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The Effects of Sodium Chloride Stress on Proline Content and Morphological Characteristics in Wheat (*Triticum aestivum* L.)

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ABSTRACT: Salinity is the most important abiotic stresses adversely affect the quality and quantity of crops, in which 20% of the world's irrigated agricultural lands are affected by salinity. Wheat is an oldest and the first crop used for bread making for human nutrition. To investigate the response of wheat root to NaCl stress, a susceptible, Arta, and resistance, Bam, wheat varieties were grown under both non-stress and stress conditions. Stress plants were exposed to 250 mM of NaCl based on a completely randomized design with four replications. After removing the roots from the soil and removing them from the shoot, the fresh and dry weight, length, volume, Numbers of main roots of each plant were recorded. Then the other roots were harvested and were immediately immersed in liquid nitrogen and stored at -80°C until used for proline measurement.

Key words: NaCl stress, proline content, morphological characteristic, root, wheat

INTRODUCTION

Plants in various ecosystems are constantly exposed to the stress of living and non-living factors such as fungi, weeds, drought and salinity contributes to the development of this limiting factor in plants (Lawlor, 2002). Desertification and Salinization phenomena increase is quick in a global scale and currently affect more than 10% of arable lands which results in a decline of the yield of major crops greater than 50% (Wang et al., 2009). Every year more and more lands become non-productive due to salt accumulation. Therefore, understanding the mechanisms of plant tolerance to salinity stress is important (Bartels and Sunkar, 2005). Salinity is one of the most important abiotic stresses that adversely affect the quality and quantity of crops, so that 20% of the world's irrigated agricultural lands are affected by salinity (Zhao *et al.*, 2007). In general salinity is excess existing the soluble salts and mineral maters in water and soil solution that resulted in accumulation salt in rhizome area and plant can't enough uptakes water from soil (Shannon *et al.*, 1994). Much salinity resulted from NaCl cause to at least three problems: 1. Osmotic pressure of external solution become more than osmotic pressure of plant cells which is require to regulating osmotic pressure to preventing dehydration by plant cells. 2. Uptake and transform of nutrition ions such as potassium and calcium, by excess sodium would make problems. 3. High Na and Cl rates would cause to direct toxic effects on enzymic and membranous systems. One of the effects of salinity stress is reducing photosynthetic activity which caused to decreasing b and a chlorophylls and reducing Co uptake and photosynthetic capacity (Francois and Maas, 1999). According to studies, Proline is used as an enzymic protector that contributes in macromolecules structure and is main source of energy and nitrogen to confront salinity. Many researchers believed that proline accumulation in plants involved in resisting to salinity stress (Patnaik and Debata, 1997; Thomas *et al.*, 1992). Present investigation in order to study harmful effect of salinity stress on wheat (*Triticum aestivum* L.) and finding mechanism and introducing more resistance variety.

MATERIALS AND METHODS

A. Plant growth and stress treatment

Plant material used in this study was two wheat (*Triricum aestivum* L.) cultivars; "Bam" and "Arta" known as tolerant and susceptible to NaCl stress, respectively. These seeds were taken from "Seed and Plant Improvement Institute", Karaj, IRAN. Experiment was conducted in completely randomized design with three replications. Treatments were combination of wheat, cultivars of wheat ("Arta" and "Bam") and two irrigation levels including normal and NaCl stress (250 mM). Stress was imposed by combination NaCl with water. Then roots were harvested and were immediately immersed in liquid nitrogen and stored at -80°C until used for proline measurement.

