Pak. J. Agri. Sci., Vol. 51(4), 983-990; 2014
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
http://www.pakjas.com.pk

PHYSIOLOGICAL CHARACTERIZATION OF WHEAT (*Triticum aestivum* L.) GENOTYPES UNDER SALINITY

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Salinity is an abiotic stress affecting the growth and development of plants like wheat and genotypes of wheat differ in their response to salinity. These genotypic variations in wheat for salinity tolerance are due to physiological and biochemical differences. The objective of this study was to assess the genotypic differences between two wheat genotypes 25-SAWSN-12 and 25-SAWSN-8. A hydroponic study with four replications was carried out; two treatments control and salinity (125 mM NaCl) were applied. Salinity reduced shoot and root growth, a reduction in water relations, photosynthetic attributes and membrane stability index (MSI) was also observed in both genotypes but this reduction was more conspicuous in 25-SAWSN-8 than in 25-SAWSN-12. The ionic composition showed that 25-SAWSN-12 had more K^+, and less Na^+ and Cl^- concentrations in its shoot and root while 25-SAWSN-8 behaved in an opposite way. It seems that the reduction in growth, water relations, photosynthetic attributes and membrane stability was due to the toxic effect of Na^+ and less uptake of K^+ caused by salt stress. The genotype 25-SAWSN-12 was able to cope with drastic effects of Na^+ therefore it showed better growth under salinity as compared to 25-SAWSN-8.
Keywords: Physiology, characterization, wheat, *Triticum aestivum*, salt stress

INTRODUCTION

Salinity is one of the abiotic stresses, in arid and semi-arid regions which limits the crop production (Sairam *et al*., 2002). Salinity affects crops in two ways i.e. either by osmotic effect or by specific ion effect (Munns and James, 2003). Osmotic effect disturbs osmotic potentials, whereas specific ion effect causes toxicity of ions (Brady and Weil, 2002). In saline soils, the concentration of Na^+ and Cl^- is more whereas concentration of K^+ is less; this severely affects the plant growth due to ion toxicity (Saqib *et al*., 2004; Ahmad *et al*., 2012; Arshad *et al*., 2012; Abbas *et al*., 2013a,b; Haq *et al*., 2013). Presence of salts in growth medium reduces its osmotic potential decreasing its availability to the roots (Munns *et al*., 2006). Outcomes of these are disturbed ionic, water homeostasis and reduced plant growth (Zhu, 2001).

Plants have developed different approaches to adapt salt stress, salt exclusion from cells and/or their compartmentalization in the vacuoles are important mechanisms which help plants to deal with salinity (Parida and Das, 2005). Preferential uptake of K^+ over Na^+ is another approach for salinity tolerance in plants (Wenxue *et al*., 2003). As discussed earlier salt tolerant plants maintain higher K^+ and lower Na^+ in their cytosol, this ionic regulation is achieved by K^+ and Na^+ transporters (Zhu, 2003). Plants try to keep a desirable K^+: Na^+ ratio within cells (Zhu, 2003).

Salt stress inhibits growth and development by reducing photosynthesis and respiration (Levine *et al*., 1990). Ability of plants to close stomata as a result of salt stress is an important component of salt tolerance (Robinson *et al*., 1997). More reduction in photosynthetic rate occurs in plants which are sensitive to salt stress (Greenway and Munns, 1980). Salt tolerant genotypes show a greater reduction in transpiration than salt sensitive genotypes because lesser transpiration is beneficial for salt tolerance in small plants (Vysotskaya *et al*., 2010). These factors adversely affect plant growth and development at physiological and biochemical levels (Munns, 2002).

Growth and development of plants like wheat is affected by soil conditions and availability of water (Iqbal *et al*., 2012). Wheat is a moderately salt tolerant crop and significant genotypic differences for salinity tolerance are evident in it (Saqib *et al*., 2005). Knowledge and understanding of physiological aspects of salinity tolerance will help to find out accurate screening techniques and this will ultimately add to crop improvement under saline conditions. Being an important staple food crop all over the world, present study was conducted to understand the salinity tolerance of wheat.

