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NaCl Effects on Accumulation of Minerals (Na^+, K^+, Cl^-) and Proline in *Triticum turgidum* L.

[1]M.R. Hadi, [1]R. Khavarinejad and [2]N.A. Ghoshkolgh-Sima
[1]Azad University of Islamic Science and Research Unit of Tehran, Iran
[2]Agricultural Biotechnology Research Institute Iran, Tehran, Iran

Abstract: In this project, the effects of elements (Na^+, K^+ and Cl^-) accumulations and proline contents on the salinity tolerance in seven genotypes of durum wheat (*Triticum turgidum* L. susp durum) were studied under greenhouse condition that has been collected from the region of Middle East. Seeds of durum wheat genotypes are including: ICDW19697 (Turkey-1), ICDW751 (Syria-1), ICDW859 (Iran-1), ICDW446 (Egypt), ICDW19697 (Syria-2), ICDW19764 (Turkey-2) and ICDW324 (Iran-2) that planted under hydroponics condition. Salt stress was initiated in three-leaf stage, by gradual adding NaCl to the nutrient solutions and applied salt treatments as 0 (control), 50, 100, 150, 200 and 300 mM NaCl. The results showed that all genotypes died in concentrations of 200 and 300 mM NaCl and also all genotypes showed decrease of growth in concentration of 150 mM NaCl respect to control (0 mM NaCl). In concentration of 150 mM NaCl, genotype of ICDW751 (Syria-1) has minimum accumulation Na^+ (40 mg Na^+ g^{-1} DW) and maximum accumulation K^+ (50 mg K^+ g^{-1} DW) and high proline content in shoot have been higher dry weight that it's known as salt tolerance respect to other genotypes and genotype of ICDW324 (Iran-2) with high accumulations of Na^+ and Cl^- and also low accumulations of K^+ and low proline content in shoot have been lower dry weight that it's known as salt sensitive respect to other genotypes. In concentration of 150 mM NaCl, genotype of ICDW19697 (Syria-2) has highest proline content at the shoot. In general, results from above measurements indicate that among genotype of Iran-2 has low resistant to salinity whereas genotype Syria-1 show better resistant to salinity than the others because it had minimum content of Na^+ and had more K^+, K^+/Na^+ ratio than the others genotypes in 150 mM NaCl in the medium.

Key words: *Triticum turgidum*, salinity, accumulations of Na^+, K^+, Cl^- and proline, tolerance genotype

INTRODUCTION

About 7% of the world's total land area is affected by salt, as is a similar percentage of its arable land (Ghassemi *et al*., 1995). Irrigation systems are particularly prone to salinisation, with about half the existing irrigation systems of the world now under the influence of salinisation or water logging, due to either low quality irrigation water, or to excessive leaching and subsequent rising water tables (Szabolcs, 1994). According to the data from the digital soil map of Iran saline to moderately salt-affected soils cover about 25.5 m ha and soils with severe salinity problems cover some 8.5 m ha (Siadat *et al*., 1997). Excessive salt accumulation in soils, in fact, has been recognized as a limiting factor for crop production of one-third of the world's limited arable land (Epstein *et al*., 1980). Hence, a detailed understanding of the basic mechanisms involved in the plant salt tolerance is an important prerequisite to improve the performance of crop plant in saline soils (Binzel and Reuveni, 1994). On the other hand, macaroni (Oleson, 2000) is as one of the most important food productions of durum wheat (*Triticum turgidum* subsp durum) that its industry development and its hard need to this product in Iran, thus for planting of durum wheat is very necessary to arable land (degree of one). For removed this problem suggests that to used soil, (degree of two) such as salinity land for planting of durum wheat. Also, high international prices and strong demand has stimulated an increase in the production of durum soils wheat, *Triticum turgidum* L. ssp. durum (Desf.) (Munns *et al*., 2000). Salt tolerance in plants varies along a continuum with genetic variation attributable to ion exclusion or accumulation, production of compatible solutes, turgor maintenance, differences in development patterns (e.g., root-shoot ratios), root anatomy and general plant vigor. Plants can be grouped on the basis of their ability either to take up and compartmentalize salt or to exclude it (Greenway and Munns, 1980). Salinity affected plant growth through ionic and osmotic effects. The difference in plant's response to a given level of

