleygrass) and *Hordeum vulgare* L. (cultivated barley). Environmental and Experimental Botany, 69:76-85.

Shafaqat A, Cai S, Zeng F, Qiu B, Zhang G. 2012. Effect of salinity and hexavalent chromium stress on uptake and accumulation of mineral elements in barley genotypes different in salt. Journal of Plant Nutrition, 35:827-839.

Tammam A, Abou Alhamd MF, Hemeda M. 2008. Study of salt tolerance in wheat (*Triticum aestium* L.) cultivar Banysoif 1. Australian Journal of Crop Science, 1:115-125.

Tavakoli F, Vazan SA, Moradi F, Shiran B, Sorkheh K. 2010. Differential response of salt-tolerant and susceptible barley genotypes to salinity stress. Journal of Crop Improvement, 24:244-2