MATERIALS AND METHODS

***Growth and treatments*:** Two wheat (*Triticum aestivum* L.) genotypes 25-SAWSN-12 and 25-SAWSN-8 were collected from the Department of Plant Breeding and Genetics,

University of Agriculture, Faisalabad. Healthy seeds of the genotypes were sown in trays containing 5 cm layer of washed sand. These sown seeds were kept moistened with distilled water and nutrient solution before and after seedling emergence. At two leaf stage, seedlings of the genotypes under study were transplanted in foam plugged holes in polystyrene sheets floating over nutrient solution in 100 litre tubs (1m x 1m x 0.25m). This hydroponics study had two treatments; control (no salts) and salinity (125 mM NaCl). A solution of 125 mM NaCl was prepared by dissolving 7.31 g of NaCl in 1L distilled water. Salinity of 125 mM NaCl was developed in three increments (one per day) in the salt stress tubs after two days of transplanting. During first day 50 mM NaCl was applied to salt stress tubs while during second day the salinity level was increased upto 100 mM NaCl, at third day full dose of 125 mM NaCl was applied to stress tubs. The pH of nutrient solutions was adjusted at 5.5±1 with dilute NaOH or HCl and the solutions were changed weekly during the period of whole study. Plants were harvested after 4 weeks of growth in the treated solutions.

***Shoot and root growth*:** Shoot and root growth was recorded by collecting the data for fresh and dry weights of shoot and roots along with their respective lengths.

***Ionic concentrations*:** Ionic concentrations for Na^+, K^+ and Cl^- in the shoot and root were determined. The samples (shoot and root) were oven dried at 65°C for 72 hours and these samples were dry ashed in muffle furnace at 550 °C for 6 hours (Ryan *et al*., 2001). These samples were then dissolved in 2.5 ml 5M HNO_3 and volume of 50 ml was made with distilled water, this material was used for ionic analysis (Saqib *et al*., 2005). Sodium and potassium in plant samples were determined by Sherwood 410 Flame Photometer with the help of standard solutions using reagent grade salts of NaCl and KCl. Chloride in these samples was determined with the standardized Sherwood-926 chloride analyzer.

Specific leaf area: Specific leaf area of the plant leaves was measured by automatic leaf area meter (AAM-8, Hayashi Denko CO., Japan) (Tsuda, 1999).

Membrane stability index (MSI): MSI was determined by recording the electrical conductivity of leaf leachates in double distilled water at 40°C and 100°C (Sairam, 1994). Leaf samples (0.1 g) were cut into discs of uniform size and taken in test tubes containing 10 ml of double distilled water in two sets. One set was kept at 40°C for 30 minutes and another set at 100°C in water bath for 15 minutes and their respective electric conductivities C_1 and C_2 were measured by conductivity meter. Membrane stability index was calculated by using the following equation (Sairam *et al*., 2002).

$$Membrane\ Stability\ Index\ (MSI) = [1 - (\frac{C1}{C2})] \times 100$$

***Water relations*:** Water relations, including leaf water potential (Ψ_W), osmotic potential (Ψ_S) and turgor potential (Ψ_P) were determined. Water potential was measured at midday by pressure-bomb technique (Tyree and Hammel, 1972), using the upper most fully expanded leaf (three to four nodes from the shoot apex). Sap was extracted from leaf samples by pressing with a glass rod after freezing these samples for two weeks. The sap so extracted was used directly for Ψ_S determination in an osmo-meter (VAPRO vapor pressure osmo-meter, Model 5520, USA) (Siddiqi and Ashraf, 2008). The Ψ_P was calculated as the difference between water and osmotic potentials (Nobel, 1991).

$$\psi_P = \psi_W - \psi_S$$

***Photosynthetic attributes*:** Photosynthetic attributes including transpiration rate (E), stomatal conductance (g_s) and net photosynthetic rate (P_N) were also determined. All these parameters were studied two days prior to mid-season harvest. For the determination of transpiration rate (E), stomatal conductance (g_s) and net photosynthetic rate (P_N) of youngest unfolded healthy leaf of main stem an Infrared Gas Analyser (IRGA) LCA-2 attached to a Parkinson Broad Leaf Chamber (Analytical Development Company, Herts, U.K.), was used. These measurements were carried out between 0900-1600 hours in the natural light conditions on a bright sunny day using flag leaves (Wahid *et al*., 2011).

***Statistical analysis*:** The data collected from this study were analysed statistically using completely randomized design (CRD) and genotypic tolerance to salinity was determined by analysis of variance (Steel and Torrie, 1980). The significance of differences among the means has been compared using the standard error.

RESULTS AND DISCUSSION

***Shoot and root growth*:** Reduction in shoot and root growth of many crops by salt stress has been reported in many studies including this study as shown in the Figs. 1 and 2, respectively. This reduction due to salt stress was also observed by Sotiropoulos *et al*. (2006) and it was more pronounced in 25-SAWSN-8 as compared to 25-SAWSN-12. The shoot fresh and dry weights along with their lengths were significantly affected by salinity, both the genotypes showed significant differences; the interactions between genotypes and treatments were also found to be significant (Figs. 1 and 2).

On the overall mean basis all the growth parameters of shoot were more in control and these parameters were significantly reduced in saline condition. In saline treatment percent decrease in shoot fresh weight, dry weight and plant height were 61, 67 and 51%, respectively, as compared to control. Genotype 25-SAWSN-12 showed non-significant differences from 25-SAWSN-8 under control treatment for shoot fresh weight however, in saline treatment significant difference was observed in both genotypes for shoot fresh weight. While in terms of shoot dry weight and plant height,

both 25-SAWSN-12 and 25-SAWSN-8 showed significant difference in all the treatments.

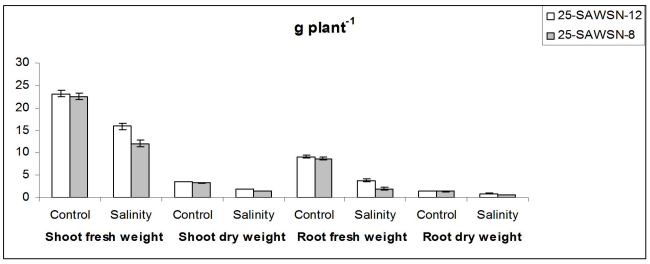

Figure 1. Effect of salinity (125 mM NaCl) on shoot and root growth (g plant^{-1}) of different wheat genotypes. The LSD values for shoot fresh and dry weight, and root fresh and dry weight are 3.97, 0.99, 0.86 and 0.11 respectively.

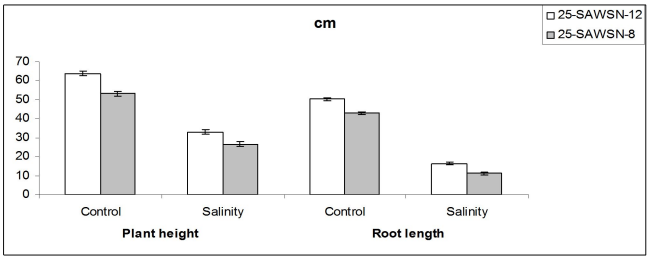

Figure 2. Effect of salinity (125 mM NaCl) on plant height and root length (cm) of different wheat genotypes. The LSD values for plant height and root length are 10.77 and 11.50 respectively.

Changes in structure and chemical composition of plasma membrane due to excessive amounts of salts in cellular tissues were observed by Wang and Zhao (1997) and disruption in cell metabolism caused by these changes was recorded by Hasegawa *et al*. (2000). The treatments and genotypes differed significantly for root fresh and dry weights along with root length (Figs. 1 and 2). The interactions between genotypes and treatments were also found to be significant. On overall mean basis all the growth parameters of root showed higher values in control and the application of salt stress resulted in significant reduction of these parameters.

In saline treatment there were 33, 58 and 30 % reductions in root fresh and dry weights, and root length respectively as recorded by Taffouo *et al*. (2004). In control both the genotypes showed non-significant differences for root fresh and dry weights but under salt stress 25-SAWSN-12 produced significantly higher root fresh and dry weight as compared to 25-SAWSN-8. Similarly, root length of 25-SAWSN-12 was significantly higher as compared to SAWSN-8 for both treatments due to high concentration of salts and the disturbances in physiological activities. Reduction of plant growth and development caused by high salt concentration and disturbances in several physiological processes of plants were observed by Taffouo *et al*. (2004), our study also proved their findings (Figs. 1 and 2).

***Ionic composition of shoot and root*:** Our data is in line with the previous studies regarding changes in ionic composition, it revealed that 25-SAWSN-8 accumulated more Na^+ than 25-SAWSN-12, whereas, reverse trend was observed for accumulation of K^+ in both the genotypes (Figs. 3 and 4).

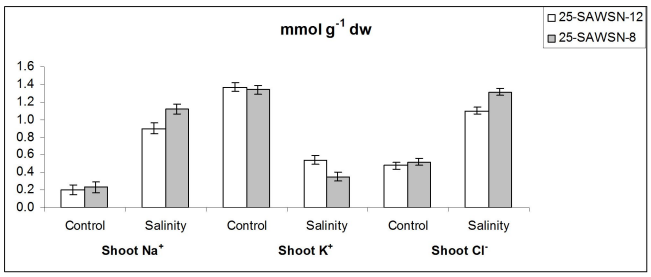

Figure 3. Effect of salinity (125 mM NaCl) on shoot ionic composition (mmol g^{-1} dw) of different wheat genotypes. Error bars show the values of LSD at P ≤ 0.05.

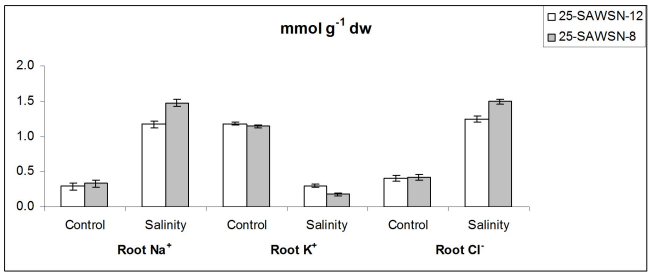

Figure 4. Effect of salinity (125 mM NaCl) on root ionic composition (mmol g^{-1} dw) of different wheat genotypes. Error bars show the values of LSD at P ≤ 0.05.

The data about sodium (Na^+), potassium (K^+) and chloride (Cl^-) concentrations in shoot are given in Fig. 3. Significance differences were observed between genotypes and treatments. On overall mean basis, there were significant increases in shoot Na^+ and Cl^-; however, a significant reduction was found in shoot K^+ under saline conditions as compared to control. The interactions between genotypes and treatments for shoot ionic compositions were also found to be significant. In control treatment the genotypes showed non-significant differences in shoot ionic composition but under salt stress 25-SAWSN-8 showed significantly higher accumulation of Na^+ and Cl^- in its shoot unlike 25-SAWSN-12 but shoot K^+ showed an opposite trend for both genotypes. Toxic concentration of Na^+ in the cytoplasm or chloroplast can affect the integrity and function of photosynthetic membranes when toxic ions are not sequestered in the

vacuole these results in sensitive genotypes like 25-SAWSN-8 were recorded by Bastias (2004).

The data regarding root Na^+, K^+ and Cl^- concentrations revealed significant differences between treatments and genotypes as shown in Fig. 4. The interactions between genotypes and treatments were also significant. On overall mean basis, there was a significant increase in root Na^+ and Cl^- whereas root K^+ was decreased significantly under saline conditions as compared to control treatment. Under salt stress 25-SAWSN-8 showed significantly higher accumulation of Na^+ and Cl^- in its roots as compared to 25-SAWSN-12 but shoot K^+ was less in case of 25-SAWSN-8 than 25-SAWSN-12.

The Na^+: K^+ data for shoot and root are given in Fig. 5, which showed significant differences between control and salinity. Both varieties showed non-significant differences in control for shoot and root Na^+: K^+. While in case of salt treatment both genotypes showed significant differences, 25-SAWSN-12 showed significantly less Na^+: K^+ for both shoot and root as compared to 25-SAWSN-8. However when this ratio was compared in roots very high values of Na^+: K^+ was found in roots of 25-SAWSN-8. These finding are in concurrence with those of Uddin *et al*. (2012a) who found that salinity decreased K^+ contents and K^+/Na^+ ratio but increased Na^+ contents in the shoot and root tissues in turfgrass species. Jungklang *et al*. (2003) also found that different salts in soils especially due to NaCl cause accumulation of Na^+ and Cl^- and a decrease in K^+ concentrations in leaves, as well as in roots. Different crops accumulate the least toxic ions (Na^+ and/or Cl^-) specially tolerant genotypes like 25-SAWSN-12 or accumulate these toxic ions at high rates like 25-SAWSN-8 (sensitive genotypes). Saqib *et al*. (2005) observed that plants with higher K^+: Na^+ ratio are considered to have better tolerance (25-SAWSN-12) than plants with low K^+: Na^+ ratio (25-SAWSN-8).

