Effect of salt stress on physiology and agronomy characteristics of barley cultivars

Farshad Sorkhi*

(Department of Agronomy and Plant Breeding, Miandoab Branch, Islamic Azad University, Miandoab, Iran.)

Abstract: In most northwest provinces of Iran, such as West Azarbaijan Province, soil salinity is a growing problem, particularly in irrigated agricultural areas. To investigate the effect of sodium chloride on two barley (*Hordeum vulgare*) cultivars, four levels of salinity (0, 4, 8, 12 dS m^{-1}) were employed in a factorial experiment in a greenhouse with controlled environment during 2017-2018. Increasing salinity stress from 0 to 12 dS m^{-1} significantly reduces emergence percentage. Two cultivars of Zarjoo and Valfajr responded differently to salinity. Zarjoo showed a significantly higher emergence rate. This cultivar had greater shoot potassium content. In two cultivars, salinity dcreases number of tillers, leaves per plant and plant height, phytomass and grain yield but increases the shoot sodium content when the salinity level was elevated. However, comparing with Valfajr, sodium content of Zarjoo was lower, probably due to Na^+ exclusion mechanisms in this cultivar. The highest grain number and phytomass were obtained from Zarjoo at the lowest salinity level. Less adverse effect of salinity on Zarjoo indicated that this cultivar might be suitable for growing in saline soils.

Keywords: emergence percentage, potassium, proline, salinity, yield components

Citation: Sorkhi, F. 2020. Effect of salt stress on physiology and agronomy characteristics of barley cultivars. Agricultural Engineering International: CIGR Journal, 22(3):69-75.

1 Introduction

In most northwest provinces of Iran, such as West Azarbaijan Province, salinity is a growing problem particularly in irrigated agricultural areas with rising water table, poor water quality and/or deficient soil drainage. Soil salinity has reduced barley yield usually when values of electrical conductivity were above 6 dS m^{-1} throughout the root zone (Kamboj et al., 2015). 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

Received date: 2019-09-07 **Accepted date:** 2019-11-29

***Corresponding author: Farshad Sorkhi,** Department of Agronomy and Plant Breeding, Miandoab Branch, Islamic Azad University, Miandoab, Iran. Email: farsorkh@gmail.com

of plant cells normally contains 100-200 mmol of K^+ and 1^{-10} mmol of Na^+ (Taiz and Zeiger, 2002), and the Na^+/K^+ ratio is optimal for many metabolic functions in cells. Physico-chemically, Na^+ and K^+ are similar cations. Under the typical NaCl-dominated salt environment in nature, accumulation of high Na^+ in the cytosol and high Na^+/K^+ ratio disrupt enzymatic functions that are normally activated by K^+ in cells (Taiz and Zeiger, 2002). 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 under NaCl stress (Maathuis and Amtmann, 1999). It has been showed that the two responses to salinity occur sequentially (Nevo and Chen, 2010). For example, comparing of two genotypes with contrasting uptake rates of Na^+ and long-term differences in salt tolerance (Munns and Gilliham, 2015), showed that both genotypes had similar growth reduction for the first four weeks in 150 mmol NaCl, and it was not clearly observed until a growth difference between the genotypes appeared (El-Monem et al., 2013). However, within two weeks, dead leaves were visible on the sensitive genotype and the death rate of old leaves were clearly greater on the sensitive genotype than on the tolerant genotype. Once the number of dead leaves increased above about 20% of the total, the plant growth would slow down and many individuals started to die (Munns and James, 2003). Improved salt tolerance of crops can lessen the leaching requirement, and lessen the costs of an irrigation scheme, both in the need to import fresh water and to dispose of saline water (Pitman and Lauchli, 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 the saline soils. In most southern provinces of Iran, where the rainfall is low and the salt remains in the subsoil, increasing salt tolerance will allow plants to extract more water. Salt tolerance may have its greatest impact on crops growing on soils with natural salinity, when all of the other agronomic constraints have been overcome (e.g. disease and nutrient deficiency). Subsoil salinity remains a major limitation to agriculture in all semi-arid regions, such as most southern provinces of Iran. Even where clearing of land in higher rainfall zones has caused water-table 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, as salt will be left in the soil when the water-table lowered (Goudarzi and Pakniyat, 2008). Barley is a moderately salt-tolerant crop (Walia et al., 2005). Zarjoo is an improved genotype recommended for saline areas in most southern provinces 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, the effect of NaCl on the chemical composition of the plant organs was investigated.

