EFFECTS OF DIFFERENT LEVELS OF SODIUM CHLORIDE ON YIELD AND CHEMICAL COMPOSITION IN TWO BARLEY CULTIVARS

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Abstract. *Salinity of agricultural lands and irrigation water is the most limiting factor for plant growth in many dry parts of the world. Twenty five million ha of agricultural lands are saline in Iran, and this is increasing due to poor irrigation management. Particularly in irrigated agricultural areas, has been found to reduce barley yields, dramatically. To investigate the effects of sodium chloride on two barley cultivars, four levels of salinity: 0, 4, 8 and 12 dS/m, were employed as a factorial experiment arranged in a randomized complete block design with four replications in a controlled environment of the greenhouse during 2007-2008. The results indicated that increasing salinity from 0 to 12 dS/m, decreased the emergence percentage, significantly. The two cultivars (Afzal & Reyhan) responded differently to salinity, so that Afzal showed a significantly higher emergence rate. This cultivar (Afzal) also had greater shoot potassium content. The number of tillers and leaves per plant and also the plant height were decreased upon increasing salinity level. The shoot sodium content was also increased by increasing the salinity level in both cultivars, however, the sodium content of Afzal cultivar, compared to Reyhan cultivar, was lower, probably due to Na^+ exclusion mechanisms in this cultivar. The results also revealed that the highest grain number and phytomass was obtained from Afzal cultivar at the lowest salinity level. Phytomass and grain yield were also decreased upon salinity, significantly. Overall, it appeared that less adverse effect of salinity on Afzal cultivar may indicate that this cultivar it might be suitable for saline soils, an object which worth more investigations.*

Keywords: Salinity, Yield components, Barley, Sodium and Potassium.

INTRODUCTION

In most southern provinces of Iran, Salinity is a growing problem particularly in irrigated agricultural areas with rising water tables, poor water quality and/or deficient soil drainage.

Salt stress is one of the most important abiotic stresses affecting natural productivity and causes significant crop loss worldwide. For plants, the sodium ion (Na+) is harmful whereas the potassium ion (K+) is an essential ion. The cytosol of plant cells normally contains 100–200 mM of K+ and 1–10 mM of Na+ (Taiz and Zeiger, 2002); this Na+/K+ ratio is optimal for many metabolic functions in cells. Physico-chemically, Na+ and K+ are similar cations. Therefore, under the typical NaCl-dominated salt environment in nature, accumulation of high Na+ in the cytosol, and thus high Na+/K+ ratios, disrupts enzymatic functions that are normally activated by K+ in cells (Bhandal and Malik, 1988; Munns et al., 2006).

Therefore, it is very important for cells to maintain a low concentration of cytosolic Na+ or to maintain a low Na+/K+ ratio in the cytosol when under NaCl stress (Maathuis and Amtmann, 1999).

It has been showed that the two responses occur sequentially, giving rise to a two-phase growth response to salinity (Munns, 1993). For example, comparisons between two genotypes with contrasting rates of Na+ uptake, and long-term differences in salt tolerance (Schachtman et al., 1991), showed that both genotypes had the same growth reduction for the 4 first weeks in 150 mM NaCl, and it was not until afterwards that a growth difference between the genotypes was clearly observed (Munns et al., 1995). However, within 2 weeks, dead leaves were visible on the more sensitive genotype and the rates of leaf death of old leaves were clearly greater on the sensitive than the tolerant genotype. Once the number of dead leaves increased above about 20% of the total, plant growth slowed down and many individuals started to die (Munns et al., 1995). Improved salt tolerance of crops can lessen the leaching requirement, and so lessen the costs of an irrigation scheme, both in the need to import fresh water and to dispose of saline water (reviewed by Pitman and Läuchli, 2002). Salt-tolerant crops have a much lower leaching requirement than salt-sensitive ones. In dry-land agriculture, improved salt tolerance can increase yield on saline soils.

In most southern provinces of Iran, where the rainfall is low and the salt remains in the subsoil, increased salt tolerance will allow plants to extract more water. Salt tolerance may have its greatest impact on crops growing on soils with natural salinity as, when all the other agronomic constraints have been overcome (e.g. disease resistance and nutrient deficiency); subsoil salinity remains a major limitation to agriculture in all semi-arid regions as most southern provinces of Iran. Even where clearing of land in higher rainfall zones has caused water-tables to rise and salt to move, improved salt tolerance of crops will have a place. The introduction of deep-rooted perennial species is necessary to lower the water-table, however, salt tolerance will be required not only for the 'de-watering' species, but also for the annual crops that follow, as salt will be left in the soil when the water-table is lowered (Francois et al.,1994).

