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Chemical Composition, Yield and Yield Components of Two Wheat Cultivars in Response to Salt Stress

H Sadeghi[1*] and Y Emam[2]

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[1]Assistant Professor of College of Agriculture, Shiraz University, Shiraz, Iran
[2] Professor of College of Agriculture, Shiraz University, Shiraz, Iran
* Coresponding author : E-mail sadeghih@shirazu.ac.ir

Abstract
In most southern provinces of Iran, soil salinity is a growing problem, particularly in irrigated agricultural areas, and has been found to reduce wheat yield, dramatically. To investigate the effect of sodium chloride on two wheat (*Triticum aestivum* L.) 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 2006-2007. The results indicated that increasing salinity from 0 to 12 dS/m, decreased the emergence percentage significantly. Two cultivars of Kavir and Shiraz responded differently to salinity, so that Kavir showed a significantly higher emergence rate. This cultivar also had greater shoot potassium content. Number of tillers and leaves per plant and, also, plant height were decreased upon increasing salinity level. The shoot sodium content was, also increased by increasing the salinity level in both cultivars. However, sodium content of Kavir in comparison with Shiraz, was lower, probably due to Na^+ exclusion mechanisms in this cultivar. The highest grain number and phytomass was obtained from Kavir at the lowest salinity level. Phytomass and grain yield were, also significantly decreased as the result of salinity. Less adverse effect of salinity on Kavir indicates that this cultivar might be suitable for saline soils, an object which worth more investigation.

Keywords: Potassium, Salinity, Sodium, Wheat, Yield components

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. Soil salinity has reduced wheat yield usually when values of electrical conductivity were above 6 dS/m throughout the root zone (Munns *et al*. 2006).

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, Tester and Davenport 2003, 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 under NaCl stress (Maathuis and Amtmann 1999).

In wheat, it has been showed that the two responses occur sequentially, giving rise to a two-phase growth response to salinity (Munns 1993). For example, comparison of two genotypes with contrasting rates of Na^+ uptake and long-term differences in salt tolerance (Schachtman *et al*. 1991), showed that both genotypes had similar growth reduction for the four 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 two 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 on 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 the 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, when all of 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).

Wheat is a moderately salt-tolerant crop (Maas and Hoffman 1977). One of the two new cultivars of wheat, used in the present study, Kavir, 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 wheat cultivars in relation to various concentrations of NaCl. In addition, NaCl effect on the chemical composition of the plant organs was investigated.

Materials and Methods

Site, treatment application and data collection

This experiment was conducted to evaluate the effect of four levels of salinity (0, 4, 8 and 12 dS/m) on two wheat cultivars (Kavir, a relatively salt tolerant genotype and Shiraz, a salt sensitive cultivar). 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 wheat crop was sown on 17 November 2006 and harvested on 29 April 2007.The experiment was carried out in a greenhouse at the College of Agriculture, Shiraz University, Shiraz, Iran (52^o 46'E, 29^o 50'N, altitude 1810 m asl), 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}, 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) soil 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 two 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, straw yield, 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

Year	OC (%)	pH	Sand (%)	Silt (%)	Clay (%)	Soil texture	EC (dSm^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	Total N (%)
2005-06	0.83	7	7	66.7	26.3	Silty loam	0.05	16.5	476	0.08

Sodium and potassium measurements

Dried samples 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 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 by application of the ninhydrin acid reagent method as described by Bates *et al.* (1973) using L-proline as a standard.

Statistical analysis

Statistical analysis for each variable was performed based on a randomized complete block design model using SAS software (SAS Institute 1985). Means were compared by Duncan's multiple range test at $p \leq 0.05$.