2 Materials and Methods

2.1 Site, treatment application and data collection

This experiment was conducted to evaluate the effect of four levels of salinity (0, 4, 8, 12 dS m^{-1}) on two barley cultivars (Zarjoo, a relatively salt tolerant genotype, and Valfajr, a salt sensitive cultivar). The desired salinity levels (0, 2.16, 4.32, 8.64 g kg^{-1} soil) were developed by mixing the required amount of NaCl and $CaCl_2$ (5:1) in soil before filling the pots. The barley crop was sown on November 17, 2017, and harvested on April 29, 2018.The experiment was carried out in a greenhouse with a fine mixed mesic Typic Calcixerpets soil at the College of Agriculture, Miandoab University. The air temperature was in the range of about 25°C to 30°C and light intensity was in the range of about 600-1000 μmol m^{-2} s^{-1}. The factorial experiment was arranged in a completely randomized design with three replications. Soil properties are shown in Table 1. Pre-germinated seeds were sown in 5

L perforated plastic pots filled with fertilized soil (50, 25, 25 mg kg^{-1}, N, P and K, respectively) and were kept in concrete tanks filled with tap water according to El-Hendawy et al. (2005). The level of water was maintained at 3 cm below the soil surface for two days. Ten seeds of each cultivar were sown in each pot, and thinned to five seedlings at two-leaf stage. The pots were kept flooded thereafter for the rest of the experiment. The emergence percentage and numbers of leaves per plant were recorded throughout the experiment. Plants were harvested and threshed manually. The data regarding grain number, grain weight, spikes per plant, tillers per plant and shoot length were recorded (Wilhelm et al., 1989).

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

Soil texture	Silt (%)	Clay (%)	Sand (%)	Total N (%)	K (mg kg^{-1})	P (mg kg^{-1})	OC (%)	EC (dS m^{-1})	pH
Value	67.1	23.5	9.4	0.09	487.23	16.71	0.79	0.06	7.13

2.2 Sodium and potassium measurements

Dried samples were ground to a fine powder and about 0.1 g of powder was transferred to a test tube containing 10 ml 0.1 normality acetic acid, and heated in a water bath at 80 °C for 2 h. The extracted tissue was cooled at room temperature overnight, and then filtered using filter paper. Sodium and potassium concentrations were measured using an atomic absorption spectrometer (Munns and James, 2003).

2.3 Proline measurements

Fresh flag leaf tissue (0.5g) was ground in liquid nitrogen and then extracted in 20 ml hot water for 30 min with moderate shaking. The homogenate was centrifuged at 5000 g for 10 min. The proline concentration was quantified by the ninhydrin acid reagent method using L-proline as a standard（Bates et al.， 1973).

2.4 Statistical analysis

Statistical analysis for each variable was performed based on a randomized complete design model using SAS software. Means were compared by Duncan's multiple range test at $P \leq 0.05$.