Barley is a relatively tolerant crop to soil salinity, and genetic variations exist among genotypes of cultivated barley. One of the two new cultivars of barley, used in the present study, Afzal, is an improved hybrid recommended for Salinity areas in most southern provinces of Iran (pakniyat et al, 2003). However, the salt tolerance mechanisms of these varieties have not been studied in detail. The objective of the present study was to quantify plant growth, yield and yield components of the two barley cultivars in relation to various concentrations of NaCl. In addition, NaCl effects on the chemical composition of the plant organs were measured. The experiment was carried out in the Fars province, one of the main barley–growing areas in southern Iran, with more than 430,000 ha barley grown as nearly continuous cropping.

Table 1

Soil properties (0-30 cm) before plant sowing

Year	OC (%)	pH	Sand (%)	Silt (%)	Clay (%)	Soil texture	EC (dSm^{-1})	P ($mg\ kg^{-1}$)	K ($mg\ kg^{-1}$)	Total N (%)
2007-2008	0.73	7.1	7.1	66.6	26.3	Silty loam	0.03	15.5	376	0.07

MATERIAL AND METHOD

Site, Treatment application and Data collection: This experiment was conducted to evaluate the effects of sodium chloride on two barley cultivars (Afzal, a relatively salt tolerant genotype and Reyhan, a salt sensitive cultivar) and four levels of salinity: 0, 4, 8 and 12 dS/m. The desired salinity levels were developed by mixing the required amount of NaCl and CaCl2 (5:1) in soil before filling the pots (0, 2.16, 4.32, 8.64 g/kg soil). The barley crop was sown on 17 November 2007 and harvested on 29 April 2008.The experiment was carried out in a greenhouse at the college of agriculture, Shiraz university, Shiraz Iran (52o 46'E, 29o 50'N, altitude 1810 m asl), 12 km north of Shiraz, on a Fine mixed, mesic Typic Calcixerpets soil with air temperature in the range of about 25 to 30 °C and light intensity in the range of about 600–1000 µmol m–2 s–1, and was conducted using as a factorial experiment arranged in a randomized complete block design with four replications. Soil properties are shown in Table 1. Pre-germinated seeds were sown in 5 L perforated plastic pots filled with fertilized (50, 25 and 25 N, P and K mg kg–1, respectively) and were kept in concrete tanks filled with tap water according to Maas et al., (1986). The level of water was maintained at 3 cm below the soil surface for 2 days. Ten seeds of each cultivar were sown in each pot, thinned to five seedlings at two-leaf stage. The pots were kept flooded thereafter for the rest of the experiment. The emergence percentage and number of leaves per plant were recorded throughout the experiment. Plants were harvested and threshed manually. The data regarding grain number and straw yields and grain weight, number of spikes per plant, number of tillers per plant and shoot length were recorded (Wilhelm et al., 1989).

Sodium and potassium measurements: Dried samples at the harvesting date were ground to a fine powder and about 0·1 g was transferred to a test tube containing 10 mL of 0·1 N acetic acid, and heated in a water bath at 80 °C for 2 h. The extracted tissue was cooled at room temperature and left overnight, and then filtered using Whiteman filter paper number 40. Sodium and potassium concentrations were then determined using an atomic absorption spectrometer (Munns and James, 2003).

Proline measurements: Fresh flag leaf tissue (0·5 g) was ground in liquid nitrogen and then extracted in 20 ml of hot water for 30 min with moderate shaking. The homogenate was centrifuged at 5000 g for 10 min. The proline concentration was quantified using the ninhydrin acid reagent method as described by Bates *et al.* (1973) using L-proline as a standard.

Statistical analysis; Statistical analysis was performed for each parameter studied based on a randomized complete block design model with four replications using SAS software (SAS Inst., 1985). Means were separated by Duncan's Multiple Range Tests at $p \leq 0.05$.

