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Genetic variability in wheat genotypes for salt tolerance, growth and physiological responses

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Abstract

In arid and semi-arid regions of the world, soil salinity is becoming a major problem for crop production due to changing climate and shortage of good quality water. In these circumstances, screening, breeding and cultivation of food crop species or genotypes with high tolerance to salinity may be considered as the most feasible and effective approach, for fulfilling the safe food requirement of increasing population. Therefore, a hydroponic study was conducted to evaluate the genetic variations in Pakistani wheat genotypes for salt tolerance. In present study, two salinity levels (100 and 200 mM NaCl) along with a control and twelve wheat genotypes were evaluated. The results showed that SARC-I (V_5), Sehar-2006 (V_8) and Shafaq-2006 (V_9) were found tolerant to salinity because of better growth, lower NaCl relative toxicities, leaf Na^+, higher tolerance indices, photosynthetic rate, total chlorophyll contents, transpiration rate, stomatal conductance and leaf K^+ concentration. Thus, these genotypes were found as valuable resource that can be used in further wheat breeding programs aimed at increasing salinity tolerance.

Keywords: Wheat genotypes, salinity, tolerance index, cluster analysis, physiological attributes

Introduction

Wheat (*Triticum aestivum* L.) is the most important cereal crop in the world, and is a staple food of about one third of the world's population. It is a main source of carbohydrates and nutrition. Wheat is grown on an area of 9.039 mha in Pakistan with total production of 25.3 million tons and an average of 2797 kg ha^{-1} (GOP, 2014). Wheat has been categorized as moderately salt-tolerant crop (Qureshi and Barrett-Lenard, 1998), but increasing concentration of salts in growth medium results in less height and tillering capacity compared to non-saline control (Shafqat *et al.*, 1998).

The increasing demand of food for increasing population necessitates the use of poor quality soils and waters to increase the wheat production. The problem of salinity is very serious in Pakistan as 6.67 Mha area in Pakistan is salt affected (Khan, 1998). Salinity harms plant growth through osmotic effects, specific ion effects and induced nutrient deficiency (Ashraf and Haris, 2013).

Salinity stress involves changes in various physiological and metabolic processes. Depending on crop species/genotypes, severity and duration of the stress, salinity ultimately inhibits crop production (Munns, 2005; Rozema and Flowers, 2008). Initially soil salinity is known to repress plant growth in the form of osmotic stress which is then followed by ion toxicity (Rahnama *et al.*, 2010; James *et al.*, 2011). During the initial phases of salinity stress, water absorption capacity of root systems decreases and water loss from leaves is accelerated due to osmotic stress caused by high salt accumulation in soil and plants, and therefore salinity stress is also considered as hyperosmotic stress (Munns, 2005). Osmotic stress in the initial stage of salinity stress causes various physiological changes, such as interruption of membranes, nutrient imbalance, impairment of the ability to detoxify reactive oxygen species (ROS), differences in the antioxidant enzymes, decreased photosynthetic activity and reduced stomatal aperture (Munns and Tester, 2008; Rahnama *et al.*, 2010). Salinity also affects physiological process in plants such as photosynthesis by decreasing stomatal conductance (SC) and transpiration and disturbing the biosynthesis of photosynthetic pigments (Sairam *et al.*, 2005). Salinity stress is also believed as a hyper-ionic stress. One of the most detrimental effects of salinity stress is the accumulation of Na^+ and Cl^- ions in tissues of plants exposed to soils with high NaCl concentrations. Entry of both Na^+ and Cl^- into the cells causes severe ion imbalance and excess uptake might cause significant physiological disorder(s). High Na^+ concentration inhibits uptake of K^+ ions which is an essential element for plant growth and development, ultimately resulting in lower productivity and may even lead to death (James *et al.*, 2011). In response to salinity stress, the production of ROS, such as superoxide, hydroxyl radical, and hydrogen peroxide, is enhanced

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(Ahmad and Umar, 2011; Ahmad and Parsad, 2012). These ROS cause oxidative damage to various cellular components such as lipids, proteins, and DNA, thereby interrupt vital cellular functions of plants (Gupta and Huang, 2014).

Many different approaches can be used to manage salt-affected soils. Though well-established techniques such as provision of adequate drainage and use of amendments are available for this purpose but due to the limitations of availability of good quality irrigation water, high cost of amendments and low soil permeability, it becomes very difficult to tackle this problem (Ghafoor *et al.,* 2004). Genetic variations in salt tolerance exist and the degree of salt tolerance varies with plant species and genotypes within a species (Gupta and Huang, 2014). Saline agriculture is another appropriate approach which involves the cultivation of salt-tolerant species/crop cultivars that produce economic yields under adverse soil conditions (Ahmad, 2011).

Identifying genotypes that have tolerance against salinity is a practical and relatively simple way of improving crop yield and profitability on saline soils. Ehsan and Wright (1998) suggested that improvement for salt tolerance might be achieved through selection from already existing wheat varieties. Due to both spatial and temporal variability in soil salinity, screening under natural saline field is not feasible (Akhtar *et al.,* 2003). To avoid this problem, crop gene stocks are often screened in nutrient solution to which NaCI is added. Screening of crop genotypes against salinity in solution culture is well established. This method is relatively quick and reliable for screening the crop genotypes against salinity (Qureshi *et al.,* 1990; Naseem *et al.,* 2000). In wheat, differences in varietal response to salt stress and various growth parameters related to such differences have been extensively studied earlier. However, it is still imperative to develop an effective methodology for detailed evaluation and identification of wheat crop germplasm for higher levels of salt tolerance in developing countries like Pakistan. In this context, the present study was unique of its nature in which tolerance indices (TI) on growth parameters as well as plant physiological functions have been simultaneously used for screening wheat genotypes.