3 Results and Discussion

3.1 Effect of sodium chloride on growth and agronomy characteristics

According to the variance analysis table, cultivar interaction and salinity stress were significant on growth and agronomic characteristics (Tables 2 and 3). Salinity had significant effect on agronomy traits of both cultivars. The results indicated that emergence percentage decreased significantly with the salinity increasing from 0 to 12 dS m^{-1}. The two cultivars (Zarjoo and Valfajr) responded differently to salinity. Zarjoo showed significantly higher emergence rate. Numbers of tillers and leaves per plant as well as the plant height decreased upon increasing salinity level (Table 4), which was in agreement with the finding of El-Sharkawy et al. (2017). It was found that Zarjoo was superior to Valfajr as far as the salinity tolerance characteristics (Tables 4 and 5). El-Madidi et al. (2004) claimed that the major difference between two lines of barley in salinity tolerance was their different response to specific ion effects, at the level of the organ, tissue, cell, and sub-cellular entities. The salt-tolerant line is superior to compart toxic ions than the sensitive line in the vacuole presumably, that might enable it to maintain its cytoplasmic metabolic apparatus in a stable and more nearly normal state. Therefore, true cytoplasmic toleration measurement of salt is needed. The first phase of the growth was affected by 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 do not build up in the growing tissues at concentrations that inhibit growth. Because the rapidly elongating cells can accommodate the salt within their expanding vacuoles that arrives in the xylem. Thus, the salt taken up by the plant does not directly inhibit the growth of new leaves (Munns and Gilliham, 2015).

Table 2 Variance analysis in emergence percent, leaves per plant, tillers per plant, plant height, spikes per plant and number of grains per spike of barley

S.O.V	df	Emergence percentage	Leaves per plant	Tillers per plant	Plant height	Spikes per plant	Number of grains per spike
Cultivar	1	57.04ns	12.07ns	0.65ns	28.44ns	9.16ns	36.03ns
Salinity	3	160.53*	25.83*	58.62**	495.75**	12.43*	121.84**
Cultivar× Salinity	3	212.32*	93.12**	37.76**	278.35*	8.65*	92.53**
Error	16	41.67	7.32	3.87	58.60	2.53	14.08
CV (%)		8.28	5.33	4.81	11.29	3.12	9.85

Note: ns, * and ** mean non-significant and significant at the 5% and 1% levels of probability, respectively. Same below.

Table 3 Variance analysis in grain weight per spike, grain yield per plant, phytomass, K^+, proline and Na^+ of barley

S.O.V	df	Grain weight per spike	Grain yield per plant	Phytomass	K+	Proline	Na+
Cultivar	1	0.19ns	45.96*	26.88*	821.84**	2.24*	448.01**
Salinity	3	1.46*	75.14**	17.69*	575.19**	3.49**	281.45**
Cultivar× Salinity	3	1.72*	81.92**	25.75*	528.12**	1.38*	321.58**
Error	16	0.35	6.83	5.19	94.14	0.27	50.17
CV (%)		2.56	4.21	6.18	10.06	3.84	8.33

The salt taken up by the plant concentrates in the old leaves. Continued transport of salt to transpiring leaves over a long time, eventually results in very high Na^+ and Cl^- concentrations, and the leaves died while it was observed in experiments (Tables 4, 5 and 6). 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 would rapidly build up in the cytoplasm and inhibit enzyme activity (Nevo and Chen, 2010). Alternatively, salts might build up in the cell walls and dehydrate the cell (Flowers et al., 1991). However, Muhling and Lauchli (2002) found no evidence for this speculation in maize cultivars that were different in salt tolerance

3.2 Relationship between salinity and yield components

The results revealed that the highest grain number and phytomass was obtained from Zarjoo at the lowest salinity level (Table 3). Phytomass and grain yield were decreased upon salinity, significantly. Yield reduction was attributed to the reduced spike weight and individual seed weight primarily rather than spike number (Table 5). Our results also suggest that estimates of grain yield might bring another complexity to the salinity response. Because the crops must not only be grown in controlled environments, but also due to the complexity of converting shoot biomass into the grain. The low level of salinity may not reduce grain weight even though phytomass reduces (Table 5). In fact, the grain yield may not decrease until reach a given level of salinity ("threshold") (Muhling and Lauchli, 2002).