Results and Discussion

Effect of sodium chloride on growth and morphological characteristics

Salinity had significant effect 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 (Kavir & Shiraz) responded differently to salinity and Kavir showed significantly higher emergence rate. 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 Kavir was superior to Shiraz 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 wheat in salinity tolerance was their different 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 maybe needed. 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. Thus, 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

Table 2. Means of main effects and their interaction for morphological traits

Treatment	Emergence percent	Leaves per plant	Tillers per plant	Plant height (cm)	Spikes per plant
Cultivar					
(V_1) Shiraz	58.58 a	5.91 a	1.33 a	30.46 a	1.16 a
(V_2) Kavir	64.41 a	7.66 a	1.99 a	32.66 a	1.41 a
Salinity (dS/m)					
(S_0) 0	94.00 a	13.83 a	3.00 a	53.17 a	2.50 a
(S_1) 4	93.67 a	10.00 b	2.50 a	44.67 b	1.83 b
(S_2) 8	55.00 b	3.33 c	1.16 b	28.50 c	083 c
(S_3) 12	3.33 c	-$^+$	-	-	-
Cultivar *Salinity					
V_1S_0	92.67 a	13.33 a	2.66 ab	48.33 ab	2.33 ab
V_1S_1	94.00 a	8.33 b	2.00 bc	48.33 ab	1.66 bc
V_1S_2	47.67 c	2.00 cd	0.66 d	25.33 d	0.66 de
V_1S_3	0.00 e	0.00 d	0.00 d	0.00 d	0.00 d
V_2S_0	95.33 a	14.33 a	3.33 a	58.00 a	2.66 a
V_2S_1	93.33 a	11.67 a	3.00 a	41.00 bc	2.00 ab
V_2S_2	61.33 b	4.66 c	1.66 c	31.67 cd	1.00 cd
V_2S_3	6.66 d	-	-	-	-

Means at each column for each source, followed by similar letters are not significantly different using Duncan's multiple range tests ($p \leq 0.05$).

$^+$ No plant growth due to salinity

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

Relationship between salinity and yield components

The results revealed that the highest grain number and phytomass was obtained from Kavir at the lowest salinity level (Table 3). Phytomass and grain yield were, also decreased upon salinity, significantly. Yield reduction was attributed, primarily to the 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 the complexity of the converting 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 3), the fact that grain yield may not decrease until a given ('threshold') salinity is reached (Maas and Hoffman 1977).

Table 3. Means of main effects and their interaction for yield and yield components of two wheat cultivars

Treatment	No. of grains per plant	Grain 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) Shiraz	9.75 a	0.18 a	1.75 b.	3.31 b	4200 b	1.38 b	2.90 b
			+				
			-+-				
(V_2) Kavir	12.66 a	0.17 a	2.15 a	4.02 a	4700 a	1.75 a	3.60 a
Salinity (dS/m)							
(S_0) 0	19.17 a	0.43 a	8.24 a	11.67 a	5700 a	3.09 a	11.20 a
(S_1) 4	15.00 ab	0.25 a	3.75 b	6.21 b	5150 b	2.21 b	5.78 b
(S_2) 8	10.67 b	0.04 b	0.43 c	1.36 c	3100 c	0.96 c	0.98 c
(S_3) 12	-[+]	-	-	-	-	-	-
Cultivars* Salinity							
V_1S_0	14.00 ab	0.54 a	7.56 a	10.75 a	5350 a	2.65 b	10.35 a
V_1S_1	14.33 ab	0.20 bc	2.86 b	5.31 b	5200 ab	2.25 b	4.91 b
V_1S_2	10.67 bc	0.0 1 c	0.11 c	0.79 c	2800 d	0.63 d	0.39 c
V_1S_3	-	-	-	-	-	-	-
V_2S_0	24.33 a	0.33 ab	8.03 a	11.89 a	5750 a	3.53 a	11.49 a
V_2S_1	15.67 ab	0.30 abc	4.70 b	7.18 b	5210 b	2.18 b	6.78 b
V_2S_2	10.67 bc	0.07 bc	0.75 c	2.11 c	3400 c	1.29 c	1.71 c

Means at each column for each source, followed by similar letters are not significantly different using Duncan's multiple range tests ($p \leq 0.05$).

[+] No plant growth due to salinity

Effect of sodium chloride on the chemical composition

Our results showed that Kavir had greater shoot potassium content (Table 4). The shoot sodium concentration was also increased by increasing the salinity level in both cultivars; however, the sodium content of Kavir in comparison with Shiraz, was lower, probably due to Na^+ exclusion mechanisms in this cultivar (Table 4). The increase in Na^+ and Cl^- and decrease in K^+ content of wheat grains suggest that the effect of salinity on the physiological phenomenon is due to changes in the ionic content of the plants (Abdullah *et al*. 1978). Other approaches to improve salt tolerance in wheat are based on the mechanisms for salt tolerance, using physiological traits to select within the germplasm. In wheat, 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 wheat (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*., 2006).

