Effect of salinity on Na^+ and K^+ compartmentation in salt tolerant and sensitive wheat genotypes

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Abstract

A factorial experiment was carried out as based on completely randomized design (CRD) in three replicates in normal and salinity conditions with 12 dS m-1 electrical conductivity (EC). In order to investigation of Na^+ compartmentation in different wheat tissues under salinity stress, 20 different wheat genotypes including salt tolerant, semi-tolerant and sensitive ones was studied in the greenhouse condition. Soil was salinized with calculated amount of NaCl salt to develop level of salinity (12 dSm^{-1}) while control has the same EC as that of original soil. 20 pure wheat genotypes were selected from 100 genotypes during the same experimental method and condition. Higher K^+/ Na^+ ratio and more dry weight were two criterions for selecting of tolerant genotypes. Na^+ and K^+ content was measured in the root, internodes, flag leaf and its sheath blade to assess the mechanism of salt exclusion. The results showed that Na^+ accumulated in the root and leaf sheath of tolerant genotypes and this mechanism prevented to transfer Na^+ to leaf blade. Whereas, K^+ could be transferred to leaf blade more than Na^+. These results were vice versa in sensitive genotypes. Leaf sheath was detected as a storage tissue that prevented to transfer of Na^+ to leaf blade and this mechanism involved in improving of salt tolerance. Moreover, significant negative correlation between Na^+ and K^+ content was announced in most tissues due to transfer of K^+ to above ground tissues and excluded Na^+ in tolerant genotypes.

Key Words: Wheat, salt stress, distribution of Na^+, K^+ content, K^+/Na^+

Introduction

Effect of salinity on plant growth combined osmotic stress, ion toxicity and mineral deficiency. (Hasegawa et al., 2000); (Munns, 1993); (Munns, 2002); (Neumann, 1997); (Yeo, 1998). Salt stress in soil or water is one of the major stresses especially in arid and semi-arid regions and can severely limit plant growth and productivity (Allakhverdiev et al., 2000); (Koca et al., 2007). Higher concentration of sodium (Moradi and Ismail, 2007); (Tester and Davenport, 2003) and Cl^- (Shabala et al., 2000) and lower concentrations of K^+ (Flowers and Hajibagheri, 2001) were announced in plant tissue due to salinity. The control of the rate of ion (mainly Na^+ and Cl^-) uptake from the soil and its transport and distribution throughout the plant, as well as the capacity to compartmentalize ionic solutes in the vacuole at the same time that organic solutes accumulate in the cytoplasm (osmotic adjustment) have been considered as important mechanisms by which plants can tolerate salinity (Ashraf and Harris, 2004). The capacity of plants to tolerate high levels of salinity depends on the ability to exclude salt from the shoot, or to tolerate high concentrations of salt in the leaf (tissue tolerance). It is widely held that a major component of tissue tolerance is the capacity to compartmentalize salt into safe storage places such as vacuoles (Ashraf, 2004); (Munns, 2007).Mechanisms that control the specific effects of salt can be divided into two main groups, the first mechanism causes a reduction in entering salt into the plant and is called avoidance mechanism, and the second mechanism which reduces the salt concentration into cytoplasm, and is called as the mechanism of tolerance (Munns et al., 2006). Some plants are able with the maintenance and accumulation of ions in their lower tissues to prevent transfer to highly sensitive organs and thereby leading to salt tolerance. For example, stop of Na^+ in roots, leaves and old leaf sheath grasses is mechanism, including salt tolerance (Netondo et al., 2004). Genotypes with salt tolerance excreted sodium from the shoots and maintained in leaf sheath (Davenport et al., 2005). Salinity tolerance in plants relates to their ability to excrete of sodium ions, thereby avoiding the accumulation of high concentration of sodium in leaves, especially in leaf blade (Munns, 2005); (Lauchli, 1984). This study investigated the distribution pattern of ions in different tissues and the effect of salinity on selective transfer of Na^+ and K^+. This study was carried out on 20 wheat genotypes with different salt tolerance levels.