Table 4 Means of main effects and their interaction in emergence percentage, leaves per plant, tillers per plant, plant height and spikes per plant of barley

Treatment		Emergence percentage	Leaves per plant	Tillers per plant	Plant height (cm)	Spikes per plant
Cultivar	(C_1) Zarjoo	63.05 a	7.69 a	1.95 a	41.65 a	1.65 a
	(C_2) Valfajr	59.35 a	5.98 a	1.36 a	35.72 a	1.32 a
Salinity (ds m^{-1})	(S_1) 0	96.07 a	13.78 a	2.99 a	76.86 a	2.84 a
	(S_2) 4	93.69 a	10.03 b	2.57 a	48.88 b	2.10 b
	(S_3) 8	51.34 b	3.55 c	1.05 b	29.01 c	1.02 c
	(S_4) 12	3.71 c	-	-	-	-
Cultivar× Salinity	C_1 S_1	96.45 a	14.03 a	3.17 a	79.66 a	2.95 a
	C_1 S_2	94.36 a	11.81 a	3.09 a	54.25 ab	2.48 ab
	C_1 S_3	53.98 b	4.93 c	1.52 c	32.67 c	1.19 cd
	C_1 S_4	7.41 d	-	-	-	-

$C_2 S_1$	95.69 a	13.53 a	2.81 ab	74.06 a	2.73 a
$C_2 S_2$	93.02 a	8.25 b	2.05 bc	43.51 b	1.71 bc
$C_2 S_3$	48.71 c	2.17 d	0.59 d	25.33 d	0.86 d
$C_2 S_4$	2.30 e	-	-	-	-

Note: different letters at each column mean significant difference, and the same letters mean no significant difference using Duncan,s multiple tests ($P \le 0.05$); "-" means no plants growth due to salinity. Same below.

Table 5 Means of main effects and their interaction in number of grains per spike, grain weight per spike, grain yield per plant, phytomass per plant of barley

Treatment		Number of grains per spike	Grain weight per spike (g)	Grain yield per plant (g)	Phytomass (g)
Cultivar	(C_1) Zarjoo	14.33 a	0.30 a	3.65 a	5.86 a
	(C_2) Valfajr	12.09 a	0.26 a	2.81b	4.90 b
Salinity (ds m^{-1})	(S_1) 0	24.99 a	0.55 a	8.01 a	12.02 a
	(S_2) 4	19.11 ab	0.43 a	4.38 b	7.50 b
	(S_3) 8	15.02 b	0.14 b	0.53 c	2.01 c
	(S_4) 12	-	-	-	-
Cultivar× Salinity	$C_1 S_1$	25.77 a	0.56 a	8.12 a	12.39 a
	$C_1 S_2$	20.89 ab	0.47 ab	5.70 b	8.40 b
	$C_1 S_3$	10.67 c	0.17 c	0.75 d	2.64 c
	$C_1 S_4$	-	-	-	-
	$C_2 S_1$	24.21 a	0.54 a	7.89 a	11.65 a
	$C_2 S_2$	17.33 b	0.38 b	3.06 c	6.59 b
	$C_2 S_3$	6.85 c	0.12 c	0.31 d	1.37 c
	$C_2 S_4$	-	-	-	-

