DOI: 10.1007/s11099-017-0699-y PHOTOSYNTHETICA 56 (2): 549-556, 2018

Effect of salt stress on photosynthesis and physiological parameters of three contrasting barley genotypes

M. MAHLOOJI[*], R. SEYED SHARIFI[+,*], J. RAZMJOO[**], M. R. SABZALIAN[**], and M. SEDGHI[*]

Department of Agronomy and Plant Breeding, Faculty of Agricultural and Natural Resources, University of Mohaghegh Ardabili, Daneshgah Street, 5619911367, Ardabil, Iran[*]
Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan University of Technology, Isfahan 8415683111, Iran[**]

Abstract

In order to understand the physiological traits important in conferring salt tolerance in three barley genotypes, this study was performed under field conditions with three water salinity levels (2, 10, and 18 dS m^{-1}). High salinity decreased net photosynthetic rate, transpiration rate, and stomatal conductance, K^+ concentration, K^+:Na^+ ratio, and grain yield, but increased electrolyte leakage and Na^+ content. Under 10 and 18 dS m^{-1} salinity, Khatam (salt-tolerant) had the maximum stomatal conductance, K^+, K^+:Na^+ ratio, and the grain yield, and a minimum Na^+ content and electrolyte leakage, whereas Morocco (salt-sensitive) had the lowest net photosynthetic rate, stomatal conductance, K^+ content, K^+:Na^+ ratio, and grain yield, and the highest Na^+ content and electrolyte leakage. This study showed that tolerant genotypes of barley may avoid Na^+ accumulation in aboveground parts, facilitating a higher photosynthetic rate and higher grain yield.

Additional key words: *Hordeum vulgare*; photosynthesis; gas exchange; salinity.

Introduction

Drought and population rise in the world have increased demands for high-quality water. The use of drainage water, sea water, and recycled water for crop production has been suggested as a part of solutions to such problems (Yordanov *et al.* 2003). However, application of reusable water and reduction in the rainfall cause salinity stress. Salinity stress significantly reduces growth and productivity of plants; it can result in various physiological disturbances, such as osmotic effects, ion-specific stress, ionic imbalance, and oxidative stress (Tabatabaei and Ehsanzadeh 2016). Osmotic stress leads to inhibitions of water uptake which in turn inhibits cell expansion and cell wall synthesis, stomatal conductance, protein synthesis, photosynthetic activity, and lateral bud development (Munns and Tester 2008). Salt is also transported from roots to leaves, leading to ion-specific stress and an increase in leaf mortality with chlorosis and necrosis, and a decrease in the activity of essential cellular metabolic pathways including photosynthesis (Yeo and Flowers 1986, Munns 2005, Witzel *et al.* 2014).

Understanding salt-tolerant mechanisms is imperative for crop improvement in salt-affected areas. Traditional screening techniques for salt tolerance are usually based on the grain yield and are expensive and time-consuming (Kiani-Pouya and Rasouli 2014). In recent years, the focus in screening has shifted towards examining specific physiological traits involved in salt tolerance (Ashrafi *et al.* 2014). Therefore, there is a need for introduction of reliable physiological markers for selection of salt-tolerant genotypes to be planted directly or used in breeding programs. One solution to salinity problem is using salt-tolerant species and cultivation of resistant cultivars within species.

Barley (*Hordeum vulgare L.*) is the most salt-tolerant species among cereals. However, there is a wide variation in salt tolerance within genotypes of this species (Niazi *et al.* 1992, Munns and Tester 2008, Wu *et al.* 2013, Pirasteh-Anosheh *et al.* 2016). Therefore, identifying specific physiological mechanisms is needed to screen for salt-tolerant genotypes within this species. Chen *et al.* (2005) studied several barley cultivars with contrasting salt tolerance and reported that the ability of barley cultivars to retain K^+ under salt stress was the key to their salt tolerance. Ligaba and Katsuhara (2010) also concluded that salt tolerance in barley cultivars was related to their greater ability to maintain K^+ in shoot tissues or transfering

Received 21 May 2016, *accepted* 20 December 2016, *published as online-first* 8 February 2017.
[+]Corresponding author; e-mail: raouf_ssharifi@yahoo.com
Abbreviations: Chl – chlorophyll; *E* – transpiration rate; EL – electrolyte leakage; GMP – geometric mean productivity; g_s – stomatal conductance; P_N – net photosynthetic rate; RWC – relative water content; SPAD – chlorophyll content.

Na^+ to subcellular compartments; however, avoidance of Na^+ accumulation in shoots was not noted. Wu *et al.* (2013) showed that osmotic adjustment was a basic mechanism for salt tolerance in barley. Vysotskaya (2010) compared salt-tolerant and salt-sensitive barley genotypes and suggested that greater tolerance was characterized by lesser inhibition of leaf area, root fresh mass, leaf water content, and chlorophyll (Chl) concentration, but more by reduction in transpiration and hydraulic conductivity. Chen *et al.* (2007) noted that accumulation of compatible solutes had no role for salt-tolerant barley cultivars, whereas a higher K^+ retention, lower NaCl content, and ROS-induced K^+ efflux had crucial roles in salt tolerance. Widodo *et al.* (2009) compared two barley cultivars with contrasting salt tolerance and concluded that increases in sugars, polyols, and a large number of organic acids were associated with the higher tissue tolerance in the salt-tolerant cultivar.

We hypothesized that the selected barley genotypes possess significant diversity in terms of physiological traits under salt stress. Hence, it is necessary to unravel the contribution of each trait to salt tolerance and rank the traits based on their relation with the grain yield. The purpose of this study was (*1*) to investigate the effects of different salt concentrations on gas-exchange characteristics (net photosynthetic rate, transpiration rate, and stomatal conductance), Chl content, electrolyte leakage, relative water content, Na^+ and K^+ contents, and the grain yield of barley cultivars with contrasting salt tolerance (tolerant, intermediate, and sensitive) under field conditions, and (*2*) to find possible mechanisms with which salt-tolerant genotypes of barley endeavor to maintain the grain yield under high salinity.