Corresponding Author: M.R. Hadi, Azad University of Islamic Science and Research Unit of Tehran, Iran

salinity is dependent upon the concentration and composition of the ions in solution as well as genotype that is exposed to the salinity. One of salinity stress effects on the plant metabolism is imbalance in cell plant nutrition (Staple and Gray, 1984). Increase of Na^+ concentration in plant tissues is one of the primary plant responses to salinity stress (Meneguzzo *et al*., 2000). It is evident that salt tolerance is associated with low uptake of Na^+(Santa-Maria and Epstein, 2001), partial exclusion (Colmer *et al*., 1995) and compartmentalization of salt in the cell and within the plant (Ashraf, 1994). Schachtman and Munns (1992) described the association of low shoot Na+ concentration with salt tolerance in wheat. Potassium represents the major inorganic constituent of plant cell and is involved in many physiological processes such as turgor potential regulation, cell elongation, growth of shoot and roots, stomatal movement, transpiration. Meneguzzo *et al*. (2000) and Santa-Maria and Epstein (2001) reported that the capacity to concentrate K+ in response to NaCl stress was accompanied. In wheat a relationship between K^+ ion accumulations and tolerance to salinity has been found (Rascio *et al*., 2001). Traits used for screening germplasm for salinity tolerance have included Na^+ exclusion, K^+/Na^+ discrimination (Asch *et al*., 2000). For the durum subspecies, low Na^+ in the leaf blade correlated well with salinity tolerance (Davenport *et al*., 2005), whereas K^+ or K^+/Na^+ had a lower regression coefficient (Munns and James, 2003). Ashraf and Kanum (1997) and Munns (2002) reported that the Cl^- accumulations in shoot of durum wheat to increasing salinity have been found. Leaf concentrations of either Na^+ or Cl^- are often correlated with differences in salt-tolerance between related varieties. For example, in citrus, lower Cl^- concentration in leaves correlates with salt tolerance (Storey and Walker, 1998) and in soybeans salt tolerance can also be correlated with the ability to exclude Cl^-. For wheat a correlation is observed between the ratio of Na^+ to K^+ in the shoot and salt tolerance, a trait that is clearly determined by a variety of different genes (Omielan *et al*., 1991). The apparently different roles of Na^+ and Cl^- ions between plants may be related to how they are sequestered into the mesophyll and epidermal cells of the leaf (Leigh and Storey, 1993) and how charge balance is maintained. Many plants accumulate high levels of free proline in response to osmotic stress. This amino acid is widely believed to function as a protector or stabilizer of enzymes or membrane structures that are sensitive to dehydration or ionically induced damage. The salt stress caused increases in proline levels. Several investigations have shown that, besides other solutes, the level of free amino acids, especially proline, increases during adaptation to various environmental stresses. For the durum subspecies, high the level of free amino acids, especially proline in the leaf correlated well with salinity tolerance (Simon-Sarkadi *et al*., 2002). On the base of these concepts, the object of present research is to evaluate which are the effects of salinity stress on the Na^+, K^+, Cl^- and proline accumulations for finding correlation between accumulation elements and salt tolerance in durum wheat and also nominates highest salt tolerant genotype.

MATERIALS AND METHODS

Seeds of seven durum wheat [*Triticum turgidum* L. subsp. Durum (Desf.) Husn.] genotypes provided by Agricultural Biotechnology Research Institute Iran (ABRII) including: ICDW19697 (Turkey-1), ICDW751 (Syria-1), ICDW859 (Iran-1), ICDW446 (Egypt), ICDW19697 (Syria-2), ICDW19764 (Turkey-2) and ICDW324 (Iran-2) that it's collected from Middle East. A factorial experiment with two factors of genotypes with seven levels and salinity with six levels (0, 50, 100, 150, 200 and 300 mM NaCl) was used. The treatment combinations were replicated three and arranged in a completely randomized design (CRD). Seed were surface sterilized in sodium hypochlorite solution 5% and rinsed with distilled water. An experimental unit consisted of four seedlings for each genotype and set up on a 18 tank containing a fourth strength Clark's solution (Clark, 1982). These units were placed in the greenhouse under day/night temperatures of 22±2/15±2°C and day length of 13 h. After 3 days the nutrient solutions were replaced with full strength Clark's solution. The solution were aired automatically 15 min per hour and were renewed every 6 days. Salt stress was initiated 21 days after seed germination, by gradual adding NaCl to the nutrient solutions. To avoid osmotic shock, NaCl was added twice daily to increments of 50 mM until the final concentrations of 100, 150, 200 and 300 mM NaCl were achieved. Plants were harvested 30 days after commencing treatments, separated into shoot and root washed with distilled water. Plants samples were oven dried (75°C to constant mass) and weighed. Plant samples were pulverized then with 10 mL mixed two acids (acetic acid 10% and nitric acid 0.1 N) shakers at 24 h and extracted the volume of each sample was standardized to 100 mL. Na^+ and K^+ concentrations of the solutions were determined using an atomic absorption spectrophotometer. Cl^- concentrations of the solutions were determined using an Ion chromatograph. Free amino acid Proline content in shoot was quantified using the Ninhydrin reagent (Bates *et al*., 1973).