After removing the roots from the soil and removing them from the shoot, the fresh weight they weighed in milligrams and put into the small paper bags and put them in the oven for 48 hours at 70°C. Root volume by putting them into a 10 ml graduated cylinder containing 5 ml of distilled water, was read. Numbers of main roots of each plant were recorded. The length of the longest root of each plant was measured in centimeters.
For measurement of Proline content, 0.5 gr vegetarian matter grinded in 10 ml 3% Solphosalicylic acid using Bates method (Bates, 1973). The solution purified and 2 ml taken off from any solutions, then 2 ml ninhydrin acid agent and 2 ml acetic acid added to theme. Tubes soaked in Ban Mary for 1h in 100 C and then kept for 30 m in ice bath, then 4 ml toluene added to tubes and two separate layers formed after shaking tubes and keeping them for 20 s. Finally colored layer absorption during 520 nm waves and praline content measured using standard curve. Analysis was carried out by SPSS software and Statistical analysis of CRD.

RESULTS AND DISCUSSION

After reviewing the normal distribution of data, analysis of variance study was conducted in a completely randomized design.

A. The mechanisms of salt tolerance

The mechanisms of salt tolerance are very complex. The metabolic sites of salt influence have not been fully investigated, in particular in the photosynthetic apparatus; thus, there are no reliable indicators of plant tolerance tom salinity that could be used by plant breeders to improve salinity tolerance in agricultural crops (Shannon, 1998). Crop yield and growth parameters have been commonly used by breeders to screen for salinity tolerance (Cramer, 1990; Noble and Rogers, 1992; Munns, 1993; Kalaji and Guo, 2008).

B. The effect of sodium chloride on root characteristics

The results showed that the difference between the treatments in root length, root fresh weight and number of roots it wasn't significant and it was concluded that salt stress had no significant effect for these traits. Probably the lack of significant effect of stress on these traits is due to short duration stress and also the effect of stress on morphological traits need more time. In addition, the high coefficient of variation for traits such as root fresh weight, root length and root volume should not be ignored.
The difference between treatments in dry weight and number of root were significant in five and one percent probability levels (Fig.1, 2; Table 1). We have contradictory reports on the effects of salinity and drought stress on root growth (Dajic, 2006). After the addition of salts or other osmotics, root growth will be recovered (Munns, 2002). There are lot of reports of many negative relationship between salt stress and the development of primary and secondary roots, root and shoot dry weight, stem diameter and plant height stress (Ashraf and Ahmad, 2000). Saqib and colleagues also reported negative effects of salinity on root growth. Of course, some salt concentrations can stimulate root growth, but prevent the growth of stem.

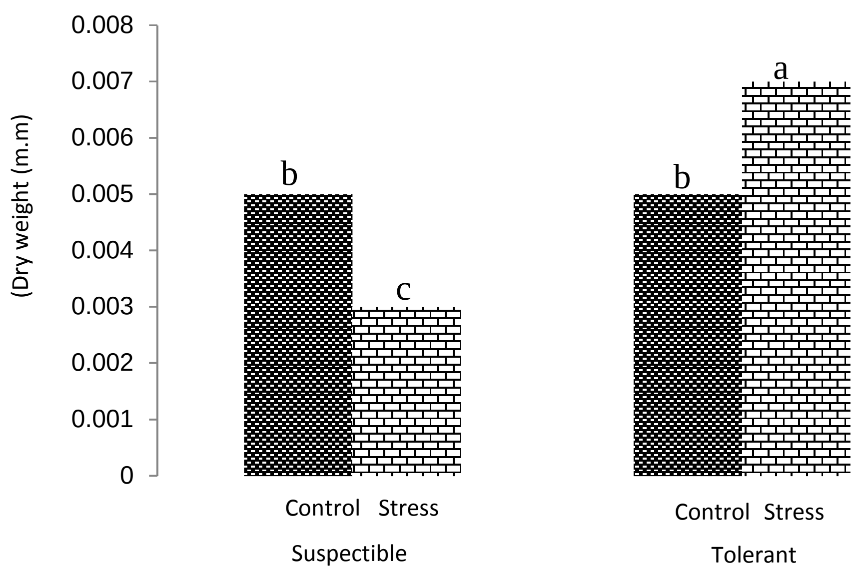

Fig. 1. Compare of root dry weight changes in susceptible and tolerant wheat varieties under normal conditions and salt stress

Non-subscribers letters indicate significant differences in the level of 5 percent probability.

