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Full Length Research Paper

Rationality of using various physiological and yield related traits in determining salt tolerance in wheat

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Improving the grain yield of wheat under stress conditions is always the major goal of plant scientist and breeder research. This study was conducted on five bread wheat genotypes with different salt tolerance potential using 8 and 15 dS m^{-1} salinity levels in pots filled with sandy clay loam soil. This study aimed to evaluate the significance of various physiological determinants commonly used for salt tolerance along with yield and yield related traits of wheat, especially grain yield. Plant relative growth rate, chlorophyll content index (CCi), water relations and biomass was recorded along with ionic (Na^+ and K^+) and yield related traits of wheat plant. The reduction in relative growth rate (RGR) was up to 34% in salt tolerant and 64% in salt sensitive wheat genotypes at 15 dS m^{-1} salinity treatment. Na^+ in leaf was increased by about 3 folds of control, while K in leaves decreased with increase in salinity and reduction was 34% in salt tolerant and 52% in the salt sensitive wheat genotype as compared to control plants. It was also observed that the number of spikelets per spike together with number of tillers per plant was the most sensitive parameters for salt tolerance. We conclude that these characteristics might be selected as desirable traits for a cross breeding programs or engineering such wheat that have ability to produce more tillers and/or number of spikelets per spike under stress to get varieties with better production in saline environments.

Key words: Wheat, salinity, yield, traits, ionic, physiological.

INTRODUCTION

A sustainable food production puts a high demand on breeding more salt-tolerant crops in the future (Cuartero et al., 2006). Saline waste land can be used for crop production with appropriate management options, with the most important one being biological options. This biological approach also deals with the improving salt tolerance in plants using conventional breeding and other molecular and genetic techniques. Therefore, improving the grain yield of wheat under stress conditions is always the major goal of plant breeding programs. This always requires the analysis of sensitivity parameters which affects the interaction between salinity and crop yield. With the identification of such physiological and yield indicators, management options may be developed to ameliorate yield reduction under salinity. Identification and evaluation of physiological processes determining grain yield and final grain yield is a critical aspect of such studies. To achieve this, we need better understanding of physiological mechanisms of salt tolerance among genotypes, to get the trait of interest and introduce it in the genotypes targeted for higher yields under stress.

For instance, reduction in the indicators of leaf water status such as relative water content (RWC) and water potential, have been reported in response to salinity stress in diverse plant species (Lutts et al., 1996; Sairam et al., 2002; Farooq and Azam, 2006) and this quickly causes reductions in growth rate, along with reduction in

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Abbreviations: **CCi**, Chlorophyll content index; **RGR**, relative growth rate.

yield (Munns, 2002). Even more striking findings came from Rivelli et al. (2002) who found that there were little differences for water relations among genotypes having contrasting behavior for Na^+ exclusion in response of salinity. In contrast, leaf water potential and leaf osmotic potential were always less negative in salt-tolerant genotypes than the salt-sensitive sorghum (Serraj and Sinclair, 2002). Generally, plants are able to withstand salinity stress by lowering leaf osmotic potential through a process called osmotic adjustment (Hazegawa et al., 2000). The osmotic adjustment in leaves contributes to uphold water uptake and cell turgor pressure, which are critical for plants to stand for salinity. Therefore, any difference among genotypes with respect to their leaf water relations may reflect differential response to salt tolerance.

One of the important aspects of salt tolerance is the capability of a plant to keep out toxic ions from the shoots. In wheat, genotypic variation for salt tolerance has been associated with low rates of Na^+ transport and high selectivity for K^+ over Na^+ (Schachtman and Munns, 1992; Husain et al., 2004), while a positive correlation between toxic ion exclusion and salt tolerance has also been observed in maize (Cramer et al., 1994). In addition, in some crops such as cotton, sorghum and wheat, Na^+ exclusion did not always predict the salt tolerance of the genotypes (Leidi and Saiz, 1997; Shabala and Cuin, 2007). The concentration of Na^+ and K^+/Na^+ in rice plants was correlated with seedling growth and grain yield under salt stress (Khatun et al., 1995; Lutts et al., 1995). Therefore, it is necessary to evaluate whether genotypes differing in salt tolerance use ion exclusion as a tