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## Salt stress effect on wheat (*Triticum aestivum* L.) growth and leaf ion concentrations

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### Abstract

Crops growing in salt-affected soils may suffer from physiological drought stress, ion toxicity, and mineral deficiency which then lead to reduced growth and productivity. A pot experiment was conducted to study the effect of different salinity levels, i.e.  $EC_e=3$  dS  $m^{-1}$  (control), 8, 12 and 16 dS  $m^{-1}$  on wheat grain yield, yield components and leaf ion uptake. Desired salinity levels were obtained by mixing adequate NaCl before filling the pots. Soil water was maintained at 70% of available water holding capacity. Results revealed that Kouhdasht and Tajan showed highest and lowest grain yield and yield components as compared to others. Leaf  $Na^+$  and  $Cl^-$  concentrations of all genotypes increased significantly with increasing soil salinity, with the highest concentrations in Tajan, followed by Rasoul, Atrak and Kouhdasht. Highest leaf  $K^+$  concentration and  $K^+ : Na^+$  ratio were observed in Kouhdasht, followed by Atrak, Rasoul and Tajan, respectively. Based on higher grain yield production, higher leaf  $K^+$  concentration,  $K^+ : Na^+$  ratio and lower leaf  $Na^+$  and  $Cl^-$  concentrations, Kouhdasht and Atrak were identified as the most salt-tolerant genotypes.

**Keywords:** Abiotic stresses; Plant ecophysiology; Stress physiology; Semi-arid agriculture.

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### Introduction

Salinization is the process by which water-soluble salts build up over the soil profile (USDA, 1998). The United Nations Environment Program

estimates that approximately 50% of cropland in the world is salt-stressed (Yokoi et al., 2002). On average, 20% of all irrigated lands are affected by salts (Yeo, 1999), but this figure increases to more than 30% in countries such as Egypt, Argentina and Iran (Zink, 2003).

Salinity has inhibitory effects on wheat phenological aspects such as leaf number, leaf rate expansion (El-Hendawy et al., 2005), root growth rate (Neumann, 1995b), root/shoot ratio (El-Hendawy et al., 2005), and total dry matter yield (Pessarakli and Huber, 1991; El-Hendawy et al., 2005). Similarly, Munns et al. (2006) found that wheat (*Triticum aestivum* L.) genotypes with the lowest  $\text{Na}^+$  concentrations produced more dry matter than genotypes with high  $\text{Na}^+$  concentrations.

A number of studies have been carried out with different wheat genotypes to look for tolerance to salinity in Iran (e.g. Milani et al., 1998; Khoshgoftar et al., 2004; Ghavami et al., 2004; Poustini and Siosemardeh, 2004; Cheraghi, 2004; Dehdari et al., 2007). However, to our knowledge no work has been done to investigate the response of the wheat genotypes that were studied for our experiments, i.e. Kouhdasht, Rasoul, Tajan and Atrak to salinity stress. Kouhdasht and Atrak have been suggested as adapted genotypes for semi-arid areas under rainfed cultivation, whereas Tajan and Rasoul produce well under irrigated cultivation conditions (Kalateh et al., 2001). Therefore, the aim of the present study was to quantify effects of different salinity levels, i.e. 3  $\text{dS m}^{-1}$  (as control), 8, 12 and 16  $\text{dS m}^{-1}$  on grain yield, yield components and leaf ion concentrations, i.e.  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$ , and  $\text{Na}^+ : \text{K}^+$  ratio of these wheat genotypes.

## Materials and Methods

### *Site Description*

A pot study was conducted in 2004-2005 in the Aghala area of northern Golestan province (37° 07' N, 54° 07' E) which is located in north of Iran. A semi-arid climate prevails in the area (Abbasabadi, 1999).

### *Treatments and Measurements*

Treatments were replicated three times in a completely randomized factorial arrangement. Treatment combinations included four salinity levels, i.e. 3  $\text{dS m}^{-1}$  (control), 8, 12 and 16  $\text{dS m}^{-1}$  and four wheat genotypes.