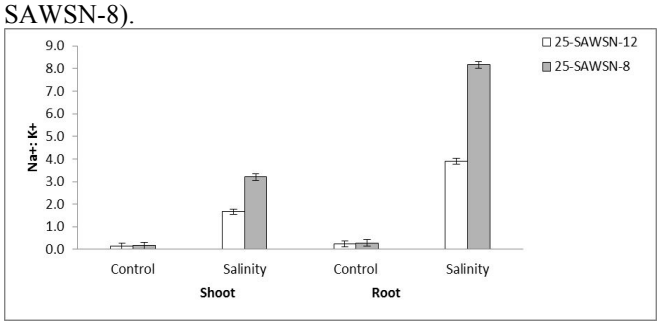

Figure 5. Effect of salinity (125 mM NaCl) on Na^+: K^+ of different wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$.

Discrimination for K^+ and against Na^+ in wheat (*Triticum aestivum* L.) was observed by Gorham (1990). The findings of Dvorak *et al*. (1994) are in agreements with our study they found that recombinant lines of wheat having K^+: Na^+ trait showed tolerance to salt stress than the line lacking this trait.

***Water relations*:** The data regarding water relations including water potential (Ψ_W), osmotic potential (Ψ_S) and turgor potential (Ψ_P) showed significant differences between genotypes and treatments as given in Fig. 6. Interactions of water relations for genotypes and treatments were also significant. Genotype 25-SAWSN-8 showed lower Ψ_W, Ψ_S (which means more negative) and Ψ_P values whereas 25-SAWSN-8 maintained higher values for these parameters under saline conditions. Munns and James (2003) found decreased availability of water to plants, due to low external water potential which can be considered as the first cause of growth restriction under saline conditions; this reduced water availability was also noticed in our genotypes (Fig. 6). All the above parameters were affected more in this genotype as compared 25-SAWN-12, shown in Figs. 6, 7 and 8. The results of water relations from this study also match the findings of Khan (2010).

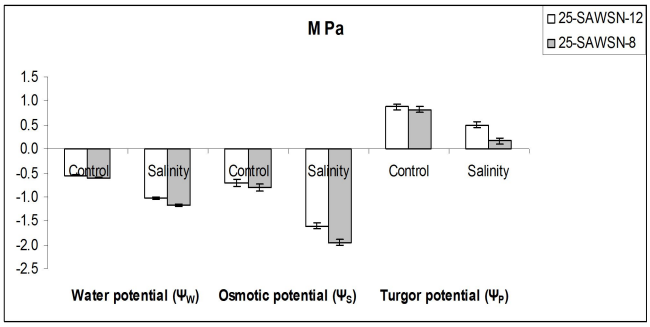

Figure 6. Effect of salinity (125 mM NaCl) on water relations of different wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$.

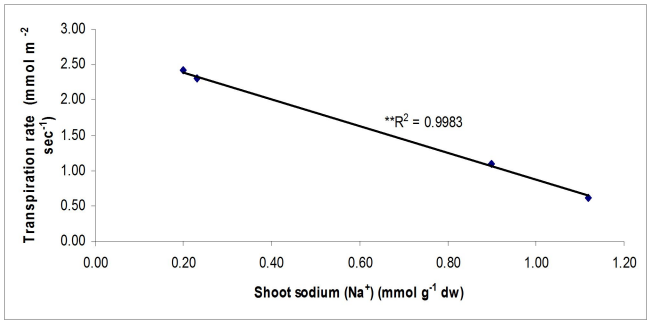

Figure 7. Relationship between transpiration rate (E) (mmol m^{-2} sec^{-1}) and shoot sodium (Na^+) (mmol g^{-1} dw) of different wheat genotypes.

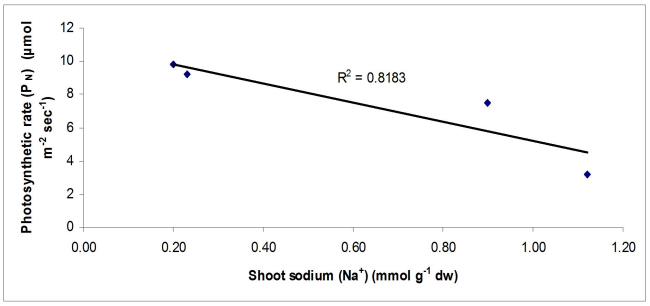

Figure 8. Relationship between photosynthetic rate (P_N) and shoot sodium (Na^+) (mmol g^{-1} dw) of different wheat genotypes.