Materials and Methods

Experimental layout

An experiment was conducted in the wire house at Saline Agriculture Research Centre (SARC), Institute of Soil and Environmental Sciences (ISES), University of Agriculture Faisalabad (UAF). The seeds of twelve wheat genotypes (V_1 = Kohistan-90, V_2 = MH-97, V_3 = SARC-IV, V_4 = Iqbal-2008, V_5 = SARC-I, V_6 = Perwas-94, V_7 = Uqab-2000, V_8 = Sehar-2006, V_9 = Shafaq-2006, V_{10} = Faisalabad-2008, V_{11} = Lasani-2008, V_{12} = AARI-2010) were taken from Ayub Agriculture Research Institute (AARI), Faisalabad. Healthy seeds of these genotypes were sown in trays containing sand. When wheat seedlings were reached to two leaf stage, after 10-days, these were transplanted in thermopole sheets having foam plugged holes and floating on ½ strength Hoagland's nutrient solution (Hoagland and Arnon, 1950). The solution was continuously aerated with the help of aeration pump. Salt treatments (100 and 200 mM NaCl) were applied six days after transplanting. The salinity levels were maintained by adding salts in three increments, at the rate of one increment per day and no salt was added in control treatment. The pH of the treated solutions was maintained between 6 ± 0.5 daily, throughout the experiment. The nutrient solution was changed after every week.

Measurement of growth, physiological processes and harvesting

Measurements regarding physiological parameters were taken before harvesting on youngest fully expanded wheat leaves. Measurements regarding the plant photosynthetic rate (A), transpiration rate (E) and stomata conductance (g_s) were taken using an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England). Chlorophyll meter (Minolta Spad-502) was used to determine total chlorophyll contents (TCC) according to Saqib et al. (2012). It is an economical approach to quantify photosynthetic capability compared to chlorophyll fluorescence (Munns *et al.,* 2006).

Plants were harvested after 42 days of the imposition of treatments and data about shoot and root lengths and fresh/dry weights were recorded. The youngest fully mature leaves were taken in eppendorf tubes and frozen for Na^+ and K^+ determination. Plant samples were dried at 65 ± 5 °C in forced air oven and then weighed for dry weight determination.

Evaluation of relative toxicity and tolerance indices

NaCl relative toxicity (RT) (Gyawali and Lekhak, 2006) and tolerance index (TI) (Zeng *et al.,* 2002) for shoot length (SL), shoot fresh weight (SFW), shoot dry weight (SDW), root length (RL), root fresh weight (RFW) and root dry weight (RDW), of wheat was calculated using following formulae:

$$RT\ (\%) = [(X - Y)/ X] \times 100$$
$$TI\ (\%) = (Y/ X) \times 100$$

Where, X = SL/SFW/SDW/RL/RFW/RDW of wheat in control; Y = SL/SFW/SDW/RL/RFW/RDW of wheat in a particular treatment

The TI values calculated for SFW, SDW, RFW, RDW, RL, leaf Na^+ concentration, leaf K^+ concentration and K^+/Na^+ were used for cluster analysis.

Determination of leaf Na^+ and K^+

After thawing, frozen leaf samples were crushed with the help of stainless steel rod having tapered end. The Gilson pipette was used to collect sap into other eppendorf tubes. The sap was centrifuged at 6500g for 10 minutes (Gorham *et al.*, 1984). The supernatant sap was taken in new eppendorf tubes, and Na^+ and K^+ was determined by flame photometry following US Salinity Lab. Staff (1954). The K^+/Na^+ was determined by dividing leaf K^+ concentration with Na^+.

Cluster analysis

In order to group genotypes into salt tolerant and sensitive categories, the cluster group analysis was used. In cluster group analysis, genotypes can be screened simultaneously on several physiological and ionic parameters. No score boundaries are set as genotypes are classified on the basis of cluster group rankings. Genotypes are ranked and grouped for their salinity tolerance. Ranking numbers are assigned to groups on the basis of cluster means. Genotypes are scored on the basis of ranking numbers (Kharis *et al.*, 1988). Salt tolerance indices were formed from the data for cluster analysis (Zeng *et al.*, 2002). In cluster analysis, methods of Jollife *et al.* (1989) and Kharis *et al.* (1988) were followed. Group ranking was obtained on the basis of Ward's minimum variance cluster analysis for salt tolerance indices of growth parameters i.e., root fresh and dry weight, root length, , shoot fresh and dry weight and ionic parameters, i.e., Na^+, K^+ and K^+/Na^+ ratio of plant leaves. The procedures are described in the SAS User's Guide (SAS Institute, 2000). Cluster groups were ranked from cluster means, in order from highest to lowest means. All the numbers of group ranking at each salinity level in each genotype were added to find the final sum. The final ranking of genotypes was done on the basis of these sums in the order that the genotypes with lowest sums were considered most salt-tolerant and those with the highest means were considered salt-sensitive when grown under salinity stress (El-Hendawy *et al.*, 2005).

Statistical analysis

Analysis of variance (ANOVA) was performed on the obtained data and significant differences among treatment means (Steel *et al*., 1997) were calculated by Least Significant Difference (LSD) test using "Statistix 8.1" statistical computer software package(s).

Results and Discussion

Wheat growth and tolerance as affected by salinity

Application of NaCl salinity treatments (T), wheat genotypes (V) and their interaction (T × V) significantly ($p \leq 0.05$) (Table 1) affected wheat growth parameters like SL, RL, SFW, RFW, SDW and RDW (Figure 1). These growth parameters of wheat genotypes decreased with applied NaCl in solution culture at both levels i.e., 100 and 200 mM as compared to their respective controls, however the higher level of salinity i.e., 200 mM was found more detrimental and resulted in higher growth reduction than 100 mM NaCl salinity. The bulk of data regarding growth and ionic parameters was converted to salt tolerance indices (% of control) to rank the twelve wheat genotypes into salt-tolerant, moderately tolerant and sensitive at two salinity levels.

Table 1: F-values of two-way ANOVA for the genetic variability in wheat genotypes for salt tolerance, growth and physiological responses

Parameter	Treatment (DF = 2)	Genotype (DF = 11)	Treatment × Genotype(DF = 22)
Shoot length	2847.04**	18.30**	12.48**
Shoot fresh weight	7789.11**	58.87**	25.47**
Shoot dry weight	877.54**	53.60**	27.99**
Root length	1732.26**	29.63**	11.53**
Root fresh weight	9620.29**	245.03**	39.01**
Root dry weight	13739.7**	121.63**	58.04**
Total chlorophyll contents	4935.99**	102.28**	31.93**
Photosynthetic rate	9612.01**	171.12**	25.87**
Transpiration rate	1655.34**	34.66**	11.91**
Stomatal conductance	2588.29**	8.26**	3.24*
Leaf Na^+ concentration	4875.68**	83.97**	18.84**
Leaf K^+ concentration	2030.38**	55.99**	3.93*
K^+/Na^+ ratio	8226.48**	73.68**	11.94**

