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Effects of salinity on some physiological traits in wheat (*Triticum aestivum* L.) cultivars

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Abstract

The effect of salt stress on some physiological traits of wheat (*Triticum aestivum* L.) was studied in a factorial experiment based on completely randomized design with three replications, under greenhouse condition. Salinity treatments carried out in four levels (1.3 dS m^{-1} as control, 5, 10, 15 dS m^{-1}) via calcium chloride and sodium chloride with 1:10 (Ca^{2+}:Na^+ ratio). Wheat genotypes included four cultivars, Sistani and Neishabour as tolerant cultivars, and Tajan and Bahar as sensitive cultivars. Chlorophyll content (CHL), Leaf relative water content (RWC), sodium and potassium contents, and also K^+/Na^+ ratio were measured at tillering and flowering stages, Total grain yield and yield components were determined. Salinity stress decreased relative water content (RWC), K^+ content, K^+/Na^+ ratio and grain yield; however Na^+ content in all the genotypes and in both stages were increased. CHL content increased at tillering stage while it is decreased at flowering stage. Sistani and Neishabour cultivars had more amounts of K^+ content, K^+/Na^+ ratio and RWC under salt conditions, at tillering stage Bahar and Tajan cultivars recorded higher CHL and sodium content at both stages. Bahar showed the highest Na^+ content and the most reduction in yield, so it can be considered as more salt sensitive than Tajan genotype. Results showed that the salinity tolerance in tolerant cultivars as manifested by lower decrease in grain yield is associated with the lower sodium accumulation and higher K^+/Na^+ compared to the sensitive cultivars.

Keywords: Relative water content (RWC), Chlorophyll content (CHL), K^+/Na^+ ratio, Salinity, Wheat

Introduction

Soil salinity is one of the major abiotic stresses affecting germination, crop growth and productivity (Sairam *et al*., 2002). The detrimental effects of high salinity on plants may be expected as the death of plants or decreases in productivity. Many plants have developed mechanisms either to exclude salt from their cells or to tolerate within the cells (Asish Kumar & Bandhu Das, 2005). Salinity significantly reduces the total chlorophyll content and the degree of reduction in total chlorophyll depending on salt tolerance of plant species and salt concentrations. In salt-tolerant species, chlorophyll content increased, while in salt-sensitive species it was decreased (Ashraf & McNeilly, 1988). According to Velegaleti *et al*. (1990), the reduction in chlorophyll content was significant for salt-sensitive species, which is correlated with Cl^- accumulation. It has been suggested that ionic status of plant to identify salt tolerance to be applicable and its relationship with salt tolerance is considered strong enough to be exploited as a selection tool in the breeding of salt tolerant cultivars (Ashraf & Khanum, 1997). It is well documented that a greater of salt tolerance in plants is associated with a more efficient system for selective uptake of K^+ over Na^+ (Wenxue *et al.,* 2003). Under salt stress, plants maintain high concentration of K^+ and low concentration of Na^+ in the cytosol. They do this by regulation the expression and activity of K^+ and Na^+ transporters and H^+ pumps that generate the driving force for transport (Zhu, 2003). Regulation of K^+ uptake, prevention of Na^+ influx, promotion of Na^+ efflux from the cell and utilization of Na^+ for osmotic adjustment are the strategies commonly used by plants to maintain desirable K^+/Na^+ ratio in cytosol. A high K^+/Na^+ ratio in cytosol is essential for normal cellular functions of the plants (Zhu, 2003).

The observable indirect effect of salinity on plant growth is reduction in water content of a soil. As salinity increases, soil water potential decreases. In general, the presence of salt in soil solution decreases the osmotic potential of soil creates water stress and makes it difficult for the plant to absorb sufficient water for growth; hence decreases leaf water potential (Munns, 1993). The decrease in leaf water potential accompanied with a decrease in leaf osmotic potential so that leaf turgor pressure of the salinized plant was maintained (Tattini *et al*., 1995). Many important physiological and morphological processes, such as leaf enlargement, stomatal opening, and leaf photosynthesis directly affected by the reduction of leaf turgor potential that accompanies the loss of water from leaf tissues (Jones & Turner, 1978). Reduced water uptake is the common response of plants subjected to water or salt stress (Munns, 2002). Relative leaf water content (RLWC) is considered to be a better indicator of water status than water potential (Sinclair & Ludlow, 1985); although the latter is also a reliable trait for quantifying plant response to water stress (Siddique *et al*., 2000).