Materials and methods

Plant material and experimental design: This experiment was conducted in a split-plot design with three replications at Isfahan Rodasht Drainage and Salinity Research Station (32º30′N, 52º9′E) during 2012 to 2015 (together three years). Three irrigation-water salinity levels [control (S_1 = 2 dS m^{-1}), common salinity in the region (S_2 = 10 dS m^{-1}), and high salinity (S_3 = 18 dS m^{-1})] were the main plots, and the barley (*Hordeum vulgare* L.) cultivars [Morocco (salt-sensitive), Nosrat (semi-salt-tolerant), and Khatam (salt-tolerant)] were the subplots. The physical and chemical characteristics of the soil and irrigation water quality are shown in the text table. Mean annual precipitation and temperature during the three years were 93.5 mm and 12°C, respectively, at the experimental site. Seeds were sown with the density rate of 450 seeds m^{-2} on 5 November by a cereal row planting machine (*Wintersteiger Plotman*, Austria). Each subplot consisted of six rows, 6 m in length, and was spaced 20 cm apart. To irrigate the plots, water was delivered from the channel (S_1), a local well (S_2), and mixed drainage water and local water well (S_3).

Soil characteristic	Amount in soil	Water characteristics	Amount in saline water		
			S_1 = 2 [dS m^{-1}]	S_2 = 10 [dS m^{-1}]	S_3 = 18 [dS m^{-1}]
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

Gas-exchange characteristics: Net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s) were measured on flag leaves at a grain-filling stage using an open-system portable infrared gas analyzer *(LCA-4 ACD*, *Analytical Development*, Huddleston, UK). Light intensity was set at 1,200–1,400 μmol(photon) m^{-2} s^{-1} with an infrared/blue light source (Azizian and Sepaskhah 2014). Gas-exchange measurements were determined on upper (adaxial) surface of the mid-portion of flag leaf from 10:00 to 14:00 h.

Nutrient concentrations: After harvesting, nutrient concentrations in the shoots were measured by digestion apparatus methods (Bauder *et al.* 2011). The content of K^+ was determined by an autoanalyzer (*Quikchem IC+FIA 8000 Series, Lachat,* USA), and the content of Na^+ was determined by an atomic absorption spectrometer (*Perkin Elmer Model 3110*, USA) (Bauder *et al.* 2014).

Leaf Chl reading: Leaf Chl content was estimated using a hand-held *SPAD-502* (*Konica-Minolta*, Japan) at the heading stage. Average SPAD Chl readings were calculated from ten individual flag leaves, measured from the tip to the leaf base, between 10:00 and 13:00 h.

Electrolyte leakage (EL): This parameter was measured using the methods of Ahmadizadeh *et al.* (2011). Ten leaf discs, 25 mm in diameter, were punched out from flag leaves at the heading stage, and thoroughly rinsed with distilled water to release material from the wounded edges. The samples were then placed in individual stoppered vials containing 10 ml of distilled water after three washes with distilled water to remove surface contamination, and incubated at room temperature (25ºC) on a shaker (100 rpm) for 24 h. Electrical conductivity (EC) of the solution (EC_1) was recorded after incubation. The same samples were then placed in an autoclave at 120ºC for 20 min, and second reading (EC_2) was taken after cooling the solution to room temperature. Electrolyte leakage was calculated using the following formula:

$$EL\ [\%] = (EC_1/\ EC_2) \times 100.$$

Relative water content (RWC): Relative water content of flag leaves was calculated at the heading stage, according to Pask *et al.* (2011). Five-cm mid-sections of ten leaves were cut from the stem, then placed into a tube and weighed to obtain the fresh mass (FM). In order to determine the turgid mass (TM), 1 cm^3 of distilled water was added to each tube, and the sample tubes were placed in a refrigerator (at 4°C in darkness) for 24 h (to reach full turgor). The leaf samples were then taken out of the tube, quickly and carefully blotted with dry with paper towel, and weighed for their TM. The leaf samples were placed in a labeled envelope and dried at 70°C for 24 h, in order to obtain the dry mass (DM). All mass measurements were made using an analytical scale, with the precision of 0,001 g. Values of FM, TM, and DM were used to calculate RWC using the equation below:

$$RWC\ [\%] = [(FM - DM)/(TM - DM)] \times 100$$

Grain yield (GY) measurement and statistical analysis: GY was measured in 0.4 × 4 m^2 plots. Analyses of variances (*ANOVA*) were conducted on the data to determine differences between the treatments using the general linear model (GLM) in *SAS 9.1* (*SAS Institute*, Cary, NC). Mean comparisons were performed using *Fisher*'s least significant differences (LSD) test at $P \leq 0.05$. Relationships between traits were examined using simple linear correlations, which were performed using *SAS*. Since there were no significant differences between the three years, averaged data were used for statistical analyses.

Salt tolerance: Salt tolerance was calculated according to geometric mean productivity (GMP) (Fernandez 1992):

$$GMP = [Y_{S1} \times Y_{S2\ or\ S3}]\ \hat{}\ 0.5$$

where Y_{S1} is the grain yield under S_1, and $Y_{S2\ or\ S3}$ are the grain yields under S_2 or S_3 stress conditions.

Results and discussion

Gas exchange: Interacting effects of salinity × genotype were found to be statistically significant for P_N and E (Table 1). Salt stress decreased or increased the gas exchange of the cultivars. The highest P_N was observed in Khatam genotype under control (S_1) and S_3, and in Nosrat under the S_2, whereas the lowest P_N was noted in Nosrat under S_1 and in Morocco under the S_2 and S_3 (Fig. 1*A*). P_N increased in Nosrat and Khatam under S_2, but decreased in Morocco and Nosrat and remained unchanged under S_3. E increased in all cultivars under S_2, but decreased in Morocco and Nosrat and remained unchanged in Khatam under S_3 (Fig. 1*B*). The g_s was reduced in all cultivars under S_2 and S_3, but the reduction was the highest in Morocco, followed by Nosrat and Khatam under S_2 and S_3, respectively (Fig. 1*C*).