Methods

This experiment was conducted under greenhouse condition at Karaj Branch, Islamic Azad university located in 35º and 45′ northern latitude and 51º and 6′ eastern longitude and 1313 meters altitude at a mean temperature of 25 ° C during a day and a temperature of 14 ° C at night, 14 h light and 60% humidity. 20 different wheat genotypes, including tolerant, semi-tolerant and sensitive ones were selected from cluster analysis of 100 wheat pure genotypes that were planted in the same conditions (12 dSm^{-1}). The selective criterions for tolerant genotypes were higher plant dry weight and K^+ / Na^+ ratio. Seeds were prepared from Agricultural Biotechnology Research Institute of Iran (ABRII) that they were planted in normal and salinity condition (12dSm^{-1}). Normal clay sandy loam soil was passed through 2.5 mm sieve and salinized with calculated amount of NaCl salt to develop level of salinity (12 dS m^{-1}) while control has the same ECe as that of original soil. The filled pots were arranged in randomized complete design (CRD) with 3 replications per treatment and 20 different wheat genotypes as a factorial experiment and irrigated with tap water having an electrical conductivity (EC) 0.4 dS m^{-1}.

Before planting, the seeds were soaked while they were in pocket paper for 2 hours, and after planting, they were irrigated immediately according to soil saturation percentage.

Soil extract saturation method was used in this experiment to calculate electrical conductivity of soil which has been known all over the world. The samples were dried slowly in air and saturated with distilled water (Gonzalez-Rosquel, 1976).

Whole plant was harvested after maturity and root was washed with tap water. It was dried for 48 hours in 70◦C in oven. Leaves, leaf sheaths, internodes and total plant dry weight was measured.

For Na^+ and K^+ determinations, samples were taken from the ground dry roots and leaves and leaf sheaths of three replicates from each line from both treatments. The 0.1 g dry matter was extracted overnight in 5 ml of 0.5 M HNO3 and then it was reserved in 100◦C for 1 hour. To a 1 ml aliquot of the sample extract were added 2 ml of distilled water. Quantitative ions estimation in plant tissues was performed using 5 standard solutions. The Na^+ and K^+ content in plant tissue were detected by a flame photometric method (Model 410, Sherwood).

The experiments were performed by using a completely randomized design. Data recorded each time were pooled for statistical analysis using SAS version 9.1 software to determine the significance of variance ($P<0.05$).

Results

Genotypes and salinity effects were highly significant on all plant characteristics. As a result, mean values of genotypes and salinity levels were different from each other. Significant genotype by salinity interaction was also found in terms of all plant characteristics. Sodium is the major cation that with increasing salinity in the plant tissue, and organs will accumulate and with significant effect of salinity and genotype interaction, it can be concluded that with increased sodium, different genotypes show different reactions (El-Hendawy et al., 2005).

Na^+ concentration

The pattern of sodium distribution in different tissue

Statistical analysis of the data revealed that Na^+ concentration in different tissues was significantly ($p<0.01$) affected by various genotypes of wheat and different soil condition and their interactions. The results of this case were showed that the highest amount of Na^+ was in tolerant and semi- tolerant genotypes relating to root and flag leaf sheath with mean of 5.75 (number of 16, 398DH) in root (Figure 4) and 5.45 µ$molg^{-1}$ concentration in tolerant genotypes in flag leaf sheath (Figure 1) and 4.45(number of 18, 386DH) in root (Figure 4) and 6.23 µ$molg^{-1}$ in semi-tolerant in flag leaf sheath (figure 1) and the lowest concentration of Na^+ related to flag leaf and the first inter node with mean of 2.82 and 2.46 µ$molg^{-1}$ concentration in tolerant and 3.66 and 3.20 µ$molg^{-1}$ concentration in the semi-tolerant genotypes (Figure 1). The genotypes with lower amounts of sodium ions are able to keep their balance of ions well. Based on data obtained from these experiments in salt stress conditions, the highest amount of sodium, in sensitive genotypes to flag leaf and the first inter node, had the concentrations of 9.70 and 9.22 µ$molg^{-1}$ (Figure 1) and lowest amount of this was in root with concentration of 2.42 µ$molg^{-1}$ (number of 20, 287S) (Figure 4). Maintenance and preferential accumulation of Na^+ in leaf sheath controls Na^+ ion concentration in leaf blades. In tolerant genotypes, Na^+ accumulation in flag leaf sheath was more in sensitive genotypes. In other words, the leaf sheath keeps Na^+ so that it can maintain the lower amount of Na^+ in leaf blade. In this study, the ion distribution of sodium in the leaf sheath compared to leaf blades in wheat (Davenport et al., 2005), maize (Netondo et al., 2004) and grain (Wei et al., 2003) has also been reported.