Better understanding of physiological and biochemical aspects of salinity stress tolerance mechanisms will not only help breeders in cloning of genes involved in salt

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stress tolerance, development of transgenic and better breeding programs, but also help scientists to determine accurate screening techniques ultimately aiding to crop improvement in saline soils (Sairam *et al*., 2002). Thus, the present study was conducted to elucidate the role of some of physiological traits in relation to salinity stress tolerance in wheat.

Materials and methods

Sowing and salinity treatments

The seeds of Four wheat (*Triticum aestivum* L.) cultivars, known as Neishabor and Sistani (salt tolerant), and Bahar and Tajan (salt sensitive) was obtained from the Iranian Seed and Plant Improvement Institute. All the seed samples were surface sterilized with 2 % sodium hypochlorite solution for 5 min and washed three times with sterilized distilled water. The experiment was conducted in the greenhouse of the Agricultural, Medical and Industrial Research School, Nuclear Science and Technology Research Institute, Karaj, Iran, during 2008-2009, where the average PAR of the entire growth period was 250 µmol m^{-2} s^{-1},average humidity 60±5% and the maximum and minimum temperatures were 25±2°C/15±2°C, respectively. The experimental plan was a completely randomized design in factorial arrangement with three replications. The treatments consisted of four wheat cultivars and four salinity levels (1.3 dS m^{-1} (control), 5, 10, 15 dS m^{-1}) with calcium chloride and sodium chloride in 1:10 ratio (Ca^{2+}/ Na^{+}). The Seeds were sown in uniformed pots (23×30 cm) filled with 4 kg loamy soil. In stage of 4-6 leaves, three plants were retained in each pot. To avoid any osmotic shock while seeds were emerging, salt enforcing was initiated in 4-6 leaf stage and continued until maturity stage.

The different growth parameters were studied at the tillering stage (45 days after sowing) and flowering stage (75 days after sowing). Samples of each treatment were immediately transferred to liquid nitrogen and maintained at -70°.

Grain yield for each treatment was counted at maturity from three pots, each having three plants. The grain yield that collected from three plants pot^{-1} recorded and converted into g pot^{-1}.

Observations

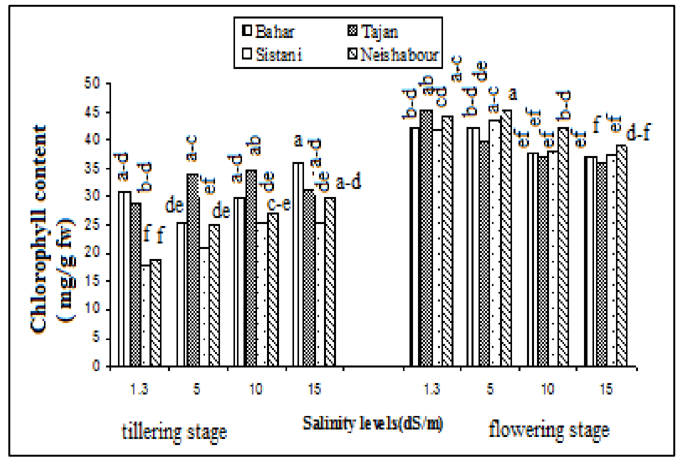
The estimation of leaf relative water content estimation conducted by incubating leaf samples (0.5 g) in 100 ml distilled water for 4 h (Weatherley, 1950). The turgid weight of leaf samples was recorded. The leaf samples were oven dried at 65 ºC for 48 h. Dry weights of the samples were taken after confirming that the samples were completely dried out.

$$\text{Relative water content (RWC)} = 100 \times \frac{\text{(Fresh wt-Dry wt)}}{\text{(Turgid wt-Dry wt)}}$$

The CHL content was determined as described by Arnon (1949). Quickly, lyophilized leaf (0.1 g) powder in 80% acetone and centrifuged at 10000×g for 10 min. Absorbance was recorded at 646, 663 nm, and CHL contents were calculated. Sodium and potassium contents were estimated flame photometerically (Tandon, 1995). For each stage, mean comparisons were separately done using SPSS software by Duncan's test (at P≤ 0.05).

Results

Fig.1. Effects of salinity levels on Chlorophyll content in wheat cultivars. The same letters indicates no significant differences at P≤ 0.05.