The effects of salt stress on gas-exchange characteristics were cultivar-, salinity-, and trait-specific. Tolerant cultivars had gas exchange traits unchanged or less affected by salinity. There were significant variations among three cultivars in P_N, E, and g_s under stress conditions, as similarly reported by Sikder *et al.* (2015). Partially in line with our results, Zheng *et al.* (2009), Kanwal *et al.* (2011), Shahbaz *et al.* (2011), Perveen *et al.* (2013), and Pirasteh-Anosheh *et al.* (2016) also reported that salt stress markedly reduced different gas-exchange characteristics, such as P_N, E, and g_s in all examined genotypes. CO_2-exchange parameters have been regarded as an important indicator of plant growth, because of their direct link to net productivity (Ashraf 2004, Piao *et al.* 2008). Stomatal conductance affects the rate of carbon dioxide uptake and water loss through the leaf stomata as determined by the degree of stomatal aperture. The g_s is related to CO_2 movement into the leaf which is controlled by stomatal regulatory processes. Moreover, reduction in gas exchange as a whole and leaf stomata closure could be due to toxic Na^+ and Cl^- ions that decrease photosynthetic electron transport and g_s, along with the reduction in absorption and metabolism of carbon, as well as the oxidative damage to PSII under salt stress, as suggested by Netondo *et al.* (2004), Azizpour *et al.* (2010), Shahbaz and Zia (2011), Shahbaz *et al.* (2011), Ashraf *et al.* (2012), Ashrafi *et al.* (2014), and Pirasteh-Anosheh *et al.* (2016).

Shoot nutrient element contents: Interacting effects of salinity × cultivars were found to be statistically significant on shoot K^+ and Na^+ contents and K^+:Na^+ ratios (Fig. 1*D–F*). The K^+ content and K^+:Na^+ ratio decreased in Morocco under S_2 and S_3, remained unchanged in Nosrat and Khatam under S_2, and decreased in all cultivars under S_3, as compared with the control. The maximum K^+ and the highest K^+:Na^+ ratio were at S_1, and the greatest reduction in these traits was observed in S_3. The Na^+

Table 1. Analysis of variance for the effect of different salt concentrations (S) and genotypes (G) on net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), sodium content in shoot (Na^+), potassium content in shoot (K^+), K^+/Na^+ ratio, chlorophyll content (SPAD), relative water content (RWC), electrolyte leakage (EL), and grain yield (GY) for three years (2012–2015) in barley genotypes. *,** – significant at the 0.05 and 0.01 probability level, respectively; df – degree of freedom; S×G – salinity × genotype interaction; Error (S) – error due to salinity and replications (block) interaction; Error (G) – error due to genotype and salinity interactions; NS – not significant.

Treatments	df	Mean squares									
		P_N	E	g_s	K^+	Na^+	K^+:Na^+ ratio	SPAD	RWC	EL	GY
Block	2	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Salinity (S)	2	**	**	**	**	**	**	**	*	**	**
Error (S)	4	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Genotype (G)	2	**	**	NS	**	**	**	**	NS	**	**
S×G	4	**	**	**	**	**	**	**	*	**	**
Error (G)	12	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