NS = Non-significant ($p > 0.05$); * = Significant ($p \leq 0.05$); ** = Highly significant ($p \leq 0.01$); DF = Degree of freedom

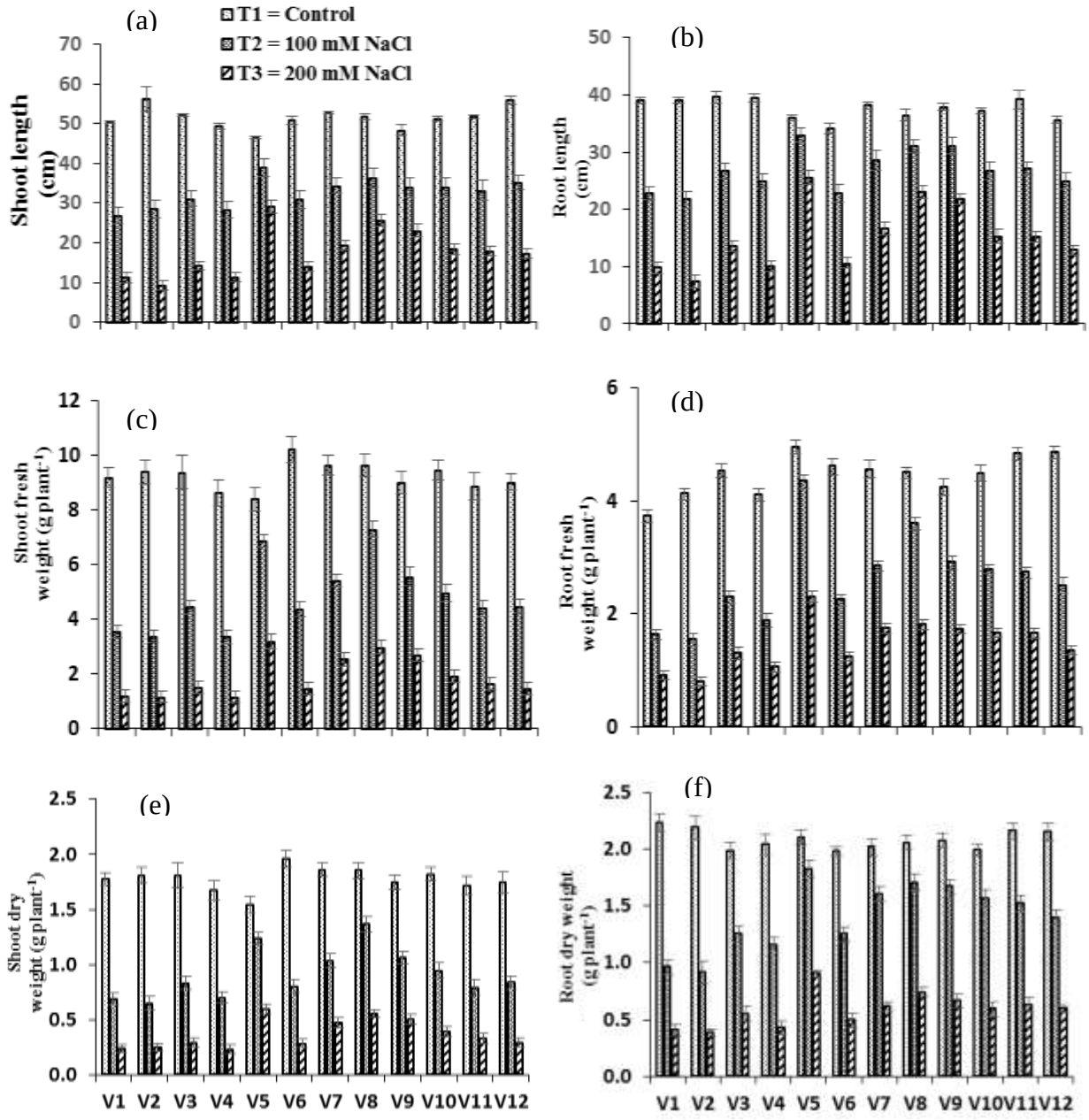

Figure 1: Effect of varying levels of salinity on growth responses: a) shoot length (SL), b) root length (RL, cm), c) shoot fresh weight (SFW), d) root fresh weight (RFW), e) shoot dry weight (SDW), and f) root dry weight (RDW, (g plant^{-1}) of wheat genotypes (Each value is a mean, n = 4, T bars represents ± standard error of means)

Wheat genotypes: V_1 = Kohistan-90, V_2 = MH-97, V_3 = SARC-IV, V_4 = Iqbal-2008, V_5 = SARC-I, V_6 = Perwas-94, V_7 = Uqab-2000, V_8 = Sehar-2006, V_9 = Shafaq-2006, V_{10} = Faisalabad-2008, V_{11} = Lasani-2008, V_{12} = AARI-2010

In twelve wheat genotypes, half the genotypes were found salt-sensitive, three genotypes were found moderately tolerant and three genotypes were ranked salt-tolerant regarding their vegetative growth and biomass production (Table 2). The dendrogram developed from the cluster analysis on the basis of growth and ionic parameters also indicates this grouping (Figure 2).

In present study, under salinity stress, the wheat genotypes were ranked in the descending order of $V_5 > V_8 > V_9 > V_7 > V_{10} > V_{11} > V_{12} > V_3 > V_6 > V_4 > V_1 > V_2$. Among all the tested wheat genotypes, under salt stress, V_5 had the better SL, RL, SFW, RFW, SDW and RDW closely followed by V_8.