K^+ Concentration

As shown in tables 2and3, salt stress significantly ($P<0.01$) caused a reduction of K^+ concentration. Interactions of genotype and salinity were statistically significant at 1% level of probability (Table2 and 3). Due to the antagonistic properties of Na^+ and K^+ with salt increase, K+ rate decreased in all tissues (Table4 and 5), but this reduction was different in genotypes. So that in first internode the highest amount of K^+ was related to tolerant and semi-tolerant genotypes with 27 % and 31% reduction compared to normal condition and lowest amount was related to sensitive genotype with 54% reduction compared to normal condition (figure2). In flag leaf sheath higher reducing of K^+ concentration was related to sensitive genotypes with 71% reduction compared to normal in tolerant and semi-tolerant this reduction was 38% and 42% compared to normal (figure2). But according to the results of tables 4and 5 the lowest reducing of K^+ rate was in flag Leaf compared to another tissues in tolerant and semi-tolerant genotypes with reduction 15% and 25% relatively to normal condition and significantly highest reducing was in leaves of sensitive genotype with 68% reduction compared to normal conditions and also was associated with increased soil salinity, K^+ rate decreased in the root of all genotypes but this reduction was in sensitive genotype (287S) about 73% compared with normal condition and in tolerant and semi-tolerant genotypes with 15% (number of 12,93K) and 26% (number of 6, 164S) reduction compared to normal (figure5).

The ratio of potassium to sodium

The results of analysis of variance showed that salinity had a significant effect ($P<0.01$) on the ratio of potassium to sodium in all of tissues. Interactions of genotype and salinity were significant at %1 level of probability (Table 2and3). The analysis of data of genotypes in salinity showed (Table 4) with increasing of salinity, the ratio of K^+ to Na^+ decreased in all tissues. The highest ration of K^+ to Na^+ related to tolerant cultivars and the lowest was related to sensitive genotypes (Figure6). In tolerant and semi tolerant genotypes under stress conditions, the minimum ratio of K^+/Na^+ was related to root with ratio of 0.16 (number of 7, 64S) and 0.25 (number of 6, 164S) respectively (figure6). Also the highest proportion in tolerant genotype related to the first inter node and flag leaf at the ratio of 2.1 and 1.66, and in the semi-tolerant genotype with ratio of 1.11 and 1.56 in the stress conditions (figure3), but in leaves and roots of sensitive genotype, it had the lowest ratio of 0.18 and 0.17 in stress condition (Table4 and 5). With studying of tolerant genotypes and sensitive to wheat, tolerant genotype had limited absorption of sodium and the lower rate of sodium to potassium related to sensitive genotype (Munns et al., 2000).

The alteration of ion ratios in the plant results from the influx of sodium through pathways that function in the acquisition of potassium. The stealth of sodium entry results from the similarity between the hydrated ionic radio of sodium and potassium, which makes it difficult for transport proteins to discriminate between the two ions (Blumwald et al., 2000). This discrimination problem also forms the basis for Na^+ toxicity, where key biochemical processes in the plant cell are inhibited by the competition by sodium for potassium-binding sites. The sensitivity to salt of cytosolic enzymes is similar in both glycophytes (salt-sensitive plants) and halophytes (salt-tolerant plants), indicating that the maintenance of a high cytosolic K^+/Na^+ concentration ratio is a key requirement for plant growth in high salt (Glenn et al., 1999). Plants could use several strategies to maintain a high K^+/Na^+ ratio in the cytosol: diminishing the entry of Na^+ ions into the cells, extrusion of Na^+ ions out of the cell, and vacuolar compartmentation of Na^+ ions.

Discussion

However, the accumulation with higher values of Na^+ in roots and leaf sheath in comparison with leaves (figure1) is one of the tolerance mechanisms in grasses, and in plants, one of the determining factors in salt tolerance is to limit the transport of sodium ions into photosynthetic cells and the growing of meristem active tissue. The maintenance of ions in the leaf sheath tissue and excretion of sodium from leaf protect sensitive photosynthetic tissue against the possible effects caused by ion toxicity. Based on the results of this experiment, sodium ion preferentially will accumulate in root and leaf sheath with high concentration, and no high accumulation of sodium ions in young leaves of tolerant genotypes expresses that root and leaf sheath respectively acted as the major obstacles to prevent the transfer of sodium ions to shoot and leaves act (Netondo et al., 2004); (Huang et al., 2006); (James et al., 2006); (Davenport et al., 2005).

These results are similar to those of (Ashraf and Khanum, 2000) in which they found that salt tolerance of wheat cultivars was associated with low transport of Na^+. It is well evident that plant adopted different strategies to limit Na^+ transport to the leaves to protect photosynthesizing tissues, a very little is known about mechanisms regulating the Na^+ transport. Recently, (Husain et al., 2004) reported that limited Na^+ transport to shoot in the salt tolerant wheat cultivar was due to a regulation of xylem loading transporters. Results of correlation coefficients between traits showed there was negative correlation between Na^+ and K^+ concentration in flag leaves,1st internode and flag leaf sheath (Table6). Plants used various mechanisms to cope with the deleterious effects of salinity stress. Of various determinants of salt tolerance, maintenance of ion homeostasis under salt stress is the most important one, which is generally achieved by exclusion of toxic ions and higher uptake of K^+ or Ca^{2+}. In the present study, Na^+ was more accumulated in the leaves of the salt stressed plants of all cultivars, and cultivars also differed in accumulation of Na^+ in the leaves (data not shown). For example, salt tolerant genotypes had lower Na^+ but higher K^+ content in their shoots and roots in comparison with other wheat genotypes. These results suggest that salt tolerant genotypes might have a key mechanism of ion exclusion or transport-restriction between the shoot and root to depress the transport of Na^+ to the upper plant parts. However, in the present study, transport of Na^+ was increased due to salt stress with a decrease in K^+ transport in all wheat genotypes, but transport of cations (Na^+, K^+, and Ca^{2+}) decreased consistently over time. Furthermore, salt tolerant wheat genotypes had the highest K+ transports with a minimum Na+ transport to the leaves under saline conditions.

Conclusion

Growth processes are especially sensitive to the effects of salt, so that growth rates and biomass production provide reliable criteria for assessing the degree of salt stress and the ability of a plant to withstand it as reported by (Ben Amor et al., 2005). This negative correlation between Na^+ in all tissues and plant length shows that increased concentration of sodium in the shoot, causing restriction reduction in plant growth and crop and thus yields. In relation to K^+, there was significant positive correlation between plant length and K^+ of flag leaf ($r = 0.526^{**}$) and between K^+ of 1stinternode ($r=585^{**}$) and between flag leaf sheath ($r=583^{**}$). But there was negative correlation between K^+ concentration in root and plant length (Table.6). The reduction in root and shoot development may be due to toxic effects of the higher level of NaCl concentration as well as unbalanced nutrient uptake by the seedlings. High level of salinity may have also inhibit the root and shoot elongation due to slowing down the water uptake for overall osmotic adjustments of the plant body under high salt stress condition.

In conclusion, the higher growth of salt tolerant genotypes under saline conditions as compared to other genotypes are associated with low transport of Na^+ and relatively higher K^+ and Ca^{2+} transport to the shoots.

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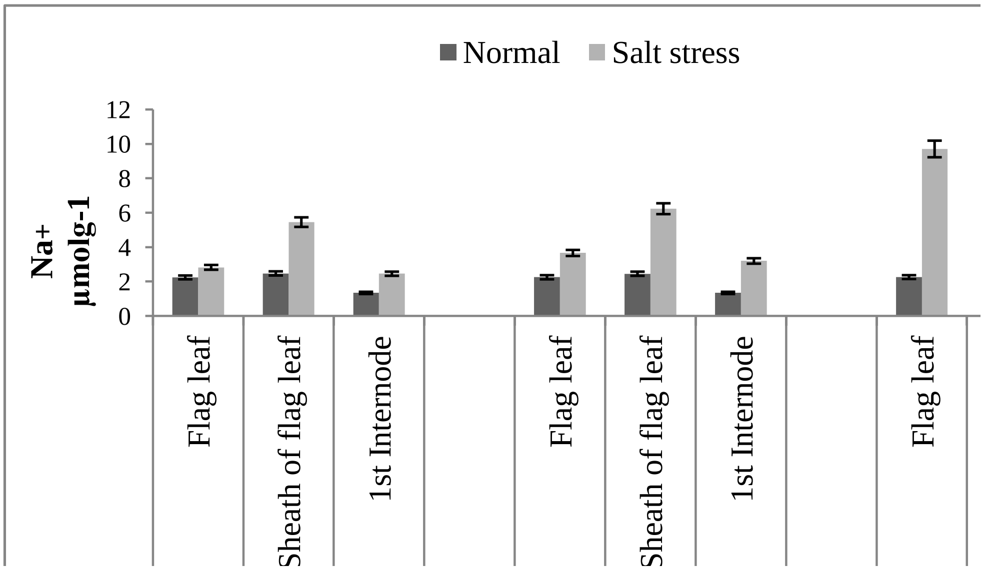