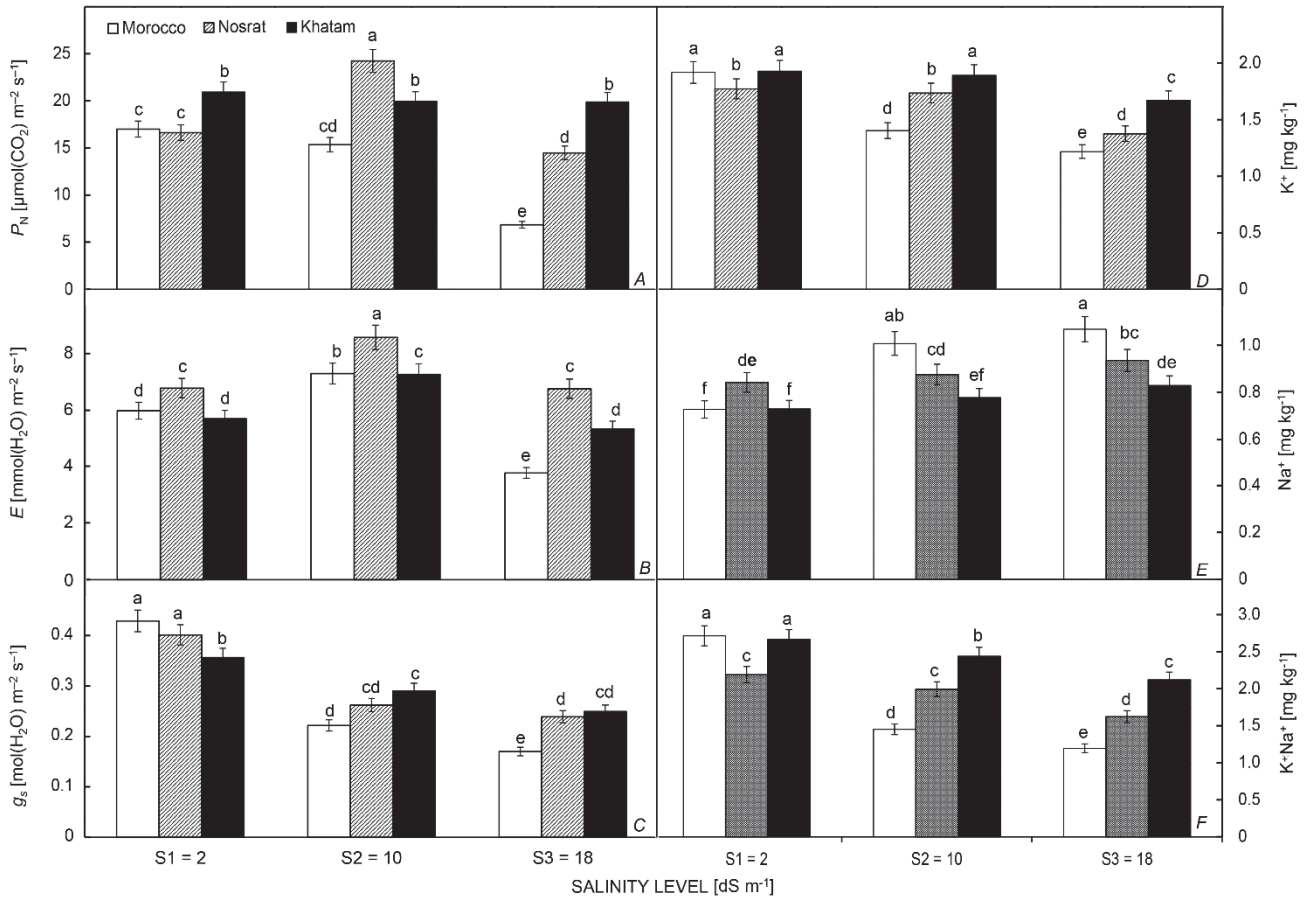

Fig. 1. Effect of salinity level (S) and genotypes (interaction) on the measured traits. All values are the mean of three replications (n = 3) and bars with *different letters* are significantly different at (P≤0.05) according to the LSD test.

content increased in Morocco under both S_2 and S_3, but the increase was not significant (Fig. 1*E*). The Na^+ content also increased in Nosrat and Khatam, but the increase was not significant between S_1 and S_2 and between S_2 and S_3.

With increasing the salinity, the Na^+ content increased, and this might cause the imbalance of the nutrient elements due to competition in uptake and toxicity in plants. It has been reported that the antagonism between the absorption of K^+ and Na^+ occurs at the root surface under salinity stress (Esechie and Rodriguez 1999, Kumar *et al.* 2008, Maggio *et al.* 2001, Ahmadi *et al.* 2009). This has been confirmed in several studies (Esechie and Rodriguez 1999, Parida *et al.* 2004, Khorshidi *et al.* 2009). A low K^+:Na^+ ratio possibly indicated that Ca^{2+}, K^+, and Mg^{2+} transports

were impaired by Na^+ under saline conditions, and this could disturb plant metabolism and reduce plant growth. The results are similar to what has been reported by other researchers (Munns and Tester 2008, James *et al.* 2008, Morshedi and Farahbakhash 2012). The concentrations of these nutrients and their ratios (*e.g.*, K^+:Na^+ and Ca^{2+}:Na^+) are reliable, useful, and widely used screening parameters in ranking cultivars by their tolerance to salt toxicity. The increasing Na^+:K^+ ratio under salt stress was confirmed by Torabi (2010). Tavakoli *et al.* (2010) reported that the salt-tolerant barley genotype 'Afzal' produced a higher dry mass compared to the salt-sensitive genotype under salt stress conditions (200 mM NaCl), and the higher tolerance in genotype Afzal was associated with the higher K^+:Na^+ ratio in the shoots. In contrast to the previous reports, in this study, it seems that the tolerant genotype (Khatam) has the ability to restrict the movement of Na^+ ions to the aboveground parts, avoiding the negative impact of salinity on physiological parameters in barley.

Chl content (SPAD): The Chl content was reduced in Morocco under salt stress, but there was no significant difference between S_2 and S_3 (Fig. 2*G*). It was also reduced in Nosrat under salt stress, but there was no significant difference between S_1 and S_2. The Chl content increased in Khatam under S_2, but remained unchanged under S_1 and S_3. The higher SPAD in Khatam (tolerant genotype) may be related to its ability to repair injury or to use an efficient mechanism for the uptake of necessary elements for Chl under saline soil (Fig. 2*G*).

These results were generally consistent with findings of Akbari Ghogdi *et al.* (2012). Therefore, we can conclude that genotypes having a higher SPAD under salt environment should produce a higher grain yield than those having the lower Chl content. Thus, the selection of cultivars based on increased or stable Chl content may prevent yield losses under salt-stress conditions. Many researchers have suggested that the SPAD measurement could be a useful selection criterion to screen a large set of genotypes for salt tolerance (Munns and James 2003, Atlassi Pak *et al.* 2009, Akhtar *et al.* 2010, Cuin *et al.* 2010). Decrease in Chl content in some of the genotypes under salt stress could be due to pigment photo-oxidation (Gomes *et al.* 2011), loss of chloroplast membranes, distortion of the lamellae vesiculation, and excessive swelling (Ceccarelli *et al.* 2010), damage to chloroplasts by ROS (Gill and Tuteja 2010), their slower synthesis, faster breakdown or dissociation (Bonales-Alatorre *et al.* 2013). Decreased or unchanged Chl contents under salt stress have been reported in several species, depending on the duration and severity of salt stress and reduction in the amount of Chl (Mohammadkhani and Heidari 2008, Pirasteh-Anosheh and Emam 2012, Talat *et al.* 2013). Many factors (genotypic differences, stage of growth, plant diseases, nutrient deficiencies, and stress) can affect Chl content readings. Chl is one of the most important chloroplast components for photosynthesis, because it harvests the light and produces reducing powers; however, Chl is susceptible to salt stress and may affect plant yield and quality (Giunta *et al.* 2002, Kianni-Pouya and Rasouli 2014). Likewise, El-Hendawy *et al.* (2007) found a significant positive relationship between SPAD values and the grain yield under salinity stress. Under saline conditions, Khan *et al.* (2009) and Azizov and Khanisheva (2010) indicated that a higher Chl content may provide an index for the superior grain yield, which is similar to the results found in our study.