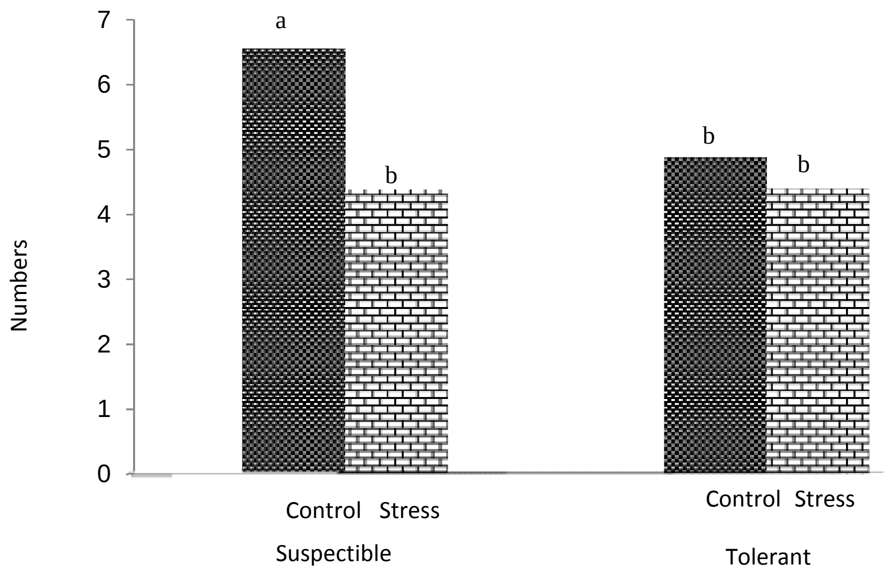

Fig. 2. Compare of root numbers changes in susceptible and tolerant wheat under normal conditions and salt stress Non-subscribers letters indicate significant differences in the level of 5 percent probability.

Salt interferes with plant growth and lead to dryness and the ion toxicity (Saqib *et al.,* 2004). Salinity cause to reduce of water potential, difficulty of water and food absorbance for plants and make to increased Na+ and Cl-, that are toxic (Tuteja, 2007). Root levels increasing, by increasing absorption, increase the efficiency of water and nutrient is important. Therefore, longer roots and provide a higher surface can help to provide salt tolerance (Kant and Kafkafi, 2005). Singh and colleagues showed that the plants longer main root and more lateral roots than plants that have this characters less, are more tolerant to salinity (Singh *et al.,* 2000). Mainand colleagues reported by experiments on winter wheat, salinity decrease fresh root weight (Main *et al.,* 1993). Sensitive to salinity stress dependent on the genotype, age and species of plant resistance to salinity (Parida *et al.*, 2004).

C. Effect of Sodium Chloride on Proline

Analysis of variance showed significant differences among treatments root proline at 1% probability (Table 1).

Compare two teams under different level of salinity showed the more level of proline in 250 mM salinity level in the Arta (sensitive) and the lowest proline in the Bam (resistance). Also, after salinity stress, proline content is more increase in sensitive cultivar than resistance (Fig. 3). The increase of proline in the susceptible cultivar under 250 mM of salinity compared to the control average was 72%. Proline and hydroxyproline in structural proteins are clearly distinguished from free proline, which serves to regulate osmotic adjustment. Proline transporter (HvProT) was highly expressed in the apical region of barley roots under salt stress (Ueda, 2007). Increasing of amino acid concentration which contributes to osmoregulation, is due to several factors such as: inhibition of proline analysis, inhibition of enter of proline to protein or increasing of protein analysis which accompany with growth reduction (Kao, 1981). Proline has conservative effect on nitrate reductase activation of cellular membrane in barely plant leaf under low-water situation seems that cellular membrane resistance is one of the best physiologic indices against with environmental stress and is as a parameter for plants selection with high resistance is high (Neto, 2004). Carbohydrate soluble aggregations in leaves under stress indicating of involve in plant consistency process (Kerepesi, 2000).