To characterize soil conditions, fifteen spots were randomly selected on an agricultural field. In each spot, 2 to 3 sub-samples of approximately 0.5-0.6 kg (wet) weight were taken from the top 0-30 cm using a 4 cm diameter Edelman auger. Specific spot sub-samples were thoroughly mixed to obtain a composite soil sample for each location.

Soil texture was determined using the hydrometer method (Bouyoucos, 1962). Soil organic matter content was determined by combustion with an elemental analyzer (Model ECS 4010, COSTECH Analytical, Valencia CA). Soil pH was measured using a WTW 330/SET-0 pH meter (Regenstauf, Germany). Electrical conductivity ( $EC_e$ ) was determined on a saturation extract using a Büchner funnel and a HI-8033 conductivity meter (Hanna Instruments, Woonsocket RI, USA) and corrected for 25 °C. Bulk density was measured using a 100 cm<sup>3</sup> core inserted to a depth of 5 cm (Blake and Hartge, 1986). Water content at field capacity and permanent wilting point were determined using pressure chambers (Soil Moisture Equipment, Santa Barbara CA, USA). Some physico-chemical properties of the soil at the study site are presented in Table 1. Prior to filling test pots, NaCl was mixed with the soil to obtain the desired salinity levels.

Table 1. Some physico-chemical properties of the experimental soil.

| Soil sampling depth (cm) | Soil texture | Clay 0-2 $\mu\text{m}$ ( $\text{g kg}^{-1}$ ) | Silt 2-50 $\mu\text{m}$ ( $\text{g kg}^{-1}$ ) | Sand 50-2000 $\mu\text{m}$ ( $\text{g kg}^{-1}$ ) | OM ( $\text{g kg}^{-1}$ ) | Saturated percent (mass%) | Field capacity (mass%) | $EC_e$ ( $\text{dS m}^{-1}$ ) | pH  |
|--------------------------|--------------|---|--|---|---------------------------|---------------------------|------------------------|-------------------------------|-----|
| 0-30                     | Si-L         | 14  | 72   | 14  | 1.52                      | 42                        | 24.0                   | 3.0                           | 7.8 |
| 30-60                    | Si-C-L       | 33  | 59   | 8   | -                         | 49                        | 28.5                   | 3.9                           | 8.0 |
| 60-90                    | Si-C-L       | 33  | 61   | 6   | -                         | 53                        | 28.8                   | 6.2                           | 7.9 |

OM is organic matter content (not measured for 30-60 and 60-90 cm depths);

$EC_e$  is electrical conductivity on a saturation extract at 25 °C;

Si-L and Si-C-L are silt loam and silty clay loam soils, respectively.

Fertilizers were applied at 120: 60: 60 kg ha<sup>-1</sup> NPK as urea, single super phosphate and potassium sulfate, respectively. Ten seeds were sown in each pot with 5 lit pot<sup>-1</sup> soil. After germination, three uniform seedlings were selected and allowed to grow whereas the rest was uprooted and discarded.

Soil water was maintained at 70% of available water holding capacity. At grain maturity, all plants from each pot were harvested. On each plant, we calculated grain yield, thousand grain weight, numbers of spikes, spikelets and etc. Wheat leaves were dried at 70 °C and then ground in a mortar with a pestle.

Ground leaves were digested with 1 N HCl for 24 hours at 40 °C, then shaken for 1.5 hours, and filtered manually. Na<sup>+</sup> and K<sup>+</sup> concentrations were determined by flame photometry (Jenway PFP-7, Essex, UK), whereas Cl<sup>-</sup> was measured with a coulometric chloride analyzer (Corning 926, Essex, U.K.).

### *Statistical Analysis*

Completely randomized design data thus obtained were statistically analyzed for differences between treatment means using SPSS (version 12.0). Treatment means were compared using Duncan's Multiple Range Test (Duncan, 1955).

