African Journal of Agricultural Research Vol. 6(20), pp. 4778-4784, 26 September, 2011
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR10.874
ISSN 1991-637X ©2011 Academic Journals

Full Length Research Paper

Use of new barley cultivar to improve salt tolerance and yield in cultivated barley (*Hordeum vulgare* L.)

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Accepted 5 January, 2011

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. 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 2008 to 2009. The results indicated that increasing salinity from 0 to 12 dS/m, decreased the emergence percentage, significantly. The two cultivars (Fajr30 and Reyhan) responded differently to salinity, so that Fajr30 showed a significantly higher emergence rate. This cultivar (Fajr30) 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 Fajr30 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 Fajr30 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 Fajr30 cultivar, may indicate that this cultivar might be suitable for saline soils, an object which is worth more investigations.

Key words: Salinity, yield components, barley, sodium, potassium.

INTRODUCTION

Salt stress is one of the most important abiotic stresses affecting natural productivity and causes significant crop loss worldwide (Pakniyat et al., 2003). For plants, the sodium ion (Na^+) is harmful whereas the potassium ion (K^+) is an essential ion (Munns et al., 1995). The cytosol of plant cells normally contains 100 to 200 mM of K^+ and 1 to 10 mM of Na^+ (Taiz and Zeiger, 2002); this Na^+/K^+ ratio is optimal (in barley is 0.29 mmol per Kg) 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 first 4 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

Table 1. Soil properties (0 to 30 cm) before plant sowing.

Year	℃(%)	pH	Sand (%)	Silt (%)	Clay (%)	Soil texture	EC (dSm^{-1})	P ($mg\ kg^{-1}$)	K ($mg\ kg^{-1}$)	Total N (%)
2008-09	0.73	7.1	7.1	66.6	26.3	Silty loam	0.03	15.5	376	0.07

scheme, both in the need to import fresh water and to dispose of saline water (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 (Pakniyat et al., 2003). One of the two new cultivars of barley, used in the present study, Fajr 30, is an improved hybrid recommended for salinity areas in most southern provinces of Iran. Fajr 30 barley cultivar has been released based on its high grain yield and yield stability, as well as its desirable agronomic characteristics, which have contributed to its wide adaptation in temperate areas of Iran. Fajr 30 barley cultivar is moderately susceptible to powdery mildew, resistant to lodging, and tolerant to low temperatures and drought, Released by Seed and Plant Improvement Institute of Iran. 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.

MATERIALS AND METHODS

Site, treatment application and data collection

This experiment was conducted to evaluate the effects of sodium chloride on two barley cultivars (Fajr 30, 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 $CaCl_2$ (5:1) in soil before filling the pots (0, 2.16, 4.32, 8.64 g/kg soil). The barley crop was sown on 10 November 2008 and harvested on 30 April 2009. The experiment was carried out in a greenhouse (15×20 m) at the college of agriculture, Shiraz university, Shiraz Iran (52° 46'E, 29° 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℃ and light intensity (photon flux density) in the range of about 600 to 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 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℃ for 2 h. The extracted tissue was cooled at room temperature (4℃) and left overnight, and then filtered using Whatman 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

Table 2. Analysis of variance for yield and yield components (Mean of squares=MS).

Source	D.F	Grain yield	1000-grain weight	Grains per spike	Fertile spikes	Straw weight (g)	Spike weight (g)
Salt (S)	3	50.17**	12.416**	2.090**	14.326**	11.57**	3.07**
Cultivars (C)	1	7.5**	17.049**	2.870**	0.050**	6.11 **	2.11**
S x C	3	7.3**	17.156**	7.122**	2.341**	1.26 **	0.86 **
Replication	3	6.9	21.298	2.40	0.073	0.59	0.25
Error	4	9.9	18.060	0.407	0.107	2.59	1.23

*and** : Significant at the 5% and 1% levels, respectively.*

Table 3. 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) Fajr 30	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

model with four replications using SAS software (SAS Inst., 1985). Means were separated by Duncan's Multiple Range Tests at $p \leq 0.05$.

RESULTS

Analysis of variance for yield and yield components are shown in Table 2. The results indicated that, interaction between salt and cultivars were significant for yield and yield component. Increasing salinity from 0 to 12 dS/m, decreased emergence percentage significantly. The two cultivars (Fajr30 and Reyhan) responded differently to salinity, so that Fajr30 showed significantly higher emergence rate. The results revealed that, the highest grain number and phytomass was obtained from Fajr30 cultivar at the lowest salinity level (Table 4). Phytomass and grain yield were also decreased upon salinity, significantly. It was found that Fajr30 was superior to Reyhan, as far as the salinity tolerance characteristics (Table 3) were concerned. Our results showed that Fajr30 cultivar had greater shoot potassium concentration (Table 5). Figure1 shows the negative relationship between leaf Na^+ concentration and salt tolerance of Fajr30 cultivar. In general, the Fajr30cultivar compared to Reyhan, with the lowest Na^+ concentrations produced greater dry matter (Table 5). The results showed that, there was a significant difference among different salinity levels for proline content of the two cultivars, and Fajr30 cultivar had greater proline content (Table 5).