There is a strong correlation between salt exclusion and salt tolerance in many species (reviewed by Läuchli, 1984; Munns and James 2003). Figure 1 shows the negative relationship between leaf Na^+ concentration and salt tolerance of Kavir. In general, Kavir, which was characterized with the lowest Na^+ concentrations, produced greater dry matter than the Shiraz cultivar (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^+. Similar relationship between shoot dry matter and leaf Na^+ was found in a population from the cross between high- and low-Na^+ 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 Kavir had greater proline content (Table 4). The proline content in both cultivars was also increased by increasing the salinity level (Table 4). 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, Colmer *et al.* (1995) suggested that the increase in proline concentration may not be associated with salinity tolerance. Indeed, elevated proline levels may also confer additional regulatory or osmo-protective 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).

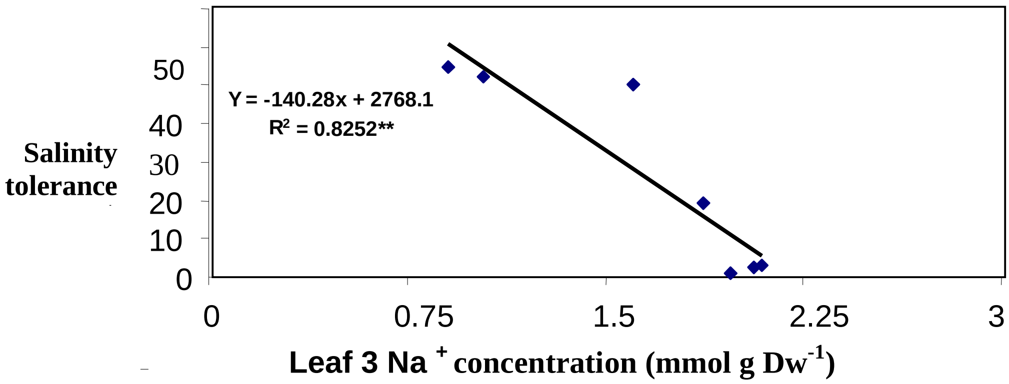

Figure 1. Relationship between salinity tolerance (% growth of the control) and leaf Na^+ concentration in the Kavir cultivar. Na^+ concentrations were measured on the third leaf after 10 d in 150 mM NaCl and shoot biomass after 24 d. Values are expressed as a percentage of shoot biomass in the control condition (R^2=0.8252). All values are based on means (*n*=5).

Table 4. Mean comparison of main and interaction effects of chemical composition of two wheat cultivars

Treatment	K^+ (mmol per Kg)	Proline (µ g/g)	Na^+ (mmol per Kg)
Cultivars			
(V_1) Shiraz	222.70 b	0.25 b	157.10 b
(V_2) Kavir	435.50 a	0.34 a	13.80 a
Salinity (dS/m)			
(S_0) 0	319.40 c	0.25 d	94.10 d
(S_1) 4	410.70 b	0.27 b	87.30 b
(S_2) 8	586.50 a	0.41 a	160.50 a
(S_3) 12	-	-	-
V_1S_0	287.20 d	0.25 d	141.14 d
V_1S_1	209.00 d	0.26 b	168.80 b
V_1S_2	394.90 c	0.33 a	318.40 a
V_1S_3	-	-	-
V_2S_0	351.70 c	0.29 d	46.80 de
V_2S_1	612.30 b	0.30 ab	5.80 e
V_2S_2	778.10 a	0.37 a	2.50 e

Means at each column for each source, followed by similar letters are not significantly different using Duncan's multiple range tests ($p \leq 0.05$).

$^+$ No plants growth due to salinity

Conclusion

Our results indicated that the two cultivars, Kavir & Shiraz, responded differently to salinity, so that Kavir showed significantly higher emergence rate. This cultivar (Kavir) also had greater shoot potassium content. Number of tillers and leaves per plant and also plant height were decreased in both cultivars upon increasing salinity. The shoot sodium content in both cultivars was also increased by increasing the salinity level; however, the sodium content of Kavir, compared to Shiraz, 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 Kavir 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 Kavir cultivar may make it more suitable for growth in saline soils. This subject is worthy of further explorations.

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