## **Results**

### *Grain yield*

Grain yield of all wheat genotypes significantly decreased with increasing salinity levels (Figure 1a). At relatively low salinity level (LS), Kouhdasht showed significantly higher grain yield than the other genotypes. At relatively moderate salinity (MS) and high salinity (HS) levels, yield differences between genotypes were more pronounced than at LS. Kouhdasht showed significantly higher grain yield than the other genotypes, whereas grain yield of Atrak was significantly higher than that of Rasoul or Tajan. Grain yield differences between the latter two genotypes were not significant at MS and HS.

### *Thousand Grain Weight (TGW)*

TGW of all wheat genotypes significantly decreased with increasing salinity level (Figure 1b). At LS, highest TGW was observed for Kouhdasht: this value was significantly higher than for the other genotypes. Non-significant differences were observed in TGW of Tajan and Atrak, whereas Rasoul showed consistently the lowest TGW values. At MS and HS, more pronounced differences in TGW were observed compared to LS. Kouhdasht showed significantly higher TGW than the other genotypes. Non-significant differences were observed in TGW of Rasoul and Tajan at MS and HS.

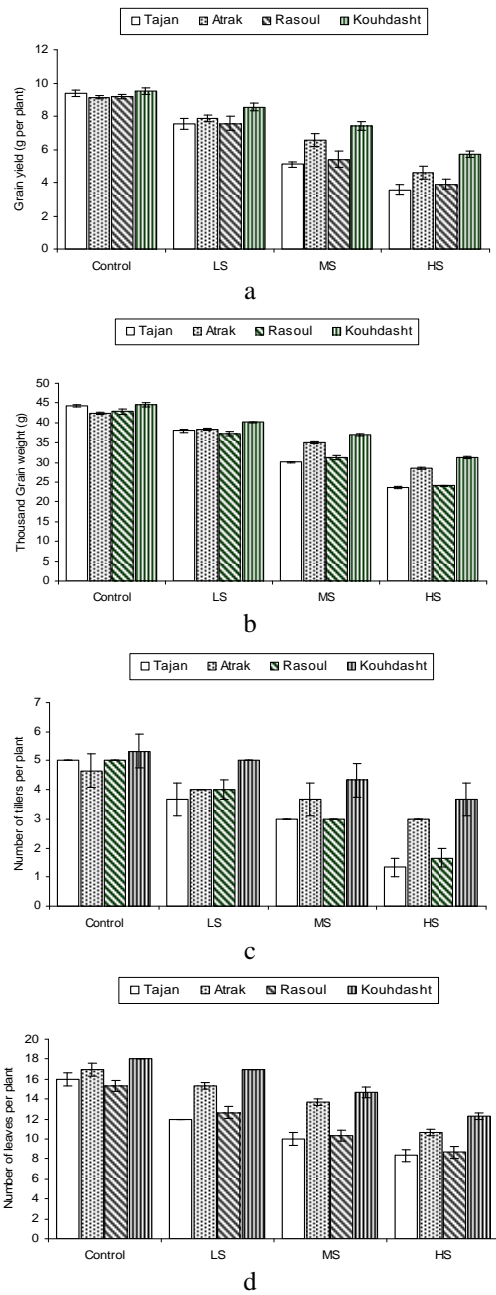


Figure 1. Effect of different salinity levels on a) grain yield (g per plant); b) 1000 grain weight (g); c) tiller numbers per plant; and d) number of leaves per plant. LS, MS and HS denote relatively low, moderate and high salinity level, respectively; error bars indicate standard deviation.

### *Number of Tillers per Plant (NTP)*

NTP of all wheat genotypes significantly decreased with increasing salinity levels (Figure 2a). NTP of Tajan and Rasoul significantly decreased at LS compared to control. Compared to LS, NTP was significantly reduced at MS and HS for all wheat genotypes. Non-significant differences in NTP were observed between Kouhdasht and Atrak and also between Rasoul and Tajan at both MS and HS.