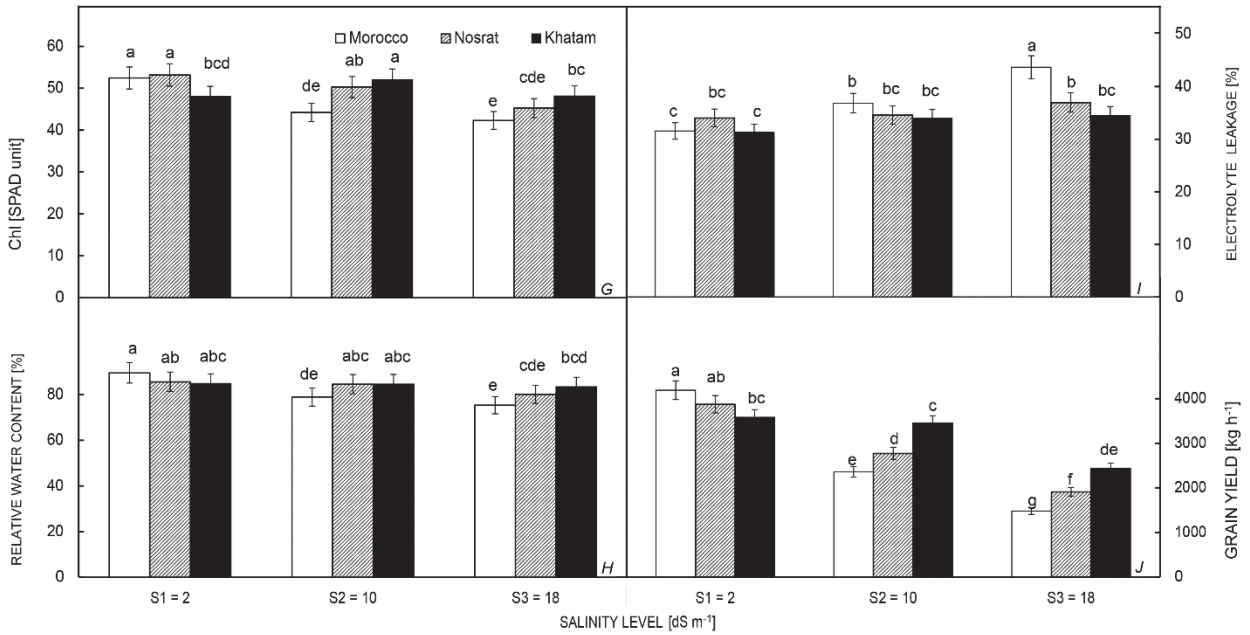

Fig. 2. Effect of salinity level (S) and genotypes (interaction) on the measured traits. All values are the mean of three replications (n = 3) and bars with *different letters* are significantly different at ($P \leq 0.05$) according to the LSD test.

RWC was reduced in Morocco with the increase of salinity. However, there was no marked difference between S_2 and S_3 (Fig. 2*H*). RWC was not affected under S_1 and S_2, but decreased under S_3 in Nosrat (Fig. 2*H*). Nevertheless, RWC in Khatam was not significantly affected under any salinity level in this study. The results demonstrated that the tolerant genotype (Khatam) rather than the sensitive genotype (Morocco) showed a higher RWC under salinity stress. This agrees with the results of El Tayeb *et al.* (2006), Farshadfar *et al.* (2003), and Geravandi *et al.* (2011).

It seems that the high Na^+ absorption under saline conditions was due to impaired water absorption and reduced RWC. Reduction in RWC may be due to the reduction in water, leaf area, E, g_s, absorption of radiation under leaf rolling, production of leaves and yield or increase in leaf senescence and abscission. Similar results were observed by Ebrahimian and Bybordi (2011) and Munns and Tester (2008). It has been shown that the ability to attract and maintain osmotic potential for higher RWC in saline soil water at various stages is an effective mechanism in salt-tolerant genotypes (Kafi *et al.* 2011).

EL: The effects of salinity levels and genotypes on EL were significant. EL increased under salt stress; however, it was cultivar- and salinity level-specific (Table 1). EL increased in Morocco as salinity level increased, while it was not affected in Nosrat and Khatam (Fig. 2*I*).

In line with our results, Kaya *et al.* (2001) and Bajji *et al.* (2001) reported that EL gradually increased under salt conditions, and the varietal differences between the genotypes may offer partial explanations for the differential tolerance to salinity stress. Using the EL, we managed to demonstrate that maintenance of membrane integrity in leaf is a major component of salt tolerance in plants. EL measurements may be correlated with several physiological parameters acclimatizing the plant responses to environmental conditions. It is reported that stress may modify the chemical composition and organic acids (Bajji *et al.* 2001), and the physical structure of biological membranes and plasma membranes (Peng *et al.* 2008, Stevens *et al.* 2006), leading to oxidative damage in plants (Chen *et al.* 2013), which has a direct impact on the rate of electrolyte leakage.

GY was significantly affected by irrigation-water quality and genotype. GY was reduced in Morocco, Nosrat, and Khatam under S_2 and S_3 (Fig. 2*J*). Morocco produced the highest yield under control (4,189 kg ha^{-1}), and Khatam under S_2 (2,772 kg ha^{-1}) and S_3 (2,434 kg ha^{-1}), whereas the lowest GY was found in Khatam under control (3,577 kg ha^{-1}) and in Morocco under S_2 (2,361 kg ha^{-1}) and S_3 (1,478 kg ha^{-1}). However, under S_2 and S_3 salinity levels, Khatam had the smallest and Morocco had the largest reduction in GY for the three years (Fig. 2*J*).

Consistent with these results, many researchers have shown that the growth of plants declined under saline conditions, but the degree of reduction depended on salt contents, environmental conditions, type of genotype, and stage of plant growth (Mahmood 2011, Shafaqat *et al.* 2012). In line with our results, Omar *et al.* (2009) reported that the grain yield and yield components of barley genotypes were reduced under salt stress, but the reduction was greater in the salt-sensitive cultivar compared to the salt-tolerant one. They concluded that this was due to the reduction in physiological and biochemical traits. Moreover, reductions in the yield could be due to the decrease in water absorption by plant tissues along with the reduction in cellular growth and development, as well as the decrease in the growth of the plants under salt stress, as suggested by Pirasteh *et al.* (2016). Similar to our results, Ashrafi *et al.* (2014) found that salinity reduced plant DM, but the reduction was cultivar- and salt content-specific due to the genetic differences and source interactions. In our experiment, the reduction in GY was perhaps due to the increase in Na^+ and reduction in K^+ content, gas exchange, SPAD, and RWC, as indicated in Fig. 2.