Reductions in shoot water contents of genotypes like 25-SAWSN-8 were also noticed in salt stress by Gholipoor *et al*. (2000). Water potential was decreased in salt sensitive plants like pea and 25-SAWSN-8 when these were subjected to salt stress as shown by Ahmad and Jhon (2005). Greenway and Munns (1980) also found that for normal growth under salinity a reduced water potential is needed. A higher reduction in osmotic potential and stomatal conductance was observed when 25-SAWSN-12 and 25-SAWSN-8 were exposed to salts for a long time but both these parameters recovered to control values, our results corroborated the findings of Hernandez and Almansa (2002). The decrease of tissue solute potential would compensate the salt induced lowering of root zone water potential. This would help the plants like 25-SAWSN-12 to maintain turgor pressure and normal functioning of cells under adverse water conditions. When different varieties like 25-SAWSN-12 and 25-SAWSN-8 were given salt stress, reduction in osmotic potential was observed and this decrease was more in 25-SAWSN-8 than in 25-SAWSN-12 Nandwal *et al*. (2000) also observed reduced osmotic potential in sensitive genotypes of mungbean. Genotypes like 25-SAWSN-12 responded to salinity by maintaining better ionic composition, water relations and photosynthetic attributes which helped these genotypes to better adapt the saline conditions.

***Photosynthetic attributes*:** Meloni *et al*. (2003) observed reduction in growth and yield which was directly related to the reduction in photosynthetic activity, these results were also shown by Sharma and Minhas (2005). Brugnoli and Lauteri (1991) stated that this reduction of photosynthetic rate due to salt stress may be related to the decreased stomatal conductance. These findings are also supported by our data for photosynthetic rate and stomatal conductance which showed that salt stress has decreased these two processes as shown in Figs. 8 and 9 respectively, however, this reduction was more pronounced in 25-SAWSN-8 than 25-SAWSN-12.

The relationships between photosynthetic attributes {transpiration rate (E), photosynthetic rate (P_N) and stomatal conductance (g_s)} and Na^+ in shoot due to presence of salts in the growth medium are shown in Figs. 7, 8 and 9 respectively. Photosynthetic attributes showed negative relationships with shoot Na^+; resulting in a decrease in transpiration, photosynthesis and stomatal conductance with an increase in shoot Na^+ concentration. Increasing salinity decreased K^+, Ca^{2+}, Mg^{2+} contents and K^+: Na^+ ratio but increased Na^+ contents in the shoot and root tissues in turfgrass species these results were recorded by Uddin *et al*. 2012a. According to Iqbal *et al*. (2002) photosynthesis was reduced by Na^+ (Fig. 7). An increase in leaf Na^+ concentration reduces leaf gas exchange under saline conditions this was observed by Walker *et al*. (1993). Similarly, high leaf Cl^- concentrations have also been held responsible for reduced photosynthetic capacity and stomatal conductance this was recorded by Banuls *et al*. (1997). High Na^+ and Cl^- uptake and accumulation with the increment of salinity in purslane was recorded by Uddin *et al*. (2012b). In the present study both the Na^+ and Cl^- seems to have played their role in reducing the stomatal conductance, transpiration rate and photosynthesis of 25-SAWSN-8.

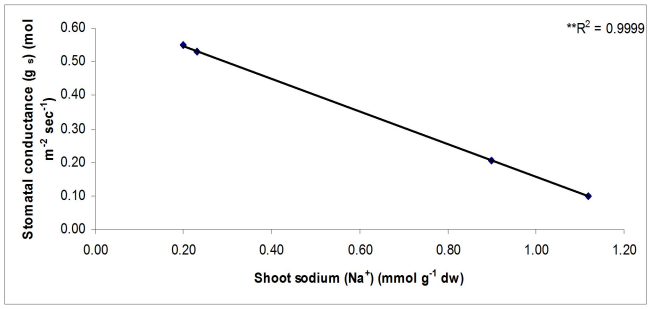

Figure 9. Relationship between stomatal conductance (g_s) (mol m^{-2} sec^{-1}) and shoot sodium (Na^+) (mmol g^{-1} dw) of different wheat genotypes.