### *Number of Leaves per Plant (NLP)*

NLP of all wheat genotypes significantly decreased with increasing salinity levels (Figure 2b). Kouhdasht showed significantly higher NLP values than the other genotypes at different levels of salinity. Atrak showed significantly higher leaf number per plant than Rasoul and Tajan, except for the control treatment. Non-significant differences were observed in NLP of Rasoul and Tajan at all salinity treatments.

### *Number of Spikes per Plant (NSP)*

NSP of all wheat genotypes decreased significantly with increasing salinity levels (Figure 2a). Non-significant differences were observed for NSP for all wheat genotypes at both control and LS. At MS and HS, Kouhdasht and Atrak showed significantly higher NSP values than Rasoul and Tajan. Rasoul and Tajan showed similar NSP.

### *Spike Length*

Spike length of all wheat genotypes was decreased significantly by applying different levels of salinity as compared to control treatment (Figure 2b). At LS, Kouhdasht showed significantly higher spike length than the other wheat genotypes. At MS and HS, Tajan showed significantly lower spike length than the other three wheat genotypes, whereas Kouhdasht showed the significantly highest spike length.

### *Number of Spikelets per Spike (NSS)*

NSS of all wheat genotypes was decreased with increasing salinity levels (Figure 2c). Except for Tajan, LS caused a non-significant decrease in NSS of all wheat genotypes as compared to control treatment. At MS and HS, Tajan

showed significantly lower NSS values compared to Kouhdasht and Atrak. Kouhdasht showed highest NSS compared to the other wheat genotypes.

#### *Straw Weight*

Straw weight of all wheat genotypes decreased with increasing salinity levels (Figure 2d). At LS, Atrak showed significantly highest straw weight compared to the other genotypes. Non-significant changes in straw weight of the other wheat genotypes were observed at LS as compared to control. At MS and HS, Tajan showed significantly lowest straw weight in comparison to the other genotypes.

#### *Harvest Index (HI)*

HI of all wheat genotypes significantly decreased with increasing salinity level (Figure 2e). Atrak showed significantly higher HI values than Tajan and Rasoul. Non-significant differences were observed for HI between Atrak and Kouhdasht at LS. At MS and HS, differences in HI became more pronounced: Kouhdasht showed significantly higher HI compared to the other genotypes, whereas, Atrak showed significantly higher HI than Rasoul and Tajan.

#### *Leaf K<sup>+</sup> Concentration*

Leaf K<sup>+</sup> concentration of all wheat genotypes significantly decreased with increasing salinity levels as compared to control treatment (Figure 3a). Under all salinity levels, genotypes were ordered in terms of leaf K<sup>+</sup> concentration as: Kouhdasht > Atrak > Rasoul > Tajan, with all values being significantly different.

#### *Leaf Na<sup>+</sup> Concentration*

Leaf Na<sup>+</sup> concentration of all wheat genotypes increased significantly with increasing salinity levels as compared to the control treatment (Figure 3b). At LS, Tajan maintained significantly higher Na<sup>+</sup> concentration than the other wheat genotypes, with the exception of Rasoul. Kouhdasht significantly maintained lowest Na<sup>+</sup> concentrations compared to the other genotypes under all salinity treatments. At MS and HS, genotypes were ordered in terms of leaf Na<sup>+</sup> concentration as: Tajan > Rasoul > Atrak > Kouhdasht.