Table 1: Root character's variance analysis of susceptible and tolerant wheat under salt stress.

		M.S					
Source of variation	**Degree of freedom**	**Proline**	**Root numbers**	**Root volume**	**Dry weight**	**Fresh weight**	**Root length**
Treatment	3	89.9 **	2.63 **	0.000090 ns	0.0000028 *	0.001 ns	34.33 ns
Error	8	0.72	0.31	0.000086	0.000001	0.001	28.266
C.V (%)		5.51	10.79	32.31	23.55	41.27	34.04

P* 0.05, P** 0.01; ns: Non-signification. Treatment is include cultivar and stress.

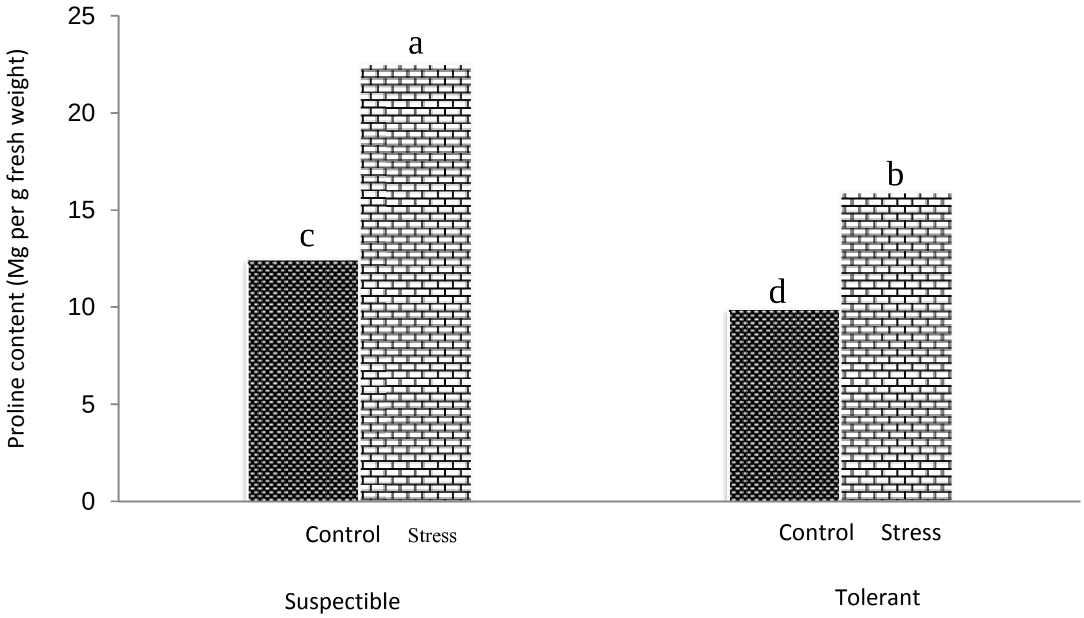

Fig. 3. Compare of proline content changes in susceptible and tolerant wheat under normal conditions and salt stress. Non-subscribers letters indicate significant differences in the level of 5 percent probability.

The reduction of photosynthesis activity is one of the main reasons in salinity influence of plants in which is due to chlorophyll reduction and also reduction of CO_2 absorption and photosynthesis capacity (Fransisco, 2002).