Salt treatment reduced the chlorophyll content at tillering stage and decreased at flowering stage (Fig.1). Tajan and Bahar cultivars had higher and lower chlorophyll contents compared to tolerant cultivars at fist and flowering stages respectively. It was found that at tillering stage, the chlorophyll content of Bahar and Tajan cultivars increased 17.10% and 9.68% up to 15 dS m^{-1} salinity level whereas Sistani and Neishabour cultivars had 42.57% and 60.45% increased at mentioned salinity level. It was observed that chlorophyll content in Tajan and Bahar were not increased so much at last stage (Fig. 1). Salinity treatments caused increased sodium content (Fig. 2a) and a reduction in potassium content (Fig. 2b). Bahar and Tajan cultivars exhibited higher sodium contents in both stages (Fig. 2a), although there were no significant difference between cultivars in control, but it was obvious along with increasing in salt levels. Accordingly, in each cultivar, there was significant difference between control and the highest salt level (i.e. 15 dS m^{-1}) and the difference between cultivars was more obvious in this saline level. So that, accumulation rate of this ion can be attributed to sensitivity of cultivars to stress. In Bahar, increasing of Na^{+} accumulation was about 143.23% and 89.2% (at tillering and flowering stages, respectively) at the highest salt level (15 dS m^{-1}) compared to control. While in Tajan cultivar, it was 138.36% (at tillering stage) and 54.69% (at flowering stage); Na^{+} accumulation was lower in two other cultivars Fig. 2a). On the other hand, Na^{+} contents were higher in Tajan and Bahar cultivars.

Fig.2. Effects of salinity levels on (a) Sodium content, (b) Potassium content, (c) K^+/Na^+ ratio in wheat cultivars. The same letters indicates no significant differences at P≤0.05.

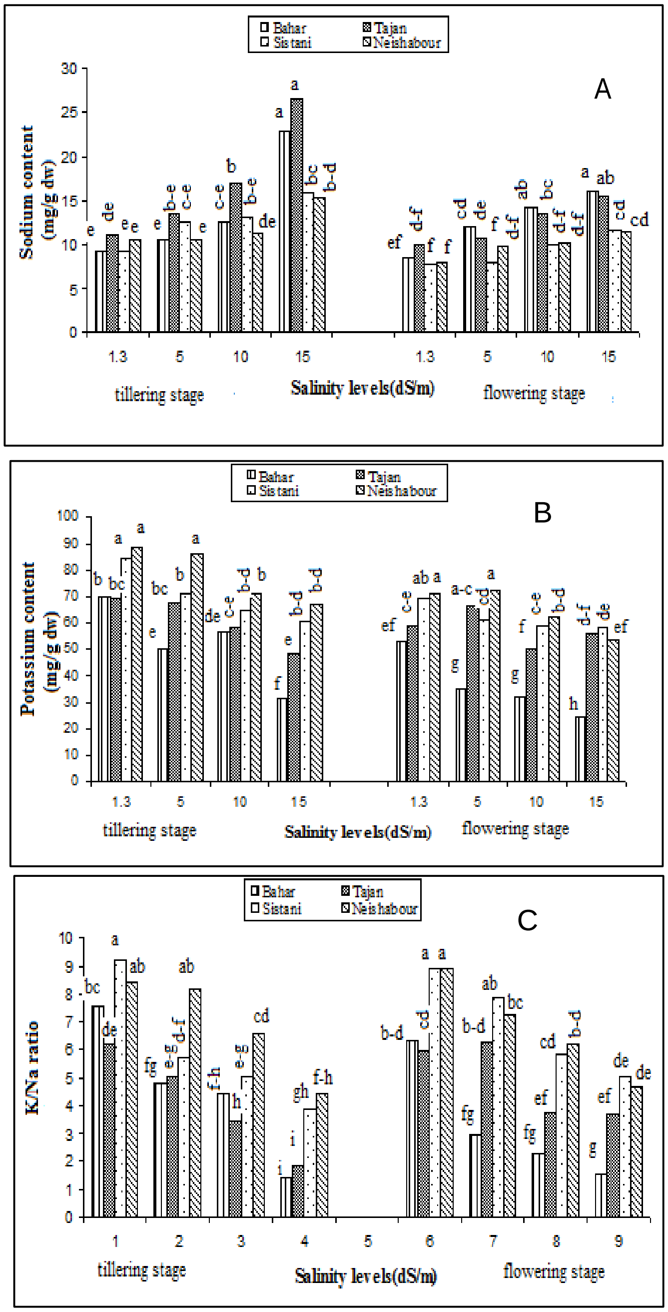
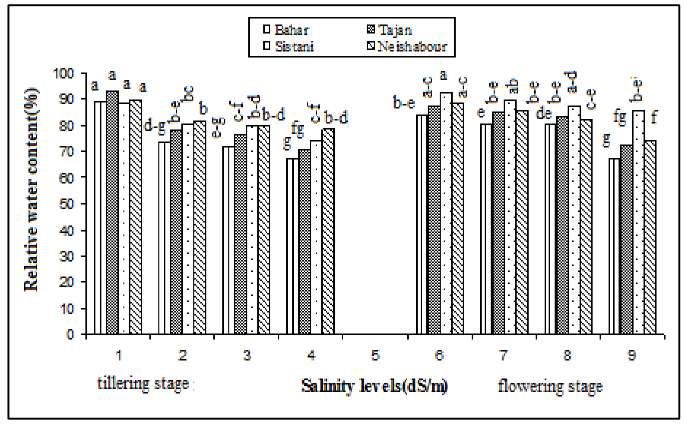