Table 2

Mean comparison of main and interaction effects of morphological traits

Treatments	Emergence percent	Leaves per plant	Tiller per plant	Plant height (cm)	Spike per plant
Cultivars					
(V_1) Reyhan	52.58 a	5.11 a	1.13 a	30.26 a	1.16 a
(V_2) Afzal	61.41 a	7.06 a	1.69 a	32.06 a	1.31 a
Salinity (dS/m)					
(S_0) 0	93.00 a	13.23 a	3.00 a	52.17 a	2.40 a
(S_1) 4	93.57 a	10.10 b	2.50 a	42.67 b	1.53 b
(S_2) 8	54.00 b	3.23 c	1.16 b	27.50 c	0.73 c
(S_3) 12	3.23 c	-$^{+}$	-	-	-
V_1S_0	91.67 a	13.13 a	2.06 ab	47.33 ab	2.13 ab
V_1S_1	92.00 a	8.23 b	2.10 bc	47.33 ab	1.26 bc
V_1S_2	45.67 c	2.10 cd	0.46 d	24.33 d	0.16 de
V_1S_3	0.00 e	0.00 d	0.00 d	0.00 d	0.00 d
V_2S_0	94.33 a	13.33 a	3.13 a	57.00 a	2.16 a
V_2S_1	92.33 a	11.57 a	3.00 a	40.00 bc	2.20 ab
V_2S_2	62.33 b	4.32 c	1.46 c	30.67 cd	1.10 cd
V_2S_3	6.16 d	-	-	-	-

Means at each column for each character, followed by similar letters are not significantly different using Duncan's multiple range tests ($p \leq 0.05$). $^{+}$ No plants growth due to salinity

RESULTS AND DISCUSSION

Effect of different sodium chloride levels on growth and morphological characteristics: Experimental treatment had significant effects on morphological traits of both cultivars. The results indicated that increasing salinity from 0 to 12 dS/m, decreased emergence percentage significantly. The two cultivars (Afzal & Reyhan) responded differently to salinity, so that Afzal showed significantly higher emergence rate. The number of tillers and leaves per plant and also the plant height were decreased upon increasing salinity level (Table 2), which is in agreement with the finding of Abdullah et al., 1978. It was found that Afzal was superior to Reyhan as far as the salinity tolerance characteristics (as shown in Table 2) were concerned. Kingsbury et al., (1984) showed that the major difference between two lines of barley in salinity tolerance was their response to specific ion effects, at the level of the organ, tissue, cell, and sub-cellular entities. Superior compartmentation of toxic ions by the more salt-tolerant line, presumably in the vacuole, might have enabled it to maintain its cytoplasmic metabolic apparatus in a stable and more nearly normal state than the sensitive line. Therefore, a measure of true cytoplasmic toleration of salt may also be needed to be considered as a factor. The first phases of the growth response results from the effect of salt outside the plant i.e. the salt in the soil solution (the osmotic stresses) reduces leaf growth as shown in Table 2. Indeed, salts themselves do not build up in the growing tissues at concentrations that inhibit growth, as the rapidly elongating cells can accommodate the salt that arrives in the xylem within their expanding vacuoles. So, the salt taken up by the plant does not directly inhibit the growth of new leaves (Munns, 1993).

The second phase of the growth response results from the toxic effect of salt inside the plant. The salt taken up by the plant concentrates in the old leaves; continued transport of salt into transpiring leaves over a long period of time eventually results in very high Na+ and Cl– concentrations, and the leaves died as it was observed in our experiment (see Table 2 and 4). The cause of the injury is probably due to the salt load exceeding the ability of the cells to compartmentalize salts in the vacuole. Salts then would rapidly build up in the cytoplasm and inhibit enzyme activity (Munns, 1993). Alternatively, they might build up in the cell walls and dehydrate the cell (Flowers et al., 1991). However, Mühling and Läuchli (2002) found no evidence for this in maize cultivars that differed in salt tolerance.

Table 3

Mean comparison of main and interaction effects of yield and yield components of two barley cultivars