Relationships between the traits: A significant and positive correlation was found between the mean grain yield for three years and g_s (r = 0.87, p = 0.01), E (r = 0.75, p = 0.01), and P_N (r = 0.62, p = 0.01), whereas a significant and negative correlation was noted between this trait and EL (r = –0.75, p = 0.01), Na^+ (r = –0.73, p = 0.01), and SPAD (r = –0.49, p = 0.01) under a medium (10 dS m^{-1}) salinity level. In addition, a significant and positive correlation was found between the mean grain yield for three year and K^+:Na^+ ratio (r = 0.90, p = 0.01), P_N (r = 0.89, p = 0.01), E (r = 0.73, p = 0.01), g_s (r = 0.78, p = 0.01), and K^+ (r = 0.50, p = 0.05), whereas a significant and negative correlation was noted between this trait and EL (r = –0.88, p = 0.01) and Na^+ (r = –0.85, p = 0.01) under the highest salinity (18 dS m^{-1}) level.

Conclusion: Based on the correlation coefficients among the measured traits under severe salt stress, the K^+:Na^+ ratio showed the highest correlation with the grain yield in barley genotypes, followed by P_N (r = 0.89, p = 0.01), EL (r = –0.88, p = 0.01), Na^+ (r = –0.85, p = 0.01), g_s (r = 0.78, p = 0.01), E (r = 0.73, p = 0.01), and K^+ (r = 0.50, p = 0.05), respectively. Under 10 and 18 dS m^{-1} salt stress, Khatam (salt-tolerant) showed the maximum g_s, K^+ content, K^+:Na^+ ratio, SPAD, and GY, and had a minimum Na^+ content and EL, whereas Morocco (salt-sensitive) had the lowest P_N, g_s, K^+ content, K^+:Na^+ ratio, SPAD, RWC, and GY, and the highest Na^+ content and EL. We indicated that tolerance in barley depends not only on a higher ratio of K^+:Na^+, but also on avoiding Na^+ accumulation in aboveground plant parts. These findings indicated that these physiological traits could be key factors and useful tools for screening many samples in a short time, and provide useful information about stress tolerance mechanisms, which could be useful to plant breeders for selecting and developing salt-tolerant genotypes.

References

Akhtar J., Saqib Z.A., Sarfraz M. *et al.:* Evaluating salt tolerant cotton genotypes at different levels of NaCl stress in solution and soil culture. – Pak. J. Bot. **42**: 2857-2866, 2010.

Ahmadi A., Emam Y., Pessarakli M.: Response of various cultivars of wheat and maize to salinity stress. – J. Food Agr. Environ.**7**: 123-128, 2009.

Ahmadizadeh M., Valizadeh M., Zaefizadeh M. *et al.*: Antioxidative protection and electrolyte leakage in *Durum* wheat under drought stress condition. – J. Appl Sci. Res. **7**: 236-246, 2011.

Akbari Ghogdi E.A., Izadi-Darbandi A., Borzouei A.: Effects of salinity on some physiological traits in wheat *(Triticum aestivum L.)* cultivars. – Indian J. Sci. Tech. **5**: 1901-1906, 2012.

Ashraf M.: Some important physiological selection criteria for salt tolerance in plants. – Flora **199**: 361-376, 2004.

Ashraf M., Ashraf A.: Salt-induced variation in some potential physiochemical attributes of two genetically diverse spring wheat (*Triticum aestivum L.*) cultivars: photosynthesis and photosystem II efficiency. – Pak. J. Bot. **44**: 53-64, 2012.

Ashrafi E., Razmjoo J., Zahedi M. *et al.*: Selecting alfalfa cultivars for salt tolerance based on some physiochemical traits. – Agron. J. **106**: 1758-1764, 2014.

Atlassi Pak V., Nabipour M., Meskarbash M.: Effect of salt stress on chlorophyll content, fluorescence, Na and K ions content in rape plants (*Brassica napus L.*). – Asian J. Agr. Res. **3**: 28-37, 2009.

Azizian A., Sepaskhah A.R.: Maize response to water, salinity and nitrogen levels: physiological growth parameters and gas exchange. – Int. J. Plant Prod. **8**: 131-162, 2014.

Azizov I.V., Khanisheva M.A.: Pigment content and activity of chloroplasts of wheat genotypes grown under saline environment. – P. Azerbaijan Nat. Acad. Sci. Bio. Sci. **65**: 96-98, 2010.

Azizpour K., Shakiba M.R., Khosh Kholg Sima N.A. *et al.*: Physiological response of spring durum wheat genotypes to salinity. – J. Plant Nutr. **33**: 859-873, 2010.

Bajji M., Kinet J.M., Lutts S.: The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. – Plant. Growth Regul. **36**: 61-70, 2001.

Bauder T.A., Waskom R.M., Sutherland P.L. *et al.*: Irrigation Water Quality Criteria. Colorado State University Extension, Fort Collins 2011.

Bonales-Alatorre E., Shabala S., Chen Z.H. *et al.*: Reduced tonoplast fast activating and slow activating channel activity is essential for conferring salinity tolerance in a facultative halophyte, quinoa. – Plant Physiol. **162**: 940-952, 2013.

Ceccarelli S., Grando S., Maatougui M. *et al.:* Plant breeding and climate changes. – J. Agric. Sci. **148**: 627-637, 2010.

Chen Z.H., Newman I., Zhou M.X. *et al.*: Screening plants for salt tolerance by measuring K^+ flux: a case study for barley. – Plant Cell Environ. **28**: 1230-1246, 2005.