The growth parameters including SL, RL, SFW, RFW, SDW and RDW are more correlated with crop salt tolerance at initial stages of growth and can serve as screening/selection criteria for salinity tolerance (Akhtar et al., 2003). Growth, physiological and ionic data of the current study indicated that genetic variation existed in the tested wheat genotypes for salt tolerance. The wheat

Table 2: Ranking of wheat genotypes for their relative salt tolerance in terms of growth (root length, root fresh and dry weight, shoot fresh and dry weight) and ionic parameters (K^+, Na^+ and K^+/Na^+ ratio) in a cluster analysis (Ward's minimum variance analysis)

Genotypes	NaCl (mM)	Cluster group ranking	Sum	Genotypic ranking	Tolerance degree
SARC-I, Sehar-2006	100	1	2	1	Tolerant
	200	1			
Shafaq-2006	100	2	3	1	Tolerant
	200	1			
Uqab-2000, Faisalabad, Lasani-2008	100	2	4	2	Moderately
	200	2			Tolerant
AARI-2010, SARC-IV	100	2	5	3	Moderately
	200	3			Sensitive
Perwa-94, Iqbal-2008, Kohistan-90, MH-97	100	3	7	4	Sensitive
	200	4			

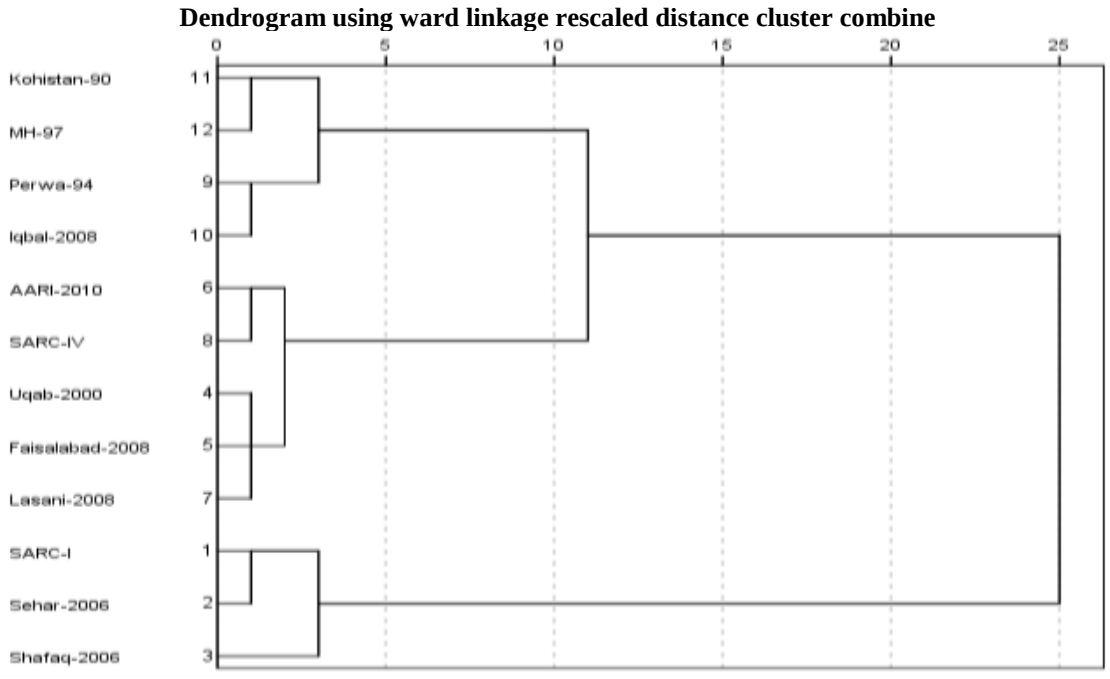

Figure 2: Dendrogram showing the separation between clusters (groups) of wheat genotypes

genotypes responded differently to varying NaCl salinity levels. Chartzoulakis and Klapaki (2000) reported that salinity affected plant growth processes including reduction in plant height, fresh and dry weights of roots, stem and leaves; lowered yield and caused deterioration of the quality of the product. The present results showed that SARC-I (V_5) had better RL, RFW and RDW in salinity treatments followed by Sehar-2006 (V_8). The root biomass of wheat reduced with increasing NaCl levels in the growth medium. Among all the tested wheat genotypes, the better SL, SFW and SDW (Figure 1) was recorded for V_5, V_8 and V_9, thus showing their high tolerance to salinity. Such tolerance of salinity by these genotypes can be due to inherent capacity and the presence of more tolerant genes to confer stress. The better growth of V_5, V_8 and V_9 than sensitive ones was attributed to reduced Na^+ accumulation and possibly mobilization of the defense mechanisms including antioxidative enzymes such as catalase, superoxide dismutase and ascorbate peroxidase, which might have suppressed the Na^+ transport to further tissues (Gupta and Huang, 2014). The increasing concentration of NaCl from 100 to 200 mM also significantly changed the shoot biomass of used wheat genotypes. The reduction in fresh and dry biomass with increasing salinity can be attributed to reduced photosynthesis rate and other physiological functions. The results are in agreement with Saqib (2002), Khan et al. (2004), Kanwal *et al.* (2011), Rao *et al.* (2013). The wheat genotypes showed differential response to salt stress might be due to their differential genetic potential for salt tolerance. Under salt stress variation in biomass production by different varieties has also been reported for other crops such as sunflower (Shahbaz *et al.,* 2011), maize (Carpici *et al.,* 2010) and barley (Mahmood, 2011).

The NaCl relative toxicity (RT) and tolerance indices (TI) on SL, RL, SFW, RFW, SDW and RDW (%) of twelve wheat genotypes are presented in Figure 3 and 4. With increasing level of applied NaCl (i.e., 100 and 200 mM), the RT increased and TI decreased. At varying levels of applied NaCl treatments, the NaCl RT (%) of wheat genotypes on SL, RL, SFW, RFW, SDW and RDW were in decreasing order as $V_2 > V_1 > V_4 > V_6 > V_3 > V_{12} > V_{11} > V_{10} > V_7 > V_9 > V_8 > V_5$. The NaCl TI (%) of wheat genotypes on SL, RL, SFW, SDW, RFW, RDW were found in decreasing order of $V_5 > V_8 > V_9 > V_7 > V_{10} > V_{11} > V_{12} > V_3 > V_6 > V_4 > V_1 > V_2$. Under NaCl salinity stress, the V_5 performed very well closely followed by V_8 and were considered salt-tolerant. However, V_2 and V_1 proved salt-sensitive in present study. The TI values calculated on the basis of growth parameters were subjected to cluster analysis for genotype ranking.