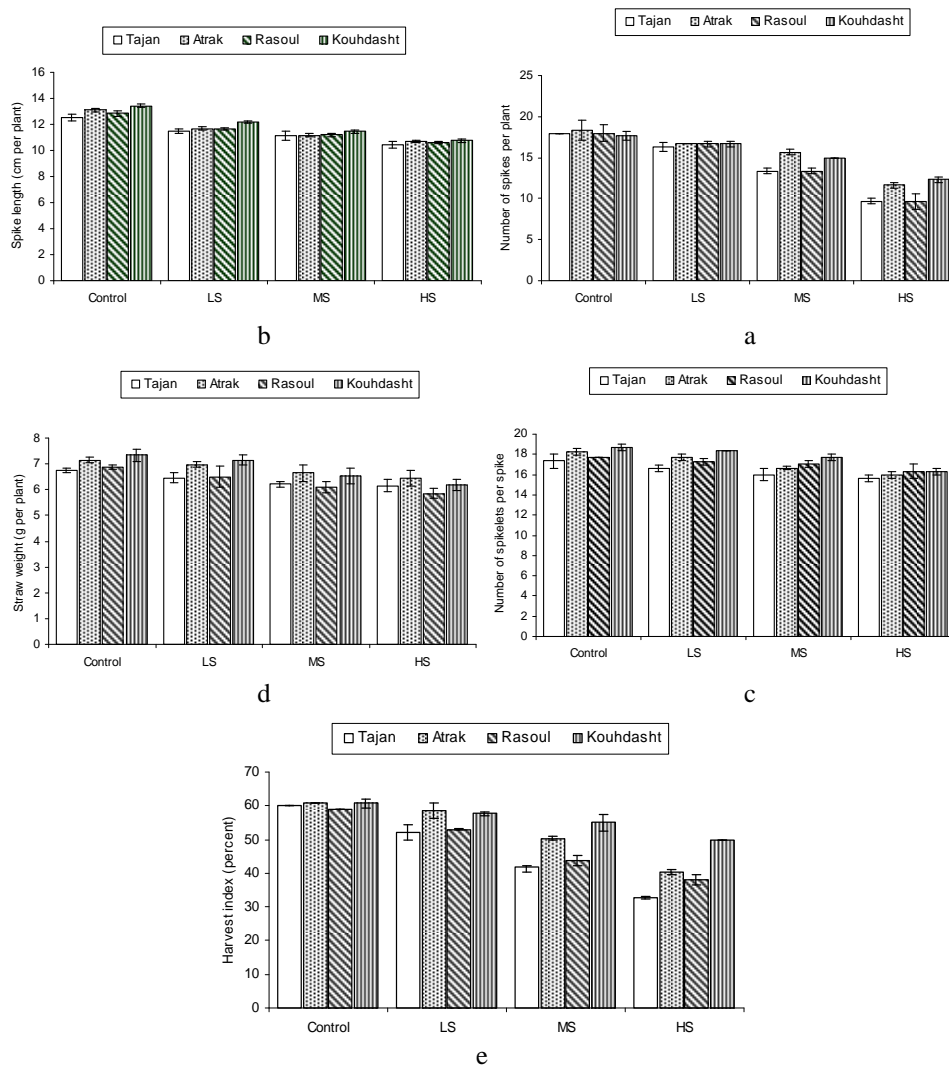


Figure 2. Effect of different salinity levels on a) spikes number per plant; b) spike length (cm per plant); c) number of spikelets per spike; d) straw weight (g); and e) harvest index (%). LS, MS and HS denote relatively low, moderate and high salinity level, respectively; error bars indicate standard deviation.

### Leaf $Cl^-$ Concentration

Chloride concentration in all wheat genotype leaves significantly increased with increasing salinity levels as compared to the control treatment (Figure 3c). Under all salinity levels, genotypes were ordered in



terms of leaf  $\text{Cl}^-$  concentration as:  $\text{Tajan} > \text{Rasoul} > \text{Atrak} > \text{Kouhdasht}$ , with all values being significantly different.

### Leaf $\text{K}^+ : \text{Na}^+$ Ratio

Leaf  $\text{K}^+ : \text{Na}^+$  ratio of all wheat genotypes decreased significantly with increasing salinity level as compared to control treatment (Figure 3d). At LS, Kouhdasht showed significantly higher  $\text{K}^+ : \text{Na}^+$  ratio than the other genotypes. Non-significant differences were observed for the other three wheat genotypes. At MS and HS, Kouhdasht showed a significantly higher  $\text{K}^+ : \text{Na}^+$  ratio compared to the other genotypes, except for Atrak. Non-significant differences were observed in  $\text{K}^+ : \text{Na}^+$  ratio of Atrak and Rasoul, and also in  $\text{K}^+ : \text{Na}^+$  ratio of Rasoul and Tajan at the latter two salinity levels.