Fig.3. Effects of salinity levels on RWC in wheat cultivars. The same letters indicates no significant differences at P≤0.05.



Salinity treatment caused a reduction in potassium content and the reduction was more at higher salinity level in the all varieties at all the stages (Fig. 2b). Sistani and Neishabor had generally higher K^+ content than two other cultivars. K^+/Na^+ ratio in all varieties (Fig. 2c) decreased by the increasing of salinity levels at both stages. Sistani and Neishabour had higher K^+/Na^+ rather than others in both stages. RWC decreased by increasing of the salinity levels (Fig. 3). There were no significant differences between cultivars under control condition but the differences were more obvious over salinity levels. Bahar significantly showed the lowest RWC in all salinity levels at both stages (Fig. 3). Based on responses of each cultivar to salinity level of 15 dS m^{-1}, Bahar significantly maintained the lowest RWC in all salinity levels at both stages and showed significant difference compared to tolerant cultivars (Fig. 3). While Tajan and Neishabour indicated the same rank with two tolerant cultivars at tillering flowering stages, respectively, Sistani and Neishabour cultivars showed higher RWC in all salinity levels at both stages. Sistani and Neishabour showed higher values in some yield components as the grain number and weight, and the spike length. Tajan maintained highest spikelet number (Table 1). Increasing salinity levels significantly reduced the grain. In all treatments, the grain yield plant^{-1} was higher in Neishabour and Sistani than two other cultivars (Table 2). The reduction of plant yield in 15 dS m^{-1} compared to control was 28.96% in Sistani, 19.03%in Neishabour, 36.11% in Tajan and 49.87% in Bahar. The lower reduction of yield in tolerant cultivars was also observed at two other salinity levels (Table 2).

Table1. Mean values of yield and different yield components for four wheat cultivars

Cultivar	Spikelet No: plant^{-1}	Grain weight (g plant^{-1})	Spike length (cm)	Grain No: plant^{-1}	1000-grain weight (g)	Yield (g plot^{-1})
Bahar	12.45^{b}	0.795^{d}	7.43^{c}	25.82^{c}	51.32^{c}	19.16^{d}
Tajan	14.22^{a}	1.164^{c}	7.93^{b}	31.38^{b}	40.05^{ab}	28.03^{c}
Sistani	13.94^{a}	1.475^{b}	8.42^{a}	41.06^{a}	36.13^{bc}	35.52^{b}
Neishabour	13.98^{a}	1.794^{a}	8.30^{a}	41.2^{a}	43.97^{a}	43.20^{a}

Within columns means followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

Table2. Effects of salinity stress on the grain yield plant^{-1}

Trait	Cultivar	Salinity levels(dS m^{-1})			
		1.3	5	10	15
Grain yield (g plot^{-1})	Bahar	27.69 ^{de}	18.06 ^{fg} (34.78)$^{\#}$	17.01 ^{fg} (38.52)	13.88 ^{g} 49.87
	Tajan	36.28 ^{bcd}	27.85 ^{de} (23.23)	24.80 ^{ef} (31.64)	23.18 ^{ef} (36.11)
	Sistani	43.58 ^{ab}	39.09 ^{bc} (10.3)	28.57 ^{de} (34.44)	30.90 ^{cde} (28.96)
	Neishabour	49.44 ^{a}	43.02 ^{ab} (12.98)	39.25 ^{bc} (20.44)	41.09 ^{ab} (19.03)