The tolerance of a crop to salinity is measured in terms of yield decrease relative to yield obtained under non-saline conditions (Ghafoor *et al.,* 2004). A significant variation in RT and TI of wheat genotypes exposed to salinity treatments has been found (Figure 3 and 4). In general, the analysis revealed that increasing levels of salinity decreased the tolerance indices and increased the relative toxicities of wheat genotypes. The results of current study displayed that at varying levels of salinity, the RT of fresh and dry, root and shoot weight were found minimum in V_5 followed by V_8, while TI were high and therefore proved salt-tolerant genotypes. Rout and Das (2002) suggested that the TI is an important indicator to screen rice genotypes for trace elements tolerance. Mahmood *et al.* (2007) also reported that the TI of wheat, rice and barley were found inversely related to NaCl treatments. This happened possibly due to greater inhibition in germination followed by decrease in root length and shoot growth in response to adverse effects of salinity. Similarly, Asmare (2013) reported that among two varieties of bean, cultivar Chercher showed higher seedling tolerance index and lower root and shoot phytotoxicity. This explains that different cultivars have genetic variability for tolerance and toxicity effects of salinity. Royo and Abio (2003) reported that genetic variations for salinity tolerance exist at critical stages in the cultivated gene pool of wheat. In another study, Ahmad (2011) reported that salt tolerance indices for germination rate under 250 and 300 mM NaCl stress showed that germination was significantly suppressed with increased NaCl concentration.

Physiological responses of wheat genotypes as affected by salinity

A significant ($p \leq 0.05$) (Table 1) effect on plant physiological responses like TCC, A, E and g_s (Figure 5) by applied NaCl treatments (T), wheat genotypes (V) and their interactions (T × V) was observed. The treatments affected these physiological processes of wheat genotypes in increasing order of $T_1 < T_2 < T_3$. These results showed that the higher level of salinity severely affected physiological functions of wheat genotypes. In present study, among different used wheat genotypes, A, TCC, E and g_s was maximum in V_5 and minimum in V_2.

The reduction in plant biomass and crop yield under salt stress has been attributed to salinity induced adverse changes in physiological and biochemical processes in plants. Among various physiological changes induced by salinity, the photosynthetic rate and other gas exchange attributes of plants are highly important as the plant growth and vigor largely depends on these characteristics (Ashraf *et al.,* 2010; Carpici *et al.,* 2010; Mahmood, 2011). The present study was aimed to evaluate relative salt tolerance of wheat genotypes using some physiological functions such as A, E, g_s and TCC (Figure 5) in addition to growth parameters.

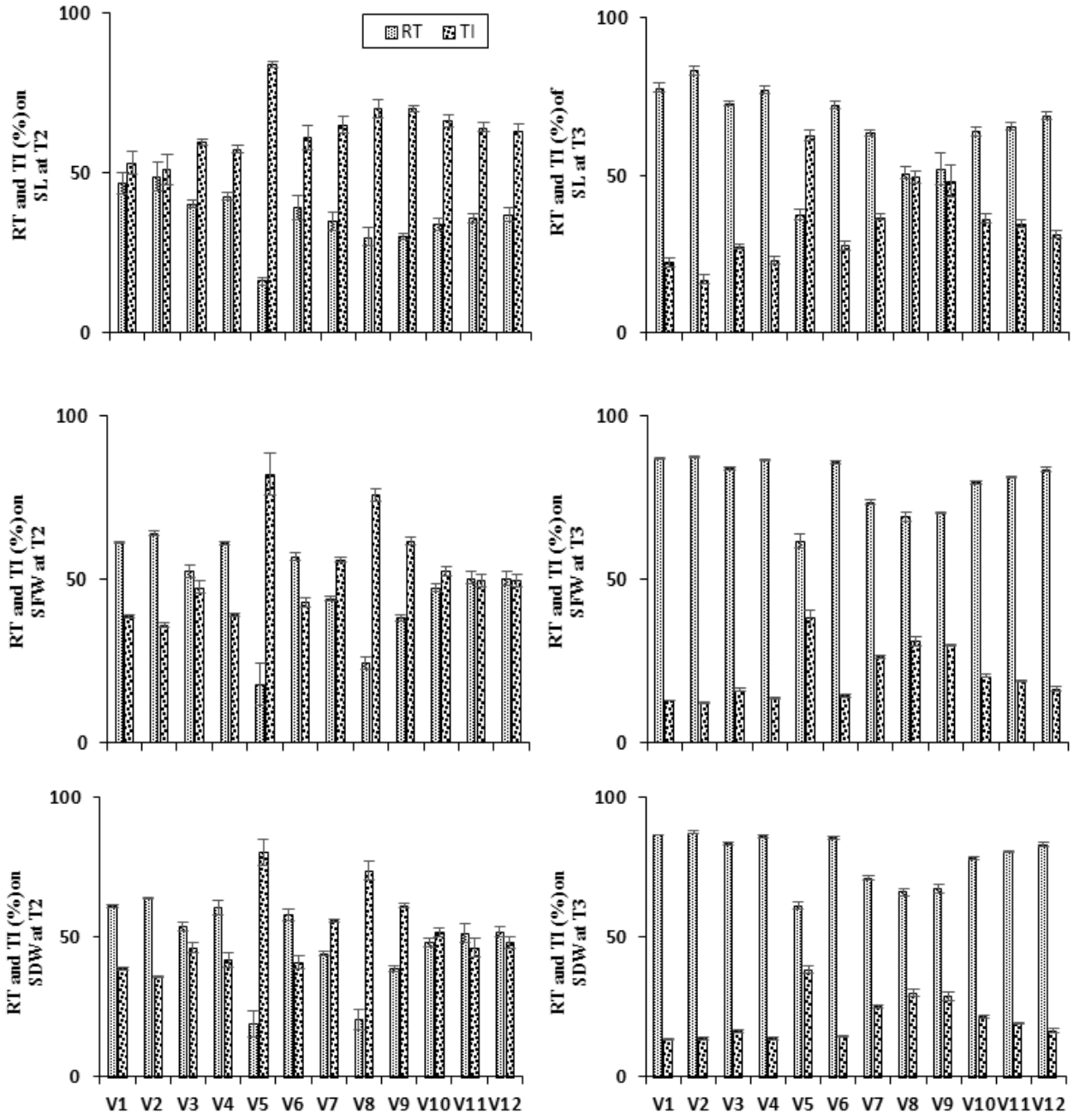

Figure 3: Effect of varying levels of salinity on relative toxicities (RT, %) and tolerance indices (TI, %) on shoot length (SL), shoot fresh weight (SFW) and shoot dry weight (SDW) of wheat genotypes (Each value is a mean, n = 4 ,T bars represents ± standard error of means)