Figure1.Na^+ content in different issues in wheat genotypes

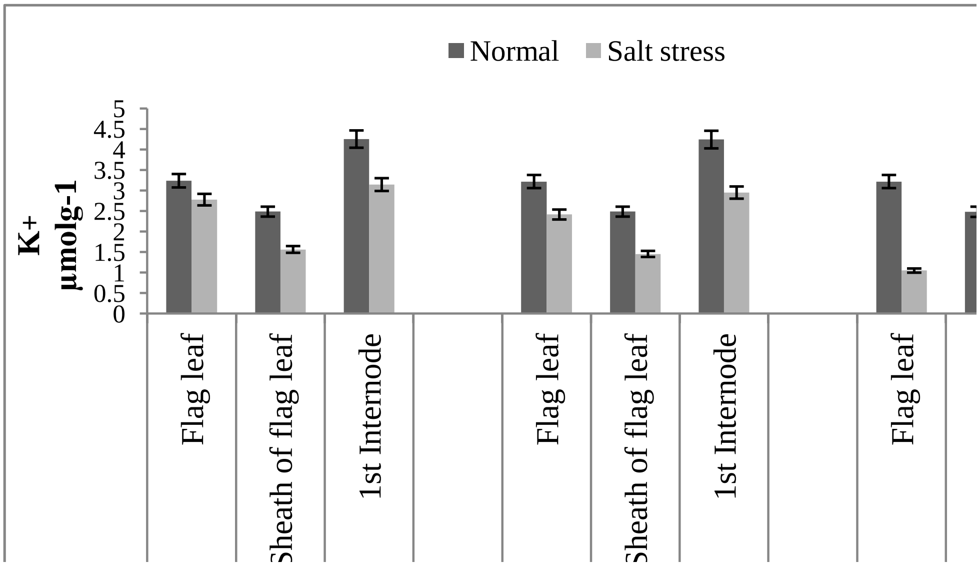

Figure2.K$^+$ content in different issues in wheat genotypes

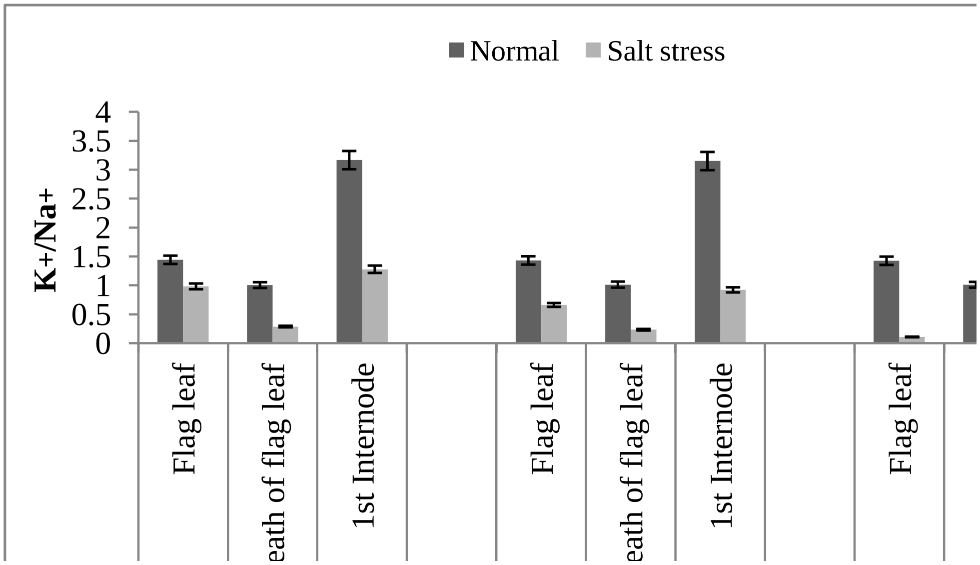

Figure3.K$^+$/Na$^+$ in different issues in wheat genotypes