RESULTS AND DISCUSSION

Shoot dry weight and root dry weight of seven genotypes of durum wheat grown on salinity in comparison with non-salinity conditions (control) used as plants growth indexes. The results show that whole seven genotypes are non-ability growing in 200 and 300 mM NaCl, but ability growing in 50, 100 and 150 mM NaCl on the hydroponics culture. Genotype of Syria-1 on 150 mM NaCl have lowest decrease percent in plant growth whilst, genotype of Iran-2 on 150 mM NaCl have highest decrease percent in plant growth respect to other genotypes (Table 1). The Na^+ accumulation to increase salinity in comparison with control has significant increased in shoots and roots. Also, the Na^+ and K^+ accumulations of shoots and roots at the seven genotypes of durum wheat grown in different concentrations of NaCl have significant difference at 1 percent level (not shown). The Syria-1 genotype grown in 150 mM NaCl concentration has minimum Na^+ accumulations whereas the Iran-2 genotype has maximum Na^+ accumulations in shoots and roots (Table 2). Therefore, Syria-1 genotype has demonstrated a better tolerance and Iran-2 genotype a lower to tolerance salt stress with respect to other genotypes. Because low Na^+ and Cl^- accumulations in laves and roots of durum wheat are lower toxicity for plant cells (Munns, 2005). In the other hand, the K^+ accumulation to increase salinity in comparison with control has significant decreased in shoots and roots (not shown). That it's according to results Almansouri *et al.* (2001). The Syria-1 genotype grown in 150 mM NaCl concentration has maximum K^+ accumulations whereas the Iran-2 genotype has minimum K^+ accumulations in shoots and roots (Table 2) that may be it due effects of antagonistic of Na^+ on the K^+ in absorption. In addition, according to Daud and Gustafson (1996), K^+/Na^+ ratio has important on the tolerant to salinity in durum wheat. Among of K^+ accumulation is high at the shoots in Syria-1 genotype while among of Na^+ accumulation it is low, therefore K^+/Na^+ ratio is maximal at the shoots in Syria-1 genotype (Fig. 1) that according to Daud and Gustafson (1996) that expressed high K^+/Na^+ ratios at the shoots in durum wheat correspond with tolerance to salinity. Therefore, seem that Syria-1 genotype in hydroponics solution to be demonstrating a better tolerance to salt stress with respect to others genotypes. In the other hand, among of proline accumulation is not low at the shoots in Syria-1 genotype (Fig. 2). Altogether, the growth analyses carried out in hydroponic solution have demonstrated a better tolerance to salt stress of the Syria-1 genotype with respect to others genotypes because, among of Na^+ accumulation at the shoots and roots in this genotype were lower than with respect to others genotypes, hence according to Munns (2002) can be this genotype demonstrates tolerance to salinity with respect to others genotypes. Also, Schachtman and Munns (1992) reported that because Na^+ exclusion as one of the mechanisms tolerance to salinity expressed in more wheat lines, hence genotype of tolerant to salinity have lower levels of Na^+ in shoot. Because, Iran-2 genotype have maximum among

Table 1: Mean values for decrease percent in plants growth from three applications in seven genotypes durum wheat grown on 150 mM NaCl concentration into control on the base of shoot dry weight, root dry weight and plant total dry weight on the LSD design

	Amount of decrease percent in plants growth in 150 mM NaCl		
Genotypes of durum wheat	Shoot (% Dry weight)	Root (% Dry weight)	Plant total (% Dry weight)
Turkey-1	86.88b	88.84bc	87.32b
Syria-1	80.21c	87.62c	82.13c
Egypt	89.32b	90.14b	89.49b
Iran-1	87.15b	89.03bc	87.61b
Syria-2	85.84b	92.76a	87.23b
Turkey-2	87.60b	93.41a	89.36b
Iran-2	94.79a	94.17a	94.59a

Mean values with the same letter(s) are not significantly different

Table 2: Means of amount elements from three applications in seven genotypes durum wheat grown on 150 mM NaCl concentration base of dry weight on the LSD design

Genotypes of durum wheat	Na^+ (mg ion g^{-1} DW*)		K^+ (mg ion g^{-1} DW)		Cl^- (mg ion g^{-1} DW)	
	Shoot	Root	Shoot	Root	Shoot	Root
Turkey-1	42.14d	39.11c	34.95c	17.99c	161.67b	74.51c
Syria-1	40.00f	39.14c	50.02a	19.37bc	124.25e	111.77b
Egypt	52.17b	40.17bc	42.43b	18.62bc	149.11c	74.51c
Iran-1	49.68c	39.53c	49.21a	26.04a	136.55d	173.90a
Syria-2	52.20b	43.21a	23.11d	17.94c	99.46f	74.51c
Turkey-2	40.94e	35.18d	42.66b	18.28c	49.90g	49.65d
Iran-2	54.08a	40.88b	33.92c	20.06b	211.07a	49.75d