Wheat genotypes: V_1 = Kohistan-90, V_2 = MH-97, V_3 = SARC-IV, V_4 = Iqbal-2008, V_5 = SARC-I, V_6 = Perwas-94, V_7 = Uqab-2000, V_8 = Sehar-2006, V_9 = Shafaq-2006, V_{10} = Faisalabad-2008, V_{11} = Lasani-2008, V_{12} = AARI-2010

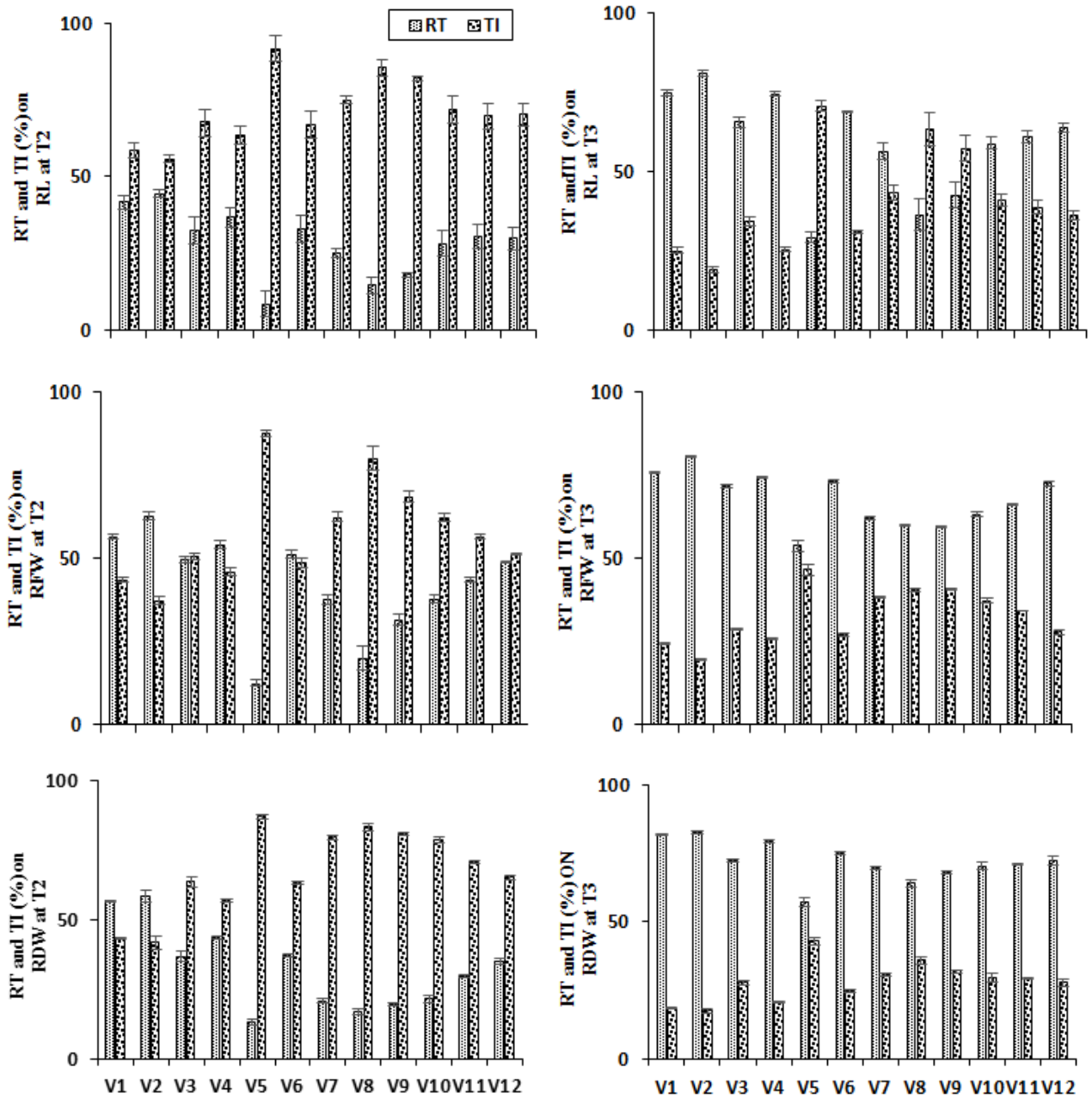

Figure 4: Effect of varying levels of salinity on relative toxicities (RT, %) and tolerance indices (TI, %) on root length (RL), root fresh weight (RFW) and root dry weight (RDW) of wheat genotypes (Each value is a mean, n = 4, T bars represents ± standard error of means)

Wheat genotypes: V_1 = Kohistan-90, V_2 = MH-97, V_3 = SARC-IV, V_4 = Iqbal-2008, V_5 = SARC-I, V_6 = Perwas-94, V_7 = Uqab-2000, V_8 = Sehar-2006, V_9 = Shafaq-2006, V_{10} = Faisalabad-2008, V_{11} = Lasani-2008, V_{12} = AARI-2010