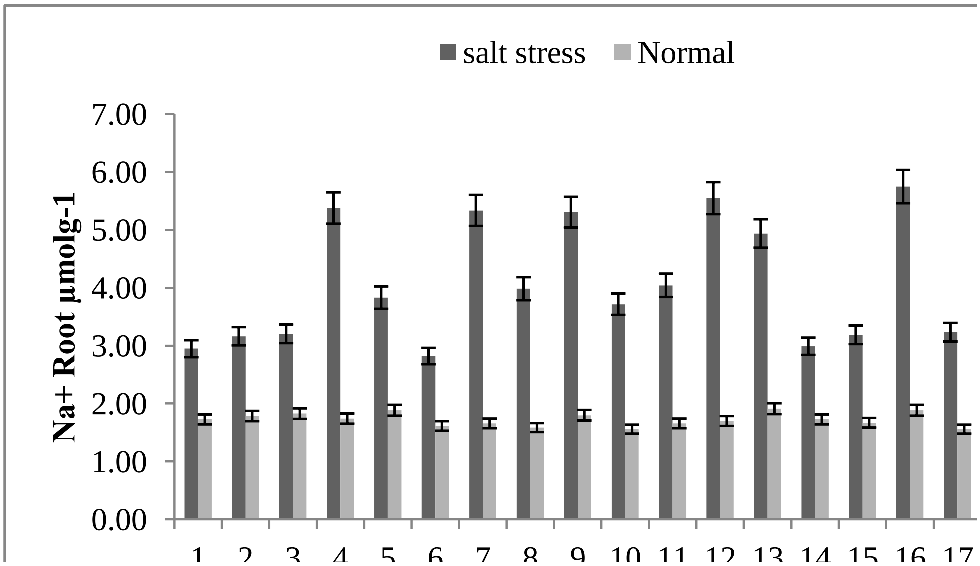

Figure4. Na$^+$ content in Root in wheat genotypes

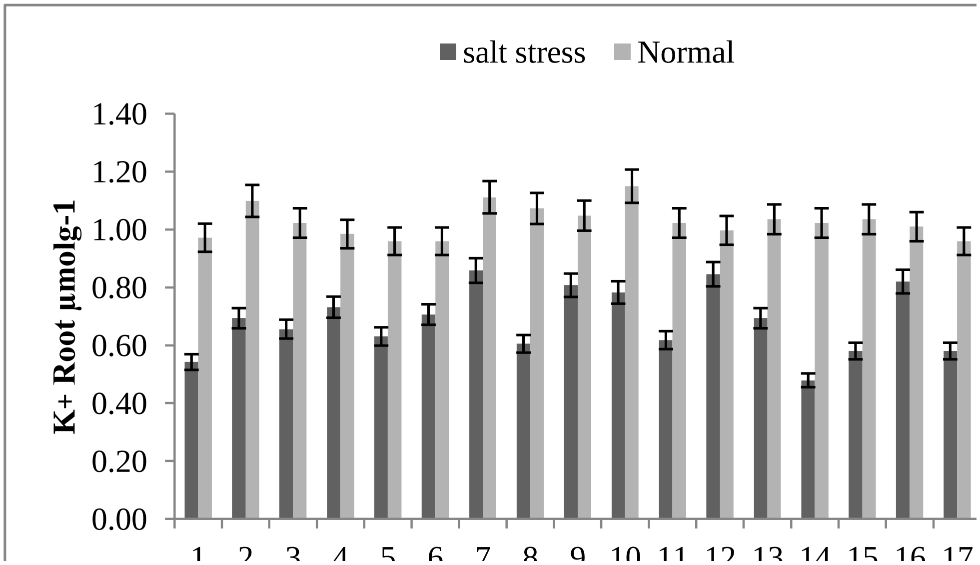

Figure5. K$^+$ content in Root in wheat genotypes

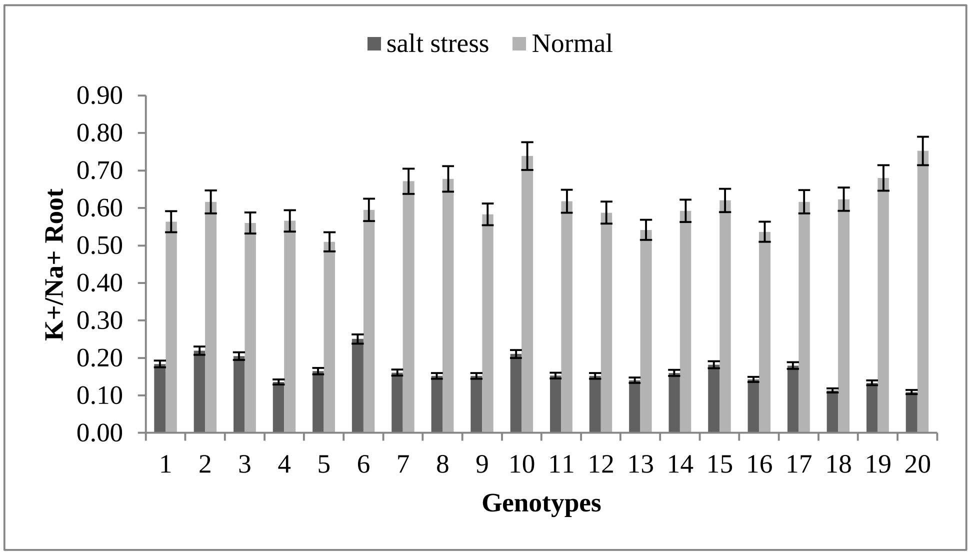

Figure6. Na^+/K^+ in Root in wheat genotypes