However the genotypes differed significantly and 25-SAWSN-8 showed lower values of E, P_N and g_s whereas 25-SAWSN-12 maintained the higher values for photosynthetic attributes. Salt treatment caused a reduction of 37, 56 and 28 % in E, P_N and g_s, respectively. Maintenance of photosynthetic activity is considered an important physiological adaptive component of salt tolerance in different plant species as stated by Dubey (2005) particularly in varieties like 25-SAWSN-12 which maintained higher photosynthetic activities as compared to genotypes like 25-SAWSN-8. In the present study significant variations for transpiration rates were also observed, 25-SAWSN-12 maintained higher transpiration rate than 25-SAWSN-8 but transpiration rate of both the genotypes was significantly reduced under salt stress as shown in Fig. 7. The toxic effect of salt stress was more pronounced in the case of 25-SAWN-8 as compared to 25-SAWSN-12. As the water uptake from

Table 1. Specific leaf area and membrane stability index (MSI) (%) of different wheat genotypes under salt stress. *Mean ± standard error.

Varieties	Specific leaf area (cm^2 g^{-1})		Membrane stability index (MSI) (%)	
	Control	Salinity	Control	Salinity
25-SAWSN-12	33.00±1.29	18.00±1.08	92.02±2.09	75.11±1.97
25-SAWSN-8	31.50±1.04	12.00±0.91	90.00±1.57	56.02±1.71

soil is reduced, a decrease in stomatal conductance occurs because of stomatal closure.

***Specific leaf area and membrane stability index*:** The data regarding specific leaf area (SLA) and membrane stability index (MSI) showed significant effect of treatments on both genotypes (Tab. 1). The interaction between genotypes and treatments in case of SLA was non-significant whereas a significant interaction between genotypes and treatments was observed in case of MSI. Under saline condition, significant reduction in SLA and MSI was recorded as compared to control. Salinity caused a reduction of 47 and 72 % in SLA and MSI respectively. In control both the genotypes showed non-significant differences, while in salt treatments 25-SAWSN-12 showed significantly higher SLA and MSI as compared to 25-SAWSN-8. Reduced leaf area (Tab. 1) and dry mass (Fig. 1) of 25-SAWSN-12 and 25-SAWSN-8 may be due to the changes in plant water relations under salt stress which cause reduction in meristematic activity as well as cell elongation; thus inhibiting leaf expansion after the loss of turgor pressure as shown in Fig. 6, which will indirectly affect SLA and MSI these results are also supported by Choluj *et al*. (2004) and Shah (2007). Elfeel and Bakhashwain (2012) found that SLA decreased with increased salt concentration same results were found in our study, the reason of less SLA in salt stress may be due less carbon invested per unit leaf area (Wright and Westoby, 2001). Membrane stability index (MSI) of two wheat cultivars like 25-SAWSN-12 and 25-SAWSN-8 was negatively influenced by salinity these results were recorded by Esfandiari (2011). Like our results Rao *et al*. (2013) also found that under salinity stress membrane stability index (MSI) of wheat genotypes were negatively influenced.

Conclusion: This study showed that the salt tolerance of wheat genotypes differs under salt stress. Certain genotypes like 25-SAWSN-12 can adapt the salt stress in a much better way by having less accumulation of toxic ions like Na^+ and by having more K^+ in their cells. It helped this genotype to keep its stomatal conductance better than 25-SAWSN-8. This in turn also helped it to maintain higher photosynthetic rate as compared to 25-SAWSN-8. The genotype 25-SAWSN-12 also maintained more water, osmotic and solute potential thus showed the ability to keep the water available to it even under stress conditions. The genotype 25-SAWSN-12 performed much better than 25-SAWSN-8 under salt stress so it may be recommended for cultivation on salt affected soils and could be used for further salt tolerant varietal development program.

***Acknowledgement*:** This experiment is a part of the corresponding author's PhD research work. He is thankful to the Higher Education Commission, Government of Pakistan for awarding him Indigenous PhD Scholarship (PIN No. 085-10943-Av5-169) without which it would have been difficult to complete this work.

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