Chen Z., Zhou M.X., Newman I. *et al.*: Potassium and sodium relations in salinized barley tissues as a basis of differential salt tolerance. – Funct. Plant Biol. **34**: 150-162, 2007.

Chen L., Ren J., Shi H. *et al.:* Physiological and molecular responses to salt stress in wild emmer and cultivated wheat. – Plant Mol. Biol. Rep. **31**: 1212-1219, 2013.

Cuin T.A., Parsons D., Shabala S.: Wheat cultivars can be screened for NaCl salinity tolerance by measuring leaf chlorophyll content and shoot sap potassium. – Funct. Plant Biol. **37**: 656-664, 2010.

Ebrahimian E., Bybordi A.: Exogenous silicium and zinc increase antioxidant enzyme activity and alleviate salt stress in leaves of sunflower. – J. Food Agric. Environ. **9**: 422-427, 2011.

El-Hendawy S.E., Hu Y., Schmidhalter U.: Assessing the suitability of various physiological traits to screen wheat genotypes for salt tolerance. – J. Integr. Plant Biol. **49**: 1352-1360, 2007.

El-Tayeb M.A.: Differential response of two *Vicia faba* cultivars to drought: growth, pigments, lipid peroxidation, organic solutes, catalase and peroxidase activity. – Acta Agron. Hung. **54**: 25-37, 2006.

Esechie H., Rodriguez V.: Does salinity inhibit alfalfa leaf growth by reducing tissue concentration of essential mineral nutrients? – J. Agron. Crop Sci. **182**: 273-278, 1999.

Farshadfar E., Mohammadi R., Aghaee M. *et al.* Identification of QTLs involved in physiological and agronomic indicator of drought tolerance in rye using a multiple selection index. – Acta Agron. Hung. **51**: 419-428, 2003.

Fernandez, G.C.J.: Effective selection criteria for assessing stress tolerance. – In: Kuo C.G. (ed.): Proceedings of the International Symposium on Adaptation of Vegetables and Other Food Crops in Temperature and Water Stress. Pp. 257-270. AVRDC Publication, Tainan 1992.

Geravandi M., Farshadfar E., Kahrizi D.: Evaluation of some physiological traits as indicators of drought tolerance in bread wheat genotypes. – Russ. J. Plant Physl+ **58**: 69-75, 2011.

Gill S.S., Tuteja N.: Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. – Plant Physiol. Bioch. **48**: 909-930, 2010.

Giunta F., Motzo R., Deidda M.: SPAD readings and associated leaf traits in durum wheat, barley and triticale cultivars. – Euphytica **125**: 197-205, 2002.

Gomes M.A.C., Suzuki M.S., Cunha M.D. *et al.:* Effect of salt stress on nutrient concentration, photosynthetic pigments, proline and foliar morphology of *Salvinia auriculata* Aubl. – Acta Limnol. Bras. **23**: 164-176, 2011.

James R.A., von Caemmerer S., Condon A.G.T. *et al.:* Genetic variation in tolerance to the osmotic stress component of salinity stress in durum wheat. – Funct. Plant Biol. **35**: 111-123, 2008.

Kafi M., Bagheri A., Nabati J. *et al.*: Effect of salinity on some physiological variables of 11 chickpea genotypes under hydroponic conditions. – J. Sci. Technol. Greenhouse Cult. **1**: 55-70, 2011.

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

Kanwal H., Ashraf M., Shahbaz M.: Assessment of salt tolerance of some newly developed and candidate wheat *(Triticum aestivum L.)* cultivars using gas exchange and chlorophyll fluorescence attributes. – Pak. J. Bot. **43**: 2693-2699, 2011.

Khan M.A., Shirazi M.U., Khan M.A. *et al.*: Role of proline, K/Na ratio and chlorophyll content in salt tolerance of wheat (*Triticum aestivum L.*). – Pak. J. Bot. **41**: 633-638, 2009.

Khorshidi M.B., Yarnia M., Hassanpanah D.: Salinity effect on nutrients accumulation in alfalfa shoots in hydroponic condition. – J. Food Agric. Environ. **7**: 787-790, 2009.

Kiani-Pouya A., Rasouli F.: The potential of leaf chlorophyll content to screen bread-wheat genotypes in saline condition. – Photosynthetica **52**: 288-300, 2014.

Kumar V., Shriram V., Nikam T. *et al.*: Sodium chloride induced changes in mineral nutrients and proline accumulation in indica rice cultivars differing in salt tolerance. – J. Plant Nutr. **31**: 1999-2017, 2008.

Ligaba A., Katsuhara M.: Insights into the salt tolerance mechanism in barley (*Hordeum vulgare*) from comparisons of cultivars that differ in salt sensivity. – J. Plant Res. **123**: 105-118, 2010.

Maggio A., Hasegawa P.M, Bressan R.A. *et al.*: Unraveling the functional relationship between root anatomy and stress tolerance. – Aust. J. Plant Physiol. **28**: 999-1004, 2001.

Mohammadkhani N., Heidari R.: Effects of drought stress on soluble proteins in two maize varieties. – Turk. J. Biol. **32**: 23-30, 2008.

Mahmood K.: Salinity tolerance in barley (*Hordeum vulgare L.*): Effects of varying NaCl, K+/Na+ and $NaHCO_3$ levels on cultivars different in tolerance. – Pak. J. Bot. **43**: 1651-1654, 2011.

Morshedi A., Farahbakhash H.: The role of potassium and zinc in reducing salinity and alkalinity stress conditions in two wheat genotypes. – Arch. Agron. Soil Sci. **58**: 371-384, 2012.

Munns R.: Genes and salt tolerance, bringing them together. – New Phytol. **167**: 645-663, 2005.

Munns R., James R.A.: Screening methods for salinity tolerance: a case study with tetraploid wheat. – Plant Soil **253**: 201-218, 2003.

Munns R., Tester M.: Mechanisms of salinity tolerance. – Annu. Rev. Plant Biol. **59**: 651-681, 2008.