3.3 Effect of sodium chloride on the chemical composition

According to the variance analysis table, effects of cultivar interaction and salinity stress were significant on the chemical composition of barley (Table 3). The results showed that Zarjoo had higher shoot potassium content (Table 6). The shoot sodium concentration was also increased by increasing the salinity level in both cultivars. However, the sodium content of Zarjoo , was lower than Valfajr which was probably due to Na^+ exclusion mechanisms of Zarjoo (Table 6). The increase of Na^+ and Cl^- content and the decrease of K^+ content in barley grains suggests that the effect of salinity on the physiological phenomenon is due to changes of the ionic content in plants (El-Madidi et al., 2004). Other approaches to improve salt tolerance in wheat are based on the mechanisms of salt tolerance, by selecting physiological traits within the germplasm. Salt tolerance of wheat is associated with low transportation rate of Na^+ to shoots, and high selectivity for K^+ over Na^+ (El-Monem et al., 2013; Munns and Gilliham, 2015). Correlations between grain yield and Na^+ exclusion from leaves, along with the associated enhanced K^+/Na^+ discrimination, have been shown in wheat (Dadrwal et al., 2018), although the relationship may not be applicable to all genotypes (El-Hendawy et al., 2005). Colmer et al. (2006) claimed that Na^+ exclusion was not the only mechanism of salt tolerance. There is a strong correlation between salt exclusion and salt tolerance in many species (El-Monem et al., 2013; Kamboj et al., 2015). In general, Zarjoo, which was characterized with the lower Na^+ concentration, produced more dry matter than the Valfajr cultivar (Table 6). Zarjoo had fewer injured leaves, and a greater proportion of living leaves, as observed in the experiment. A better carbon balance may have effect on growth in the genotype with less Na^+. Similar relationship between shoot dry matter and Na^+ content of leaves was found in high and low Na^+ content genotypes (Munns and James, 2003). The results showed that there was a significant difference among different salinity levels for proline content of the two cultivars, and Zarjoo had higher proline content (Table 6). The proline content in both cultivars was also increased by increasing the salinity level (Table 6). Moradi and Ismail (2007) stated 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, Muuns and Gilliha (2015) and Dadrwal et al. (2018) suggested that the increase of proline concentration may not be associated with salinity tolerance. Indeed, elevated proline levels may also confer additional regulatory functions under salt stress, such as controlling the activity of plasma membrane transporters involved in cell osmotic adjustment in barley roots (Cuin and Shabala, 2005).

Table 6 Means of main effects and their interaction in K^+, proline and Na^+ of barley

Treatment		K+ (mmol Kg^{-1})	Proline (μg g^{-1})	Na+ (mmol Kg^{-1})
Cultivar	(C1) Zarjoo	255.29 a	0.24 a	76.48 a
	(C2) Valfajr	215.17 b	0.20 b	49.95 b
Salinity (ds m^{-1})	(S1) 0	212.81 a	0.21 a	37.28 a
	(S2) 4	286.82 b	0.30 b	75.34 b
	(S3) 8	441.29 c	0.37 c	140.23 c
	(S4) 12	-	-	-
Cultivar× Salinity	C1 S1	216.37 e	0.22 d	32.76 de
	C1 S2	319.69 c	0.34 b	58.62 e
	C1 S3	485.11 a	0.41 a	108.40 e
	C1 S4	-	-	-
	C2 S1	209.25 e	0.21d	41.80 d
	C2 S2	253.95 d	0.26 c	92.07 b
	C2 S3	397.48 b	0.34 b	172.06 a
	C2 S4	-	-	-

4 Conclusion

The results indicated that the two cultivars, Zarjoo and Valfajr, responded differently to salinity; Zarjoo showed significantly higher emergence rate and higher shoot potassium content. Number of tillers and leaves per plant as well as plant height decreased while the sodium content of shoots increased with increasing salinity level in both cultivars. However, the sodium content of Zarjoo, was lower than Valfajr, which was probably due to Na^+ exclusion mechanisms in Zarjoo cultivar. The results also revealed that the highest grain number and phytomass was obtained from Zarjoo at the lowest salinity level. Phytomass and grain yield were also decreased because of salinity significantly. Overall, it appeared that less adverse effect of salinity on Zarjoo cultivar may make it more suitable for growing in saline soils. This subject is worthy of further explorations.

References

Bates, L. S., R. P. Waldren, and I. D. Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil*, 39: 205-207.

Colmer, T. D., T. J. Flowers, and R. Munns. 2006. Use of wild relatives to improve salt tolerance in wheat. *Journal of Experimental Botany*, 57(5): 1059-1078.

Cuin, T. A., and S. Shabala. 2005. Exogenously supplied compatible solutes rapidly ameliorate NaCl-induced potassium efflux from barley roots. *Plant and Cell Physiology*, 46(12): 1924-1933.