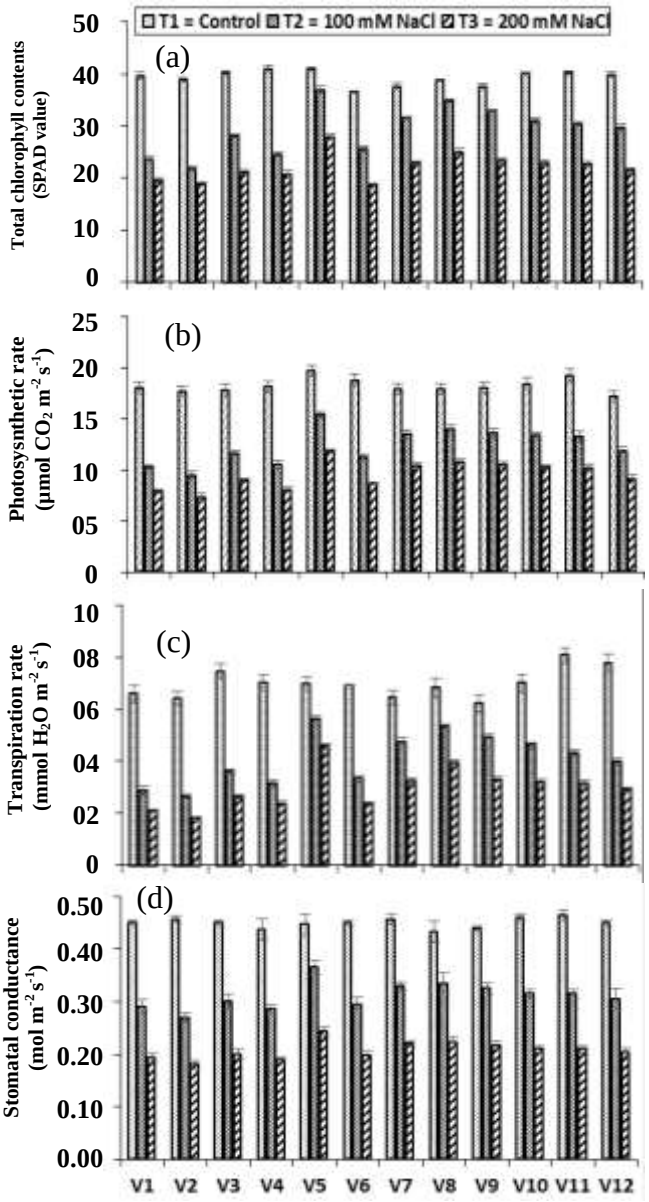

Figure 5: Effect of varying levels of salinity on physiological responses: a) total chlorophyll contents (TCC, SPAD-value), b) photosynthetic rate (A, µmol CO_2 m^{-2} s^{-1}), c) transpiration rate (E, mmol H_2O m^{-2} s^{-1}) and d) stomatal conductance (g_s, mol m^{-2} s^{-1}) of wheat genotypes (Each value is a mean, n = 4 statistically significant at $p \leq 0.05$, T bars represents ± standard error of means)

Wheat genotypes: V_1 = Kohistan-90, V_2 = MH-97, V_3 = SARC-IV, V_4 = Iqbal-2008, V_5 = SARC-I, V_6 = Perwas-94, V_7 = Uqab-2000, V_8 = Sehar-2006, V_9 = Shafaq-2006, V_{10} = Faisalabad-2008, V_{11} = Lasani-2008, V_{12} = AARI-2010

From the results of the present study it was evident that wheat genotypes responded differently to salt stress in terms of gas exchange characteristics and biomass production. Biomass production is the function of various biochemical and physiological features. The plant gas exchange characteristics play highly significant role in this regard. The salt stress results in the inhibition of these gas exchange characteristics (Ashraf and Ali, 2008; Ashraf *et al.,* 2010) and subsequently the biomass production is decreased. The reduction in plant photosynthetic rate under salt stress is attributed to the stomatal closure (Ashraf, 2009). A number of other factors including plant electron transport system, respiration system, buildup of stress metabolites, ATP synthesis in the mitochondria (Zhang *et al.,* 1999), and protein synthesis (Lawlor and Tezara, 2009) also contribute to the hostile effects of salt stress on plant photosynthetic rate. However, the plant species and cultivars have variable potential in relation to these physiological features under salt stress.

In the current study, wheat genotypes responded variably to salt stress with respect to plant photosynthetic rate. The lower biomass production of wheat genotypes was clearly associated with the reduced A, E and that were positively associated with reduced g_s and TCC. Under conditions of salt stress, variations in chlorophyll contents has been previously observed in several crops including *Brassica juncea* (Hayat *et al.,* 2011), mustard (Ahmad *et al.,* 2012), wheat (Ghogdi *et al.,* 2012) and basil genotypes (Heidari, 2012). The salt stress either impedes synthesis and/or accelerates the degradation of existing chlorophyll molecules (Iyengar and Reddy, 1996). Wani *et al.* (2013) also reported significant decrease in SPAD-value in the *Brassica Juncea* in response to soil salinity. More reduction in A, TCC, E and g_s (Figure 5) was observed in Kohistan-90 (V_1) and MH-97 (V_2) and minimum in V_5 and V_8. The photo-oxidative damage to the photosynthetic machinery occurs due to excess light excitation energy when there is reduced plant photosynthetic rate under salt stress, resulting in inhibited plant photosynthesis and a progressive reduction in plant carbon assimilation rate (Cornic, 2000). Kanwal *et al.* (2011) reported that the salt tolerance of the wheat cultivars was found associated with their higher photo-synthetic rate and photosystem II efficiency. Therefore, the wheat genotypes V_5, V_8 and V_9 were found tolerant to salt stress particularly at the early growth stages on the basis of chlorophyll contents and plant gas exchange attributes. Similarly, Rao *et al.* (2013) reported that salt-tolerant varieties including Sehar-2006 had higher K^+ and chlorophyll contents, K^+: Na^+ ratio and yield under salinity stress. However, to ascertain whether the high salt tolerance observed in these genotypes (V_5, V_8 and V_9) would be maintained at the later growth stages, further research is needed.