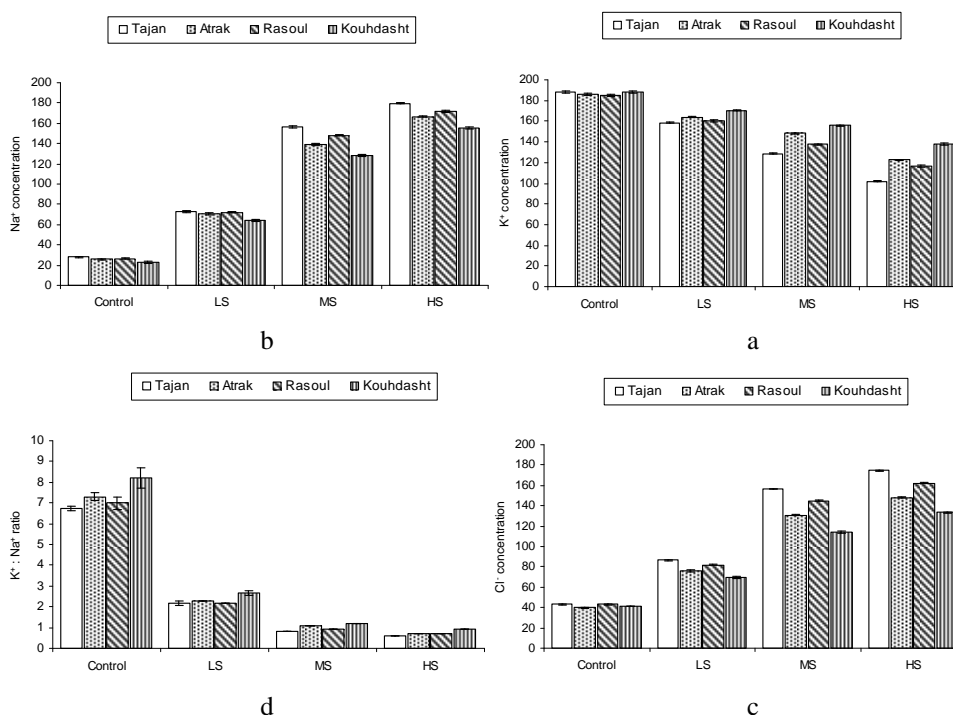


Figure 3. Effects of different salinity levels on a) leaf  $\text{K}^+$  concentration ( $\text{mol m}^{-3}$ ); b) leaf  $\text{Na}^+$  ( $\text{mol m}^{-3}$ ); c) leaf  $\text{Cl}^-$  concentration concentration ( $\text{mol m}^{-3}$ ); and d) leaf  $\text{K}^+ : \text{Na}^+$  ratio. LS, MS and HS denote relatively low, moderate and high salinity level respectively; error bars indicate standard deviation.

## Discussion

Improving grain yield is an important target in wheat plant breeding. Therefore, the evaluation of final grain yield and growth parameters determining grain yield is a critical aspect of breeding programs. Final yields of wheat are determined by the number of spikes per plant and yield components such as spikelet number, grain number and grain weight (El-Hendawy et al., 2005).

Grain yield and TGW of the four wheat genotypes tested here significantly decreased with increasing salinity levels. Kouhdasht and Tajan, respectively, showed highest and lowest grain yield and TGW values compared to the other wheat genotypes. For example, grain yield and TGW per plant at HS treatment were reduced by an average 39.7 and 29.8% (respectively, for grain yield and TGW) for Kouhdasht, whereas they were reduced by an average 62.2 and 46.9% for Tajan. Similarly, El-Hendawy et al. (2005) concluded that at final harvest, grain yield per plant at 150 mM NaCl (17 dS m<sup>-1</sup>) was reduced by an average 22% for the most tolerant genotypes, whereas it was reduced by an average 61% for the least tolerant genotypes.