Treatments	No of grains per plants	Grains weight per plant (g)	Grain yield per plant (g)	Phytomass (g)	Leaf area at anthesis (cm^2)	Straw weight (g)	Spike weight (g)
Cultivars							
(V_1) Reyhan	10.75 a	0.19 a	1.55 b	3.11 b	4700 a	1.28 b	2.70 b
(V_2) Afzal	12.96 a	0.18 a	2.25 a	4.01 a	3800 b	1.45 a	3.50 a
Salinity (dS/m)							
(S_0) 0	19.17 a	0.33 a	8.04 a	11.57 a	4900 a	3.07 a	10.20 a
(S_1) 4	15.00 ab	0.35 a	3.55 b	6.11 b	3950 b	2.11 b	5.65 b
(S_2) 8	10.67 b	0.03 b	0.33 c	1.26 c	2800 c	0.86 c	0.88 c
(S_3) 12	-$^{+}$	-	-	-	-	-	-
V_1S_0	14.00 ab	0.44 a	6.26 a	10.25 a	4350 a	2.45 b	10.15 a
V_1S_1	14.33 ab	0.21 bc	2.66 b	5.11 b	2500 ab	2.15 b	4.51 b
V_1S_2	10.67 bc	0.01 c	0.12 c	0.59 c	1900 d	0.43 d	0.34 c
V_1S_3	-	-	-	-	-	-	-
V_2S_0	24.33 a	0.13 ab	7.03 a	12.49 a	4750 a	3.43 a	11.29 a
V_2S_1	15.67 ab	0.32 abc	4.50 b	8.18 b	4210 b	2.18 b	6.48 b
V_2S_2	10.67 bc	0.05 bc	0.35 c	3.11 c	2700 c	1.19 c	1.21 c

Means at each column for each character, followed by similar letters are not significantly different using Duncan's multiple range tests ($p \leq 0.05$).$^{+}$ No plants growth due to salinity

Relationship between salinity and yield components: The results revealed that the highest grain number and phytomass was obtained from Afzal cultivar at the lowest salinity level (Table 3). Phytomass and grain yield were also decreased upon salinity, significantly. Yield reduction was attributed primarily to reduced spike weight and individual seed weight rather than spike number (Table 3). This finding confirms the results of Francois, *et al*., (1989). The straw yield was more sensitive to salinity than was the grain yield **(**Table 3). Our results also suggest that estimates of grain yield might bring another complexity to the salinity response, not just because the crops must be grown in controlled environments for long periods of time, but also due to complexity of the conversion of shoot biomass in to the grain. A low level of salinity may not reduce grain weight even though the leaf area and phytomass is reduced **(**Table 3). The fact that grain yield may not decrease until a given (‘threshold’) salinity is reached (Maas and Hoffman, 1977).

Effect of different sodium chloride levels on chemical composition: Our results showed that Afzal cultivar had greater shoot potassium concentration (Table 4). The shoot sodium concentration was also increased by increasing the salinity level in both cultivars; however, the sodium content of Afzal cultivar, compared to Reyhan, was lower probably due to Na^+ exclusion mechanisms in this cultivar (Table 4). The increase in Na^+ and Cl^- and decrease in K^+ contents of barley grains suggest that the effect of salinity on the physiological phenomenon studied is due to changes in the ionic content of the plants (Abdullah, *et al*., 1978).

Table 4

Mean comparison of main and interaction effects of chemical composition of two barley cultivars

Treatments	K^+ (mmol per Kg)	Na^+ (mmol per Kg)	Proline (μ g/g)
Cultivars			
(V_1) Reyhan	220.70 b	151.10 a	0.24 b
(V_2) Afzal	415.50 a	13.80 b	0.32 a
Salinity (dS/m)			
(S_0) 0	319.40 c	94.10 d	0.25 d
(S_1) 4	410.70 b	87.30 b	0.27 b
(S_2) 8	586.50 a	160.50 a	0.41 a
(S_3) 12	-	-	-
V_1S_0	287.20 d	141.14 d	0.25 d
V_1S_1	209.00 d	168.80 b	0.26 b
V_1S_2	394.90 c	318.40 a	0.33 a
V_1S_3	-	-	-
V_2S_0	351.70 c	46.80 de	0.29 d
V_2S_1	612.30 b	5.80 e	0.30 ab
V_2S_2	778.10 a	2.50 e	0.37 a

Means at each column for each character, followed by similar letters are not significantly different using Duncan's multiple range tests ($p \leq 0.05$).$^+$ No plants growth due to salinity

Other approaches to improving salt tolerance in barley are based on mechanisms for salt tolerance, using physiological traits to select germplasm. In barley, salt tolerance is associated with low rates of transport of Na^+ to shoots, with high selectivity for K^+ over Na^+ (Gorham *et al.*, 1987, 1990). Correlations between grain yield and Na^+ exclusion from leaves, along with the associated enhanced K^+/Na^+ discrimination, have been shown in barley (Chhipa and Lal, 1995; Ashraf and O'Leary, 1996; Ashraf and Khanum, 1997), although the relationship may not hold across all genotypes (Ashraf and McNeilly, 1988; El-Hendawy *et al*., 2005), showing that Na^+ exclusion is not the only mechanism of salt tolerance (Colmer *et al*., 2005).