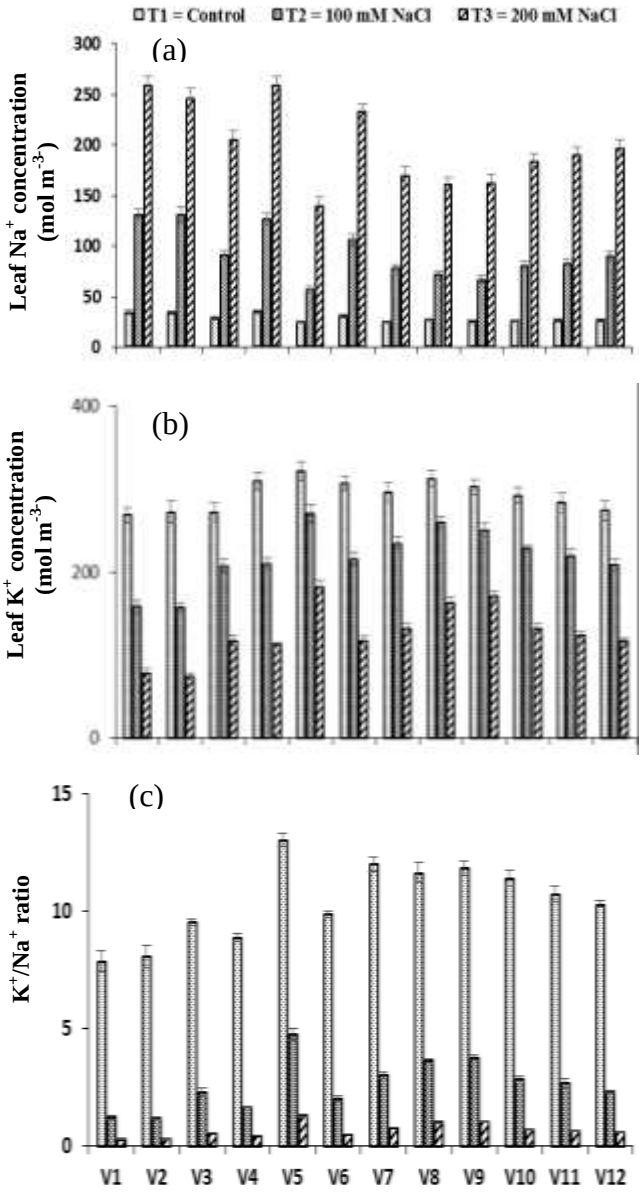

Figure 6: Ionic composition of wheat genotypes: a) leaf sodium (Na^+, mol m^{-3}), b) potassium (K^+, mol m^{-3}) concentration and c) K^+/Na^+ ratio, as affected by varying levels of salinity (Each value is a mean, n = 4 statistically significant at $p \leq 0.05$, T bars represents ± standard error of means)

Wheat genotypes: V_1 = Kohistan-90, V_2 = MH-97, V_3 = SARC-IV, V_4 = Iqbal-2008, V_5 = SARC-I, V_6 = Perwas-94, V_7 = Uqab-2000, V_8 = Sehar-2006, V_9 = Shafaq-2006, V_{10} = Faisalabad-2008, V_{11} = Lasani-2008, V_{12} = AARI-2010

Leaf ionic concentration of wheat genotypes as affected by salinity

Application of NaCl salinity with increasing rates (T), wheat genotypes (V) and their interaction (T × V) had significantly ($p \leq 0.05$) (Table 1) affected leaf Na^+ , K^+ concentration and K^+/Na^+ ratio (Figure 6) in hydroponics. The treatments effects on leaf Na^+ concentration of wheat genotypes was ranked in increasing order of $T_1 < T_2 < T_3$ and vice versa for leaf K^+ concentration and K^+/Na^+ ratio. Among tested wheat genotypes, maximum leaf K^+ concentration and K^+/Na^+ ratio was observed in V_5 followed by V_8. The leaf Na^+ concentration was found greater in V_2 than all other tested wheat genotypes.

Data regarding Na^+, K^+ and K^+/ Na^+ ratio in the leaves of wheat genotypes was converted to tolerance indices (% of control) which were subjected to cluster analysis for genotype ranking along growth parameters. The grouping of wheat genotypes obtained in the cluster analysis on the basis of growth and ionic parameters is shown in the Figure 2. A significant negative relationship was found between growth and physiological parameters and increase in leaf Na^+ concentration of the wheat genotypes grown under NaCl salinity treatments. It is well known that Na^+ is toxic to plants and disrupts different metabolic activities when present at high concentrations. The genotypes which were able to retain Na^+ in their roots were found tolerant (Akram *et al.*, 2007). The wheat genotypes V_5, V_8 and V_9 maintained low concentration of Na^+ in their leaves and therefore had higher tolerance to salinity. The dendrogram obtained in cluster analysis on the basis of growth and ionic parameters also indicate that these three genotypes are closely related in terms of their salt tolerance (Figure 2). It is reported that salt tolerance and intracellular Na^+ homeostasis are modulated by Ca^{2+} and K^+ acquisition (Munns *et al.*, 2002). There is competition between Na^+ and K^+ for uptake and Na^+ competes efficiently since the concentration of Na^+ is usually very high than that of K^+ in saline environments. The inability of some crops to keep Na^+ and Cl^- out of transpiration streams is the main cause of sensitivity to salinity (Gorham *et al.*, 1990). Plants maintaining normal nutrient ion contents and limiting the uptake of toxic ions could show greater tolerance. Uptake mechanism that discriminates similar ions such as Na^+, K^+ can be used as selection criteria for salinity tolerance in wheat.

In the present study, with increasing salinity in the growth medium, the leaf K^+ concentration decreased in all wheat genotypes (Figure 6). The concentration of K^+ was decreased due to the presence of excessive Na^+ in the growth medium because an antagonistic effect is found between K^+ uptake in plant and external Na^+ contents

(Sarwar and Ashraf, 2003). Salt tolerance is reported to be associated with K^+ contents in plants (Ashraf and Sarwar, 2002), because of the involvement of K^+ in osmotic regulation and competition with Na^+ (Ashraf *et al.*, 2005). In the present study, wheat genotypes V_5, V_8 and V_9 comparatively accumulated higher K^+ than the sensitive genotypes. The commonly used strategies by plants for maintaining desirable K^+/Na^+ ratio in the cytosol include prevention of Na^+ entry, its efflux from cell and regulation of K uptake (Ahmad, 2011).

Conclusion

In present solution culture study, growth, tolerance and physiological responses of tested twelve wheat genotypes were significantly affected by applied NaCl salinity and its increasing levels (0, 100 and 200 mM). The wheat genotypes showed variations for salinity tolerance and Na^+ and K^+ accumulation. The NaCl relative toxicities increased and tolerance indices reduced on growth parameters with all applied NaCl levels. According to the results of cluster analysis, among all the used wheat genotypes, SARC-I (V_5), Sehar-2006 (V_8) and Shafaq- 2006 (V_9) were ranked as tolerant, Uqab-2000 (V_7), Faisalabad-2008 (V_{10}) and Lasani-2008 (V_{11}) as moderately tolerant, AARI-2010 (V_{12}) and SARC-IV (V_3) as moderately sensitive and Perwaz-94 (V_6), Iqbal-2008 (V_4), Kohistan-90 (V_1) and MH-97 (V_2) as sensitive genotypes. The wheat genotypes V_5, V_8 and V_9 were found tolerant to salinity because of better growth, lower NaCl relative toxicities, leaf Na^+, higher tolerance indices, plant photosynthetic rate, total chlorophyll contents, transpiration rate, stomatal conductance and leaf K^+ concentration. Therefore, these genotypes were found as a valuable resources that can be used in further wheat breeding programs aimed at increasing salinity tolerance. Moreover, in addition to wheat growth parameters, the physiological functions such as photosynthetic and transpiration rates, stomatal conductance, chlorophyll contents, low fluxes of Na^+ and high of K^+, were also found to be useful parameters related to salinity tolerance.

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