Netondo G.W., Onyango J.C., Beck E.: Sorghum and salinity: II. Gas exchange and chlorophyll fluorescence of sorghum under salt stress. – Crop Sci. **44**: 806-811, 2004.

Niazi M.L.K., Mahmood K., Mujtaba S.M. *et al.*: Salinity tolerance in different cultivars of barley (*Hordeum vulgare L.*). – Biol. Plantarum **34**: 465-469, 1992.

Omar M.N.A., Osman M.E.H., Kasim W.A. *et al.*: Improvement of salt tolerance mechanisms of barley cultivated under salt stress using *Azospirillum brasilense*. – In: Ashraf M., Ozturk M., Athar H.R. (ed.): Salinity and Water Stress. Chapter 15. Pp. 133-147. Springer Science + Business Media B.V, Giza 2009.

Parida A.K., Das A.B., Mittra B.: Effects of salt on growth, ion accumulation photosynthesis and leaf anatomy of the mangrove, *Bruguiera parviflora*. – Trees-Struct. Funct. **18**: 167-174, 2004.

Pask A.J.D., Pietragalla J., Mullan D.M. *et al.*: Physiological Breeding II: a Field Guide to Wheat Phenotyping. CIMMYT, 2011.

Peng Y.L., Gao Z.W., Gao Y. *et al.*: Eco-physiological characteristics of alfalfa seedlings in response to various mixed salt-alkaline stresses. – J. Integr. Plant Biol. **50**: 29-39, 2008.

Perveen S., Shahbaz M., Ashraf M.: Influence of foliar-applied triacontanol on growth, gas exchange characteristics, and chlorophyll fluorescence at different growth stages in wheat under saline conditions. – Photosynthetica **51**: 541-551, 2013.

Piao S., Ciais P., Friedlingstein P. *et al.*: Net carbon dioxide losses of northern ecosystems in response to autumn warming. – Nature **451**: 49-52, 2008.

Pirasteh-Anosheh H., Emam Y.: Manipulation of morphophysiological traits in bread and durum wheat by using PGRs at different water regimes. – J. Crop Prod. Process. **5**: 29-45, 2012.

Pirasteh-Anosheh H., Ranjbar G., Pakniyat H. *et al.:* Physiological mechanisms of salt stress tolerance in plants: An overview. – In: Mahgoub Azooz M., Ahmad P. (ed.): Plant-Environment Interaction: Responses and Approaches to Mitigate Stress. Chapter 8. Pp. 141-160. John Wiley & Sons, Ltd, Jammu and Kashmir 2016.

Shafaqat A., Cai S., Zeng F. *et al.:* Effect of salinity and hexavalent chromium stress on uptake and accumulation of mineral elements in barley genotypes different in salt. – J. Plant Nutr. **35**: 827-839, 2012.

Shahbaz M., Ashraf M., Akram N.A. *et al.*: Salt-induced modulation in growth, photosynthetic capacity, proline content and ion accumulation in sunflower (*Helianthus annuus L.*). – Acta Physiol. Plant. **33**: 1113-1122, 2011.

Shahbaz M., Zia B.: Does exogenous application of glycine-betaine through rooting medium alter rice (*Oryza sativa L.*) mineral nutrient status under saline conditions? – J. Appl. Bot. Food Qual. **84**: 54-60, 2011.

Sikder S., Foulkes J., West H. *et al.*: Evaluation of photosynthetic potential of wheat genotypes under drought condition. – Photosyntheica **53**: 47-54, 2015.

Stevens J., Senaratna T., Sivasithamparam K.: Salicylic acid induces salinity tolerance in tomato (*Lycopersicon esculentum* cv. Roma): associated changes in gas exchange, water relations and membrane stabilization. – Plant Growth Regul. **49**: 77-83, 2006.

Tabatabaei S., Ehsanzadeh P.: Photosynthetic pigments, ionic and antioxidative behaviour of hulled tetraploid wheat in response to NaCl. – Photosyntheica **54**: 340-350, 2016.

Talat A., Nawaz K., Hussian K. *et al.*: Foliar application of proline for salt tolerance of two wheat cultivars. – World Appl. Sci. J. **22**: 547-554, 2013.

Tavakoli F., Vazan S.A., Moradi F. *et al.*: Differential response of salt-tolerant and susceptible barley genotypes to salinity stress. – J. Crop Improve. **24**: 244-260, 2010.

Torabi M.: Physiological and Biochemical Responses of Iranian Alfalfa (*Medicago sativa L.)* Ecotypes to Salt Stress. – PhD. Thesis, Putra University, Putra 2010.

Vysotskaya L., Hedley P.E., Sharipova G. *et al.*: Effect of salinity on water relations of wild barley plants differing in salt tolerance. – AoB Plant **2010**: plq006, 2010.

Widodo, Patterson J.H., Newbigin E. *et al.*: Metabolic responses to salt stress of barley (*Hordeum vulgare L.*) cultivars, Sahara and Clipper, which differ in salinity tolerance. – J. Exp. Bot. **60**: 4089-4103, 2009.

Witzel K., Matros A., Strickert M. *et al.*: Salinity stress in roots of contrasting barley genotypes reveals time-distinct and genotype-specific patterns for defined proteins. – Mol. Plant. **7**: 336-355, 2014.

Wu D., Cai S., Chen M. *et al.*: Tissue metabolic responses to salt stress in wild and cultivated barley. – Plos One **8**: e55431, 2013.

Yardanov I., Velikova V., Tsonev T.: Plant responses to drought and stress tolerance. – Bulg. J. Plant Physiol. **SI**: 187-206, 2003.

Yeo A.R., Flowers T.J.: Salinity resistance in rice *(Oryza sativa L.)* and a pyramiding approach to breeding varieties for saline soils. – Aust. J. Plant Physiol. **13**: 161-173, 1986.

Zheng Y.H., Xu X.B., Wang M.Y. *et al.*: Responses of salt tolerant and intolerant wheat genotypes to sodium chloride: Photosynthesis, antioxidants activities, and yield. – Photosynthetica. **47**: 87-94, 2009.