Table 2 shows that spike length and spikelet number per spike have also a positive and highly significant correlation with grain yield under moderate and high salinity treatments. However, non-significant correlations were observed under low salinity treatment. TGW has a positive, and very strong and significant correlation with grain yield under moderate and high salinity, and a positive and strongly significant correlation under low salinity.

Maas and Grieve (1990) observed reduction of spikelet and kernel number per spike under the influence of root zone salinity. Grieve et al. (1992) found a reduction in tillering capacity, spike length, number of spikelets and kernels per spike of moderately salt-stressed wheat. In the present study, values of parameters mentioned above, i.e. tiller number and leaf number, decreased with increasing salinity. Tiller numbers per plant of the salt-sensitive genotype (Tajan) showed a higher reduction (by about 37%) than tolerant genotypes (Kouhdasht) (i.e. by about 19%). This may indicate that tiller number under salinity can be used as a simple and non-destructive measurement to evaluate salinity stress tolerance of wheat genotypes in breeding programs (El-Hendawy et al., 2005).

Table 2. Relationship between grain yield and yield components and leaf ion concentrations (Pearson correlation).

|                                       | GY      | TGW     | NTP     | NSP     | NSS     | SL      | SW      | HI      | Na <sup>+</sup> | Cl <sup>-</sup> | K <sup>+</sup> |
|---------------------------------------|---------|---------|---------|---------|---------|---------|---------|---------|-----------------|-----------------|----------------|
| TGW                                   | 0.90**  |         |         |         |         |         |         |         |                 |                 |                |
| NTP                                   | 0.85**  | 0.80**  |         |         |         |         |         |         |                 |                 |                |
| NSP                                   | 0.81**  | 0.75**  | 0.79**  |         |         |         |         |         |                 |                 |                |
| NSS                                   | -0.72** | -0.70** | -0.76** | 0.64**  |         |         |         |         |                 |                 |                |
| SL (cm)                               | -0.65** | -0.55** | -0.64** | 0.54**  | 0.75**  |         |         |         |                 |                 |                |
| SW<br>(g per plant)                   | -0.57** | -0.68** | -0.72** | 0.54**  | 0.68**  | 0.77**  |         |         |                 |                 |                |
| HI (%)                                | 0.86**  | 0.83**  | 0.84**  | 0.84**  | 0.65**  | -0.71** | 0.68**  |         |                 |                 |                |
| Na <sup>+</sup>                       | -0.79** | -0.75** | -0.78** | -0.74** | -0.56** | -0.65** | -0.55** | -0.78** |                 |                 |                |
| Cl <sup>-</sup>                       | -0.45** | -0.56** | -0.65** | -0.59** | -0.67** | -0.69** | 0.61**  | -0.78** | 0.79**          |                 |                |
| K <sup>+</sup>                        | 0.80**  | 0.78**  | 0.78**  | 0.74**  | 0.69**  | -0.74** | 0.76**  | 0.82**  | -0.77**         | -0.80**         |                |
| K <sup>+</sup> :Na <sup>+</sup> ratio | 0.86**  | 0.81**  | 0.76**  | 0.78**  | 0.65**  | -0.63** | 0.56**  | 0.83**  | -0.73**         | -0.62**         | 0.83**         |

\*\* Highly significant at P=0.01 probability.

GY, TGW, NTP, NSS, NSP, SL, SW, HI, Na<sup>+</sup>, Cl<sup>-</sup> and K<sup>+</sup> are respectively grain yield, 1000 grain weight, number of tillers per plant, number of spikelets per spike, number of spikes per plant, spike length, straw weight, harvest index, leaf Na<sup>+</sup> concentration, leaf Cl<sup>-</sup> concentration and leaf K<sup>+</sup> concentration, respectively.