Within columns, means followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

 # signifies the percent reduction in grain yield in each salinity level compared to control

Discussion

Different results have been reported in case of effect of salt and water stress on chlorophyll content. (Jiang & Hung 2001) in their water stress study on two species of grasses reported that chlorophyll increased during first period of stress (6 days after stress initiation) and after that decreased. Increase in chlorophyll content due to salinity has already been reported (Asish Kuma & Bandhu Das, 2005). The higher number of chloroplast per leaf area unit may be probably attributed for the decreasing of leaf area in response to salt stress. The lower increase of chlorophyll in sensitive cultivars may indicate their higher influence harmful effects of salt stress, including high sodium accumulation. High accumulation of sodium in plant tissues have been reported as one of the effective factors in reduction of photosynthetic pigments and rate of photosynthesis (Sairam *et al*., 2002; Ashraf, 2004).

The higher chlorophyll amounts in tolerant cultivars may be related to their ability in repairing salt-dependent damage at flowering stage (Fig. 2a). Because of chlorophyll importance as one of necessary factors in plant photosynthesis, it is possible that long-term salt stress has limited photosynthetic capacity and finally plant yield in Tajan and Bahar cultivars. Therefore, their lower chlorophyll contents in flowering stage can be resulted in their sensitivity and more destruction of photosynthetic pigments in response to salt conditions.

Salinity tolerance is related to the maintenance of net photosynthetic rate and stomatal conductance to elevate chlorophyll concentration (Winicov & Seemann; 1990; Salama *et al*., 1994). Similarly, Sairam *et al.* (2002) reported that reduction of chlorophyll content in a tolerant wheat cultivar was lower than in a sensitive one. High salt uptake competes with the uptake of other nutrient ions, especially K^+, leading to K^+ deficiency (Khan *et al*., 2000). Increase in sodium and depletion of potassium contents under salinity stress in case of wheat have been reported earlier (Ashraf & Oleary, 1996; Sairam *et al*., 2002). Sodium content was reported as an indicator of salt tolerance in cereals (Ashraf & Khanum, 1997). In glycophytes such as wheat, salt tolerance correlates with sodium exclusion and cultivars, having low ability in this case, can be introduce as sensitive cultivars (Poustini & Siosemardeh, 2004). So, higher increase of Na^+ content in Bahar indicates more sensivity of this cultivar in salt stress condition. Salinity increased sodium content in salt sensitive wheat cultivars (Sairam *et al*., 2002; Poustini & Siosemardeh 2004). Similarly, (Ashraf & Oleary 1996) reported that salt tolerance could be correlated with lower leaf accumulation of Na^+. According to this report, it can be concluded that Tajan and Bahar cultivars lacked the ability of excluding Na^+ and it can be the main reason for their salt sensivity.

The result of higher potassium content in tolerant cultivars of this study, under salinity stress, is consistent with Ashraf (1997) concluded that K^+ content was higher in tolerant S24 genotype than Yecoro Rojo salt sensitive at tillering and flowering stages. Beneficial effect of higher osmolyte concentrations (such as potassium in this study) is reflected in maintenance of higher RWC and finally, high seed yield of Sistani and Neishabour in comparison with two other cultivars.