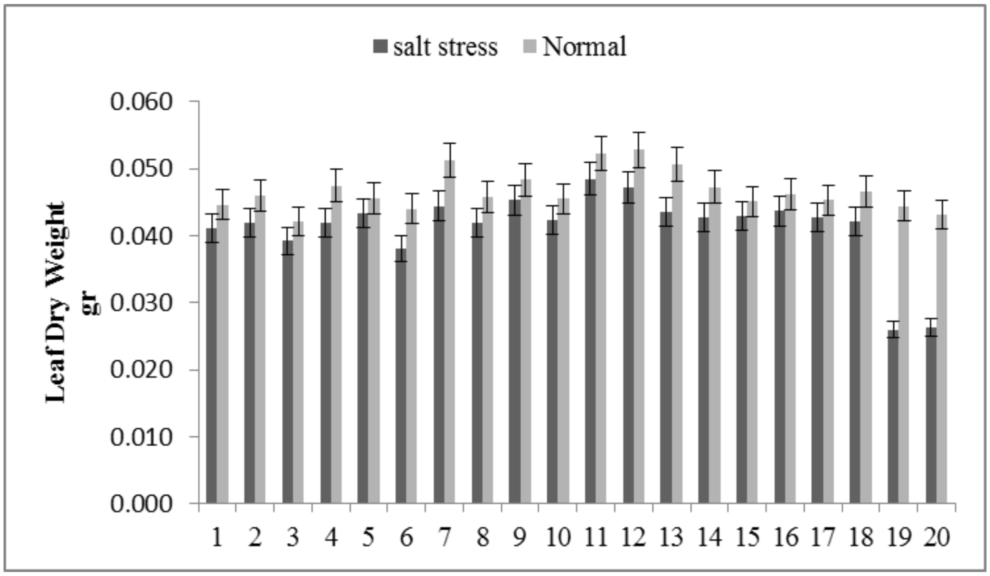

Figure7. Changes in dry weigh at different genotypes

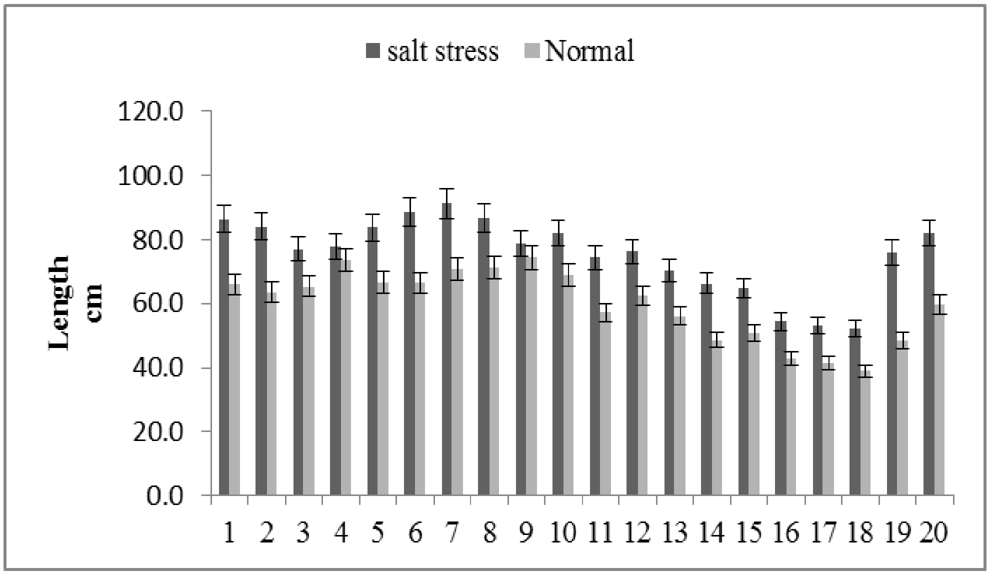

Figure8. Changes related to plant length at different genotypes

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