In the case of leaf number per plant, Kouhdasht and Tajan on average showed a reduction of 17.5 and 37%, respectively, under different salinity levels. Munns (1993) indicated that salt in plants reduces growth by causing premature senescence of old leaves and hence reduced supply of assimilates to growing regions. Sensitive cultivars accumulate ions more quickly than tolerant cultivars and this ion accumulation leads to leaf death and, progressively, death of the plant (Munns, 2002).

Significant decrease in spike length, spike number and spikelets number per spike of all wheat genotypes was observed with increasing salinity levels. Table 2 shows that spike length and spikelet number per spike have a positive and highly significant correlation with grain yield. Leaf Na<sup>+</sup> and Cl<sup>-</sup> concentrations were increased by salinity, whereas the opposite was observed for leaf K<sup>+</sup> concentration and accordingly, the K<sup>+</sup>: Na<sup>+</sup> ratio. K<sup>+</sup> had a positive and highly significant correlation with grain yield.

Figure 3b shows that leaf Na<sup>+</sup> concentration of Kouhdasht under all salinity levels was significantly lower than that observed in other genotypes. Moreover, Kouhdasht accumulated highest K<sup>+</sup> concentration and had higher K<sup>+</sup>: Na<sup>+</sup> ratio than that of other wheat genotypes. By contrast, Tajan

maintained significantly higher leaf  $\text{Na}^+$  concentration than the other genotypes (except Rasoul) under different salinity levels. In contrast, Table 2 shows that there is a highly significant positive correlation between grain yield and  $\text{K}^+$  concentrations as well as with  $\text{K}^+:\text{Na}^+$  ratio.

Within a species, or even within a genus, leaf  $\text{Na}^+$  concentrations can be used as an indicator of relative ability to 'exclude'  $\text{Na}^+$ . In addition, genotype tolerance to high  $\text{Na}^+$  concentrations in leaves may differ, assumedly due to differences in compartmentation efficiency in leaf vacuoles; this trait has been characterized as 'tissue tolerance' (Munns, 2005; Flowers, 2004). The ability to maintain low  $\text{Na}^+$  and high  $\text{K}^+$  concentrations in leaves (Dvorak et al., 1994) and  $\text{K}^+:\text{Na}^+$  discrimination can be found amongst wheat genotypes, wheat progenitors, and wild relatives (Gorham, 1993).

Kouhdasht and Atrak either restricted the absorption of  $\text{Na}^+$  or excluded  $\text{Na}^+$  from leaves, whereas Tajan and Rasoul were not able to exclude/restrict  $\text{Na}^+$  from their leaves. Higher leaf  $\text{Na}^+$  concentrations in Tajan and Rasoul might have inhibited  $\text{K}^+$  uptake, and therefore, these genotypes maintained a low concentration of  $\text{K}^+$  and consequently a low  $\text{K}^+:\text{Na}^+$  ratio in their leaves. By contrast, Kouhdasht showed a restricted uptake of  $\text{Na}^+$  and  $\text{Cl}^-$ , and a better maintenance of the proper  $\text{K}^+:\text{Na}^+$  ratio. Correlations between grain yield and  $\text{Na}^+$  exclusion from leaves, along with the associated enhanced  $\text{K}^+:\text{Na}^+$  discrimination, have been shown to occur in wheat (El-Hendawy et al., 2005; Iqbal et al., 2001; Zhu, 2001a), proving that  $\text{Na}^+$  exclusion is not the only mechanism of salt tolerance in this species (Munns et al., 2006).

## Conclusion

As Kouhdasht and Atrak were identified as the most salt-tolerant genotypes, they can be utilized through selection and breeding programs for further improvement in salt tolerance of Iranian wheat genotypes. Furthermore, as Atrak and Kouhdasht are also drought-tolerant (Kalateh et al., 2001), they may possibly be considered as drought/saline-tolerant genotypes in Iran.

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