Anything that decrease the water potential cause to accumulation of proline (Kuznetsov and Shevyakova, 1999). In plants under water or salt stress, proline content increases more than other amino acids, and this effect has been used as a biochemical marker to select varieties aiming to resist to such conditions (Bates, 1973). A large number of plant species accumulate proline in response to salinity stress and that accumulation may play a role in defense against salinity stress. However, data do not always indicate a positive correlation between osmolytes accumulation and an ability to adapt to stress (Mansour *et al.,* 2005). The role of proline in cell osmotic adjustment, membrane stabilization and detoxification of injurious ions in plants exposed to salt stress is widely reported (Hare *et al.,* 1999; Kavi Kishor *et al.,* 2005; Ashraf and Foolad, 2007).Postini and colleagues also reported an increase in proline content due to salinity and This increase in the susceptible cultivar was more than the resistance cultivar and concluded that proline can't have a protective effect against stress (Poustini *et al.,* 2007). Silveria and colleagues reported proline content is more increase in wheat verities that are more sensitive to salinity.

These changes are different in various stages of shoot and root growth and different from each other (Silveria *et al.,* 2003). Meighani and Ebrahimzadeh also has been reported proline content under salit stress compare with non-stressed have reached 2.9. Proline and proline dehydrogenase enzyme activity decreased Increasing concentration in wheat leaves Jerusalem (salt-sensitive) than Bolani (tolerant), respectively (Meighani and Ebrahimzadeh, 2002). By the effect of salinity on plants and plants response to salt stress can assume that the free amino acid of organic osmotic regulator solutions, have claimed more vital role. That serine, glycine, arginine, valine, leucine and proline are the most important of them (Ashraf and McNeilly, 2004). In general, free amino acids are increased with increasing salinity level (Parida and Das, 2005). The accumulation of proline in different plant species under environmental stresses has various reports (Kavi Kishor *et al.,* 2005). Proline in response to many environmental stresses such as drought, salinity (Delauney and Verma, 1993), high temperature (Kuo *et al.,* 1986), cold (Naidu *et al.,* 1991), heavy metal poisoning (Bassi and Sharma, 1993), food shortages (Vaucheret *et al.,* 1992), atmospheric pollution (Anbazhagan *et al.,* 1988) and UV radiation (Pardha *et al.,* 1995) accumulates in plants. In plant cells, proline acts as a regulator of osmotic balance between the cytoplasm and the vacuole (Flowers *et al.,* 1977). In addition osmolytes role of proline, this combination is a source of carbon and nitrogen.

Proline also provides plant protection against free radicals damage (Matysic *et al.,* 2002). Reducing protein consumption for protein synthesis during stress may be due to the cumulative of proline (Stewart, 1972). Ali and colleagues has been reported that proline accumulates in tolerant plants is more than susceptible plants. The plants that accumulate more proline have better growth, because of osmotic potential adjustment that as a result, Torger pressure cell and leaf water potential increases (Ali *et al.,* 2008). Also numerous reports have concluded that the use of proline as a spraying on leaves plants under salt stress cause increased tolerance (Claussen, 2005; Ali *et al.,* 2007). Some reports also suggests that there is a high correlation between increased tolerance to salt with the proline accumulation (Ahmad *et al.,* 2010). Sairam and colleagues showed an increase of proline cause to increase plant resistance to salinity but the changes depends on the plant species (Sairam *et al.,* 1998). Proline in in vitro has play the ROS sweeping role, increases the cellular synthesis and reduces degradation during stress (Kavikishor *et al.,* 2005; Verbruggen and Hermans, 2008). Several reports showed the increased proline under salt stress on plants such as oats, soybeans, corn (Karimi *et al.,* 2005; Keles and Oncel 2004), lentils (Bandeoglu *et al.,* 2004), purslane (Yazici *et al.,* 2007), rice (Demiral and Turkan, 2005), sesame (Koca *et al.,* 2007), beans (Molassiotis *et al.,* 2006).

CONCLUSION

We couldn't find significant effect on morphological characters of plants that are under the salt stress due to the short time duration and the effect of stress on morphological trait needs more time. A large number of plant species accumulate proline in response to salinity stress and that accumulation may play a role in defense against salinity stress. Proline content under salt stress is more increase in sensitive cultivar than resistance.

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