The selective uptake of K^+ as opposed to Na^+ is considered one of the important physiological mechanisms contributing to salt tolerance in many plant species (Gupta & Srivastava, 1990). Therefore, less Na^+ accumulation (Fig. 2a) and more K^+ content (Fig. 2b) in Sistani and Neishabor cultivars at the highest salt level, confirm salt tolerance of these cultivars. It seems these cultivars have mechanisms for restricting Na^+ inclusion and transporting it to shoot tissues, resulted in higher yield in these cultivars (Table 2). It has been suggested that the plant tolerance response is characterized by distinctly lower sodium/potassium ratio, which may be used to predict tolerance or sensitivity in wheat varieties (Sairam *et al*., 2002).It was observed that Tajan and Bahar cultivars, which had the lowest K^+/Na^+ ratio (Fig. 2c) have maintained the highest sodium content (Fig.2a) and these two cultivars showed lower yield compared to the tolerant cultivars (Table1). It has been reported that reduction of seed yield, correlated highly with K^+/Na^+ ratio in wheat leaves (Gupta & Srivastava, 1990). So, lower K^+/Na^+ in Tajan and Bahar (especially in the last one) can be related to their inability to restrict or control ion accumulation in shoot tissues (Ashraf & McNeilly, 1988). As a result, it tends to the more appearance of harmful salt stress effects on morphological and biochemical features and finally, the lower tolerance of them to salinity condition. Similarly, it has been observed that the reduction of wheat growth related to the sodium accumulation and reduction of potassium content (Sharma**,** 1996). Therefore, it may be concluded the lower sodium accumulation and higher K^+/Na^+ in Sistani and Neishabour cultivars can be contributed to their salinity stress tolerance.

Tajan and Bahar cultivars had the lowest grain yield and compared with other wheat cultivars had the highest Na^+ accumulation (Fig.2a), it could be concluded that in these cultivars, the higher Na^+ accumulation may cause to ion toxicity and finally, the lower growth and plant yield. Bahar showed the highest Na^+ content and the most reduction in the yield, so it can be considered as more salt sensitive than Tajan. It was observed that in the cultivars which had more sodium contents (Tajan and Bahar) (Fig. 2a), Bahar indicated lower RWC compared to Tajan. This result was observed at 15 dS m^{-1}, too (Fig. 3). Therefore, it can be concluded that Bahar is more sensitive to both osmotic and toxic effects as this cultivar showed the highest yield reduction. However, in comparison with Bahar, Tajan showed lower sodium content and higher RWC, and had no significant difference with tolerant cultivars (Fig. 3). It indicates lower sensitivity of this cultivar compared to Bahar. In addition, Tajan maintained lower yield than tolerant cultivars (Table1). According to (Farooq & Azam 2006), high amount of Na^+ accumulation and drastic reduction in RWC was found in salt sensitive cultivars of wheat.

Tolerance to stress condition defined as an ability of plants to grow in low water potential and in this way, high RWC is one of tolerance mechanisms to stress condition (Sinclair & Ludlow, 1985). Sistani and Neishabour genotypes showed higher RWC at 15 dS m^{-1}(Fig. 3) and finally, the highest yield belonged to them, it can be suggested that these two cultivars have avoided osmotic stress resulted from salt stress. Similarly, Sairam *et al.* (2002) reported that under salt stress, RWC was higher in salt tolerant wheat cultivar than sensitive one.

In Tajan and Bahar, maintenance of the highest spikelet number indicated the lower grain number and weight when compared to tolerant cultivars (Table1). It seems that in these cultivars, salt stress had more destructive effect, resulted in flower sterility, and decreased the transportation of assimilates to seeds. Consequently, other yield components such as the grain number and weight were decreased under salt conditions (data not shown). It is reported that reduction of yield under salt stress against control condition was used as an indicator of tolerance to salt stress (Ochiai & Matoh, 2001). Based on reduction in yield (Table 2), it is clear that yield of sensitive cultivars (Tajan and Bahar) has affected drastically by salt stress. Relating to this, Sairam *et al.* (2002) reported that reduction in yield of Kharchia 65 (tolerant cultivar) was lower than KRL 19 (moderately sensitive). In 15 dS m^{-1} compared to control, the highest yield and lowest reduction in plant yield were observed in Sistani and Neishabour (Table 2). It can use as a reference to determine validity of these cultivars in case of salt tolerance.

Conclusion

Considering data obtained on plant yield and some physiological parameters, it was clear that Sistani and Neishabour were more tolerant to salinity stress, than Tajan and Bahar. According to higher Na^+ content and more reduction in yield and RWC in Bahar genotype, this cultivar can be considered as more salt sensitive than Tajan. Higher potassium concentration in tolerant cultivars (Sistani and Neishabour), resulting in lower sodium accumulation and RWC, contributes to their salinity stress tolerance. The use of the mentioned physiological determinants is so suitable for screening salt tolerant wheat genotypes. In conclusion, it can be suggested that considering more physiological traits related to the salt tolerance can be useful in our better understanding on physiological aspects of salinity tolerance mechanisms in wheat.

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