*DW = Dry weight, Mean value with same letter(s) are not significantly different

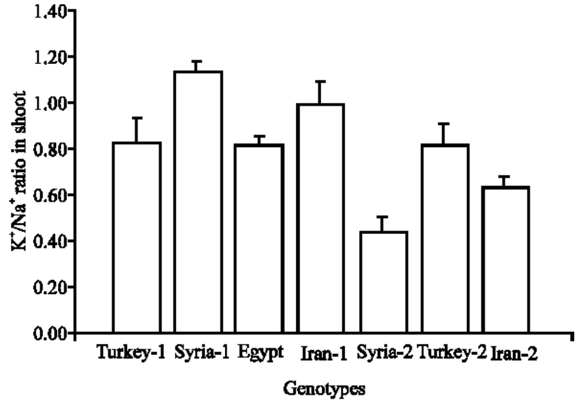

Fig. 1: The effect of salinity (150 mM NaCl) on the K^+/Na^+ ratio at shoot in seven durum wheat genotypes. Vertical bars indicate±Standard error. LSD (5%) = 0.3673

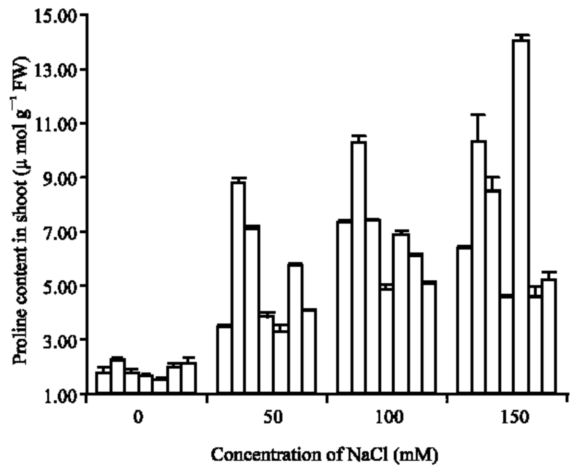

Fig. 2: The effect of salinity (150 mM NaCl) on the proline content at shoot in seven durum wheat genotypes. In each salt treatment from left to right, genotypes including: Turkey-1, Syria-1, Egypt, Iran-1, Syria-2, Turkey-2 and Iran-2 respectively. Vertical bars indicate±Standard error. LSD (5%) = 0.3493 and FW= Fresh Weight

of Na^+ accumulations in shoots, thus seems that this genotype shows a lower tolerance to salinity with respect to others genotypes. Also, among of K^+ accumulation is lower at the shoots in Iran-2 genotype, thus K^+/Na^+ ratio is minimal (Fig. 1) and according to Daud and Gustafson (1996) that expressed low K^+/Na^+ ratio it correspond with sensitive to salinity. In the other hand, among of proline accumulation is not high at the shoots in Iran-2 genotype. Also, among of proline accumulation is highest at the shoots in Syria-2 genotype (Fig. 2). Thus, proline did not seem to be an important osmoticum in Syria-2 genotype but has been found to be essential for the stress recovery (Liu and Zhu, 1997). There is evidence that under saline conditions, the symptoms of potassium deficiency will persist despite high concentrations of this nutrients in wheat leaves because some of the absorbed K^+ act as counter ions to the Na^+ ions that accumulate in vacuoles, causing a certain percentage of K^+ not to perform any vital role. Therefore, salt induced toxicity may be effectively reduced and yield enhanced by potassium ions. Furthermore uptake K^+ ions causing an antagonistic to uptake Na^+ ions decrease in salinity conditions. Thus by increasing K^+ concentration in shoots, the plants tolerance to salt stress would increase. Reducing the rate at which salt accumulates in leaves will prolong their photosynthetic activity and ensure there is sufficient assimilate to fill the grain that is set (Munns, 2005), so they are optimistic that the introduction of the mechanism for low Na^+ uptake and high K^+/Na^+ selectivity (Gorham *et al*., 1997), will confer salt tolerance to current cultivars in terms of biomass and yield. Thus, it seems that genotype Syria-1 uses avoidance strategy as a response to salinity. Thus, it may be defined as a generally osmotolerant type. Therefore, Syria-1 genotype may represent promising material to study the genetic basis and to identify the gene(s) responsible of salt stress tolerance in tetraploid wheat. The availability of large and useful genetic variation indicates that the introduction of low Na + accumulation into modern cultivars should be possible as part of a durum wheat breeding program.

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