Dadrwal, B. K., R. S. Dadarwal, G. L. Choudhary, P. Dadarwal, S. Choudhary, B. Lal, A. Kumar, S. Shivran, and M. D. Tetarwal. 2018. To study the effect of salt stress on growth of wheat. *International Journal. of Chemical Science*, 6(5): 482-484.

El-Hendawy, S. E., Y. Hu, and U. Schmidhalter. 2005. Growth, ion content, gas exchange, and water relations of wheat genotypes differing in salt tolerances. *Australian Journal of Agricultura Research*, 56(2): 123-134.

El-Madidi, S., B. El-Baroudi, and F. B. Aameur. 2004. Effects of salinity on germination and early growth of barley (Hordeum vulgare L.) cultivars. *International Journal of Agriculture and Biology*, 6(5): 767-770.

El-Monem, A. A., S. F. El-Habbasha, and M. Hozayn. 2013. Mitigation salinity stress effects on barley (Hordeum vulgare L.) growth, yield and some physiological aspects by hemin. *Journal of Applied Sciences Research*, 9(3): 2411-2421.

El-Sharkawy, M. S., T. R. El-Beshsbeshy, S. M. Hassan, E. K. Mahmoud, N. I. Abdelkader, R. M. Al-Shal, and A. M. Missaoui. 2017. Alleviating salt stress in barley by use of plant growth stimulants and potassium sulfate. *Journal of Agriculture Sciences.*, 9(4): 136-154.

Flowers, T. J., M. A. Hajibagheri, and A. R. Yeo. 1991. Ion accumulation in the cell walls of rice plants growing under saline conditions: evidence for the oertli hypothesis. *Plant, Cell & Environment*, 14(3): 319-325.

Goudarzi, M., and H. Pakniyat. 2008. Evaluation of wheat cultivars under salinity stress based on some agronomic and physiological traits. *Journal of Agriculture & Social Sciences*, 4(1): 35-38.

Kamboj, A., M. Ziemann, and M. Bhave. 2015. Identification of salt-tolerant barley varieties by a consolidated physiological and molecular approach. *Acta physiologiae plantarum*, 37: 1716.

Maathuis, F. J. M., and A. Amtmann. 1999. K^+ nutrition and Na^+ toxicity the basis of cellular K^+/Na^+ ratios. *Annals of Botany*, 84(2): 123-133.

Moradi, F., and A. M. Ismail. 2007. Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seedling and reproductive stages in rice. *Annals of Botany*, 99(6): 1161-1173.

Muhling, K. H., and A. Lauchli. 2002. Effect of salt stress on growth and cation compartmentation in leaves of two plant species differing in salt tolerance. *Journal of Plant Physiology*, 159(2): 137-146.

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

Munns, R., and M. Gilliha. 2015. Salinity tolerance of crops–what is the cost? *The New Phytologist*, 208(3): 668-673.

Nevo, E., and G. Chen. 2010. Drought and salt tolerances in wild relatives for wheat and barley improvement. *Plant, Cell & Environment*, 33(4): 67-68.

Pitman, M. G., and A. Lauchli. 2002. Global impact of salinity and agricultural ecosystems. In *Salinity: Environment – Plants – Molecules*, eds. A. Läuchli and U. Lüttge, 3-20. Dordrecht: Kluwer.

Taiz, L., and E. Zeiger. 2002. *Plant Physiology*. 4th ed. Sunderland, Massachusetts: Sinauer Associates Inc.

Walia, U. S., M. Singh, and L. S. Manpreet. 2005. Weed control efficacy of herbicides in zero-till wheat. *Indian Journal of Weed Science*, 37(3&4): 167–170.

Wilhelm, W. W., H. Bouzerzour, and J. F. Power. 1989. Soil disturbance-residue management effect on winter wheat growth and yield. *Agronomy Journal*, 81(4): 581-588.