There is a strong correlation between salt exclusion and salt tolerance in many species (reviewed by Läuchli, 1984; Munns and James, 2003). Figure 1shows the negative relationship between leaf Na^+ concentration and salt tolerance of Afzal cultivar. In general; the Afzal cultivar compared to Reyhan, with the lowest Na^+ concentrations produced greater dry matter (Table 4). This low-Na^+ genotype had fewer injured leaves, and a greater proportion of living to dead leaves, as observed during the experiment. The effect on growth was probably due to a better carbon balance in the genotype with less Na^+ and also a similar relationship between shoot dry matter and leaf Na^+ was found in a population from a cross between high- and low-Na^+ genotypes (Munns and James, 2003).

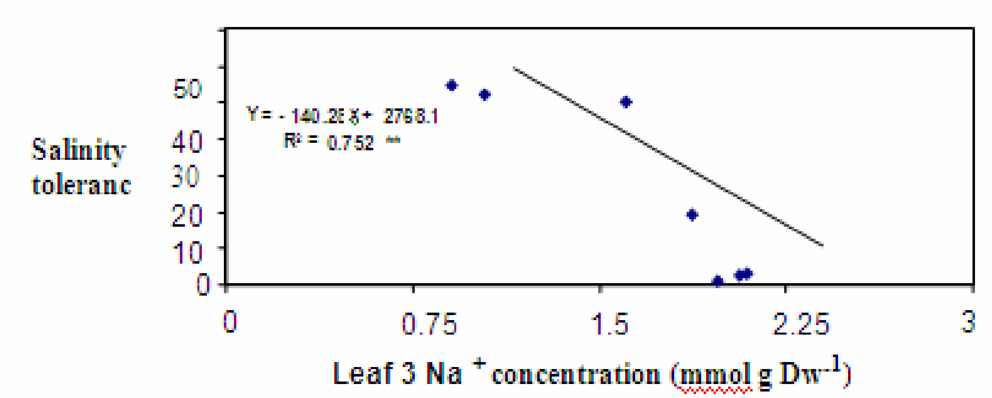

Fig. 1. Relationship between salinity tolerance (% growth of controls) and leaf Na^+ concentration in Afzal cultivar. Na^+ concentrations were measured on leaf 3 after 10 d in 150 mM NaCl and shoot biomass after 24 d. Values are expressed as a percentage of shoot biomass in control conditions (R^2=0.752).All values are means (*n*=5). Salinity tolerance measured as biomass in salt % control.

The results showed that there was a significant difference among different salinity levels for proline content of the two cultivars, and Afzal cultivar had greater proline content (Table 4). The proline content was also increased by increasing the salinity level in both cultivars (Table 4). Moradi and Ismail (2007) reported that it has been repeatedly inferred, but not yet proven, that there might be a relationship between salt tolerance and the accumulation of proline and other metabolites for osmotic adjustment. However, the Colmer *et al.*, (1995) suggest that the increase in proline concentration may not be associated with salinity tolerance. Indeed, elevated proline levels may also confer additional regulatory or osmoprotective functions under salt stress, such as its role in the control of the activity of plasma membrane transporters involved in cell osmotic adjustment in barley roots (Cuin and Shabala, 2005).

CONCLUSIONS

Our results indicated that the two cultivars (Afzal & Reyhan) responded differently to salinity, so that Afzal showed significantly higher emergence rate. This cultivar (Afzal) also had greater shoot potassium content. The number of tillers and leaves per plant and also the plant height were decreased in both cultivars upon increasing salinity level. The shoot sodium content was also increased by increasing the salinity level in both cultivars; however, the sodium content of Afzal cultivar, compared to Reyhan, was lower probably due to Na^+ exclusion mechanisms in this cultivar. The results also revealed that the highest grain number and phytomass was obtained from Afzal cultivar at the lowest salinity level. Phytomass and grain yield were also decreased upon salinity significantly. Afzal, which is a tolerant cultivar, originates from Ardekan (yazd) which is a dry, saline area in the centeral of Iran. Therefore, it may concluded that not surprisingly harsh environment due to salinity may contain tolerant genotypes as a result of natural selection. Overall, it appeared that less adverse effect of salinity on Afzal cultivar may make it more suitable for growth in saline soils. This subject is worthy of further explorations.

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