DISCUSSION

Effect of different sodium chloride levels on growth and morphological characteristics

Experimental treatment had significant effects on morphological traits of both cultivars. The number of

Table 4. 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	10.25 a	0.19 a	1.55 b	3.11 b	4600 a	1.28 b	2.70 b
(V_2)Fajr 30	12.16 a	0.18 a	2.25 a	4.01 a	3700 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.

Table 5. Mean comparison of main and interaction effects of chemical composition of two barley cultivars.

Treatments	K^+ (mmol per Kg)	Na^+ (mmol per Kg)	Proline (µg/g)
Cultivars			
(V_1) Reyhan	210.70 b	15.10 a	0.24 b
(V_2) Fajr 30	410.50 a	13.70 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.

tillers and leaves per plant and also the plant height were decreased upon increasing salinity level (Table 3), which is in agreement with the finding of Abdullah et al. (1978). 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

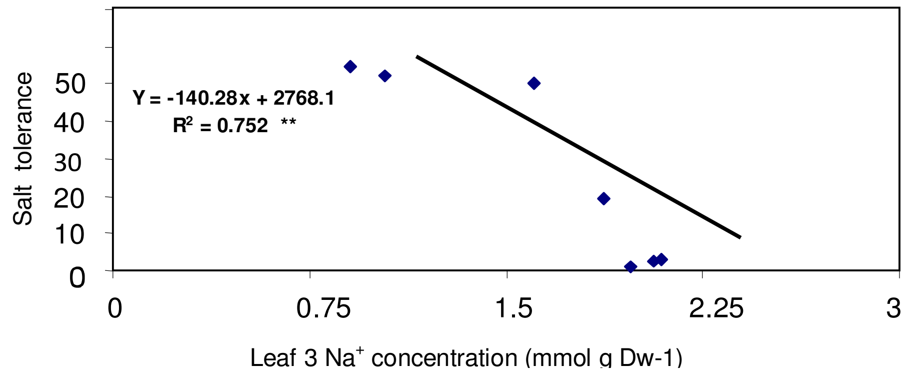

Figure 1. Relationship between salinity tolerance (% growth of controls) and leaf Na^+ concentration in Fajr30 cultivar. Na^+ concentrations were measured on leaf 3 after 10 days in 150 mM NaCl and shoot biomass after 24 days. Values are expressed as a percentage of shoot biomass in control conditions (R^2=0.752).All values are means (n=5). Salinity tolerance measured as biomass in salt % control.

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, that is, 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). There is a strong correlation between salt exclusion and salt tolerance in many species (Läuchli, 1984; Munns and James, 2003).

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 a very high Na^+ and Cl^- concentrations, and the leaves died as it was observed in our experiment (Tables 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.

Relationship between salinity and yield components

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 4). 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 into the grain. A low level of salinity may not reduce grain weight, even though the leaf area and phytomass is reduced (Table 4). 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

The shoot sodium concentration was also increased by increasing the salinity level in both cultivars; however, the sodium content of Fajr30 cultivar compared to Reyhan, was lower probably due to Na^+ exclusion mechanisms in this cultivar (Table 5). 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). 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 (Läuchli, 1984; Munns and James, 2003). Figure 1 shows the negative relationship between leaf Na^+ concentration and salt tolerance of Fajr30 cultivar. Fajr30 cultivar compared to Reyhan, with the lowest Na^+ concentrations produced greater dry matter (Table 4). It seems that this low-Na^+ genotype had fewer injured leaves, and a greater proportion of living to dead leaves, as observed during the experiment the same result showed by Munns and James (2003). 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).

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, 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).

Conclusion

Our results indicated that the two cultivars (Fajr30 and Reyhan) responded differently to salinity, so that Fajr30 showed significantly higher emergence rate. This cultivar (Fajr30) 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 Fajr30 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 Fajr30 cultivar at the lowest salinity level. Phytomass and grain yield were also decreased upon salinity significantly. Fajr30, which is a tolerant cultivar, originates from Ardekan (yazd) which is a dry, saline area in the center of Iran. Therefore, it may be 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 Fajr30 cultivar, may make it more suitable for growth in saline soils. This subject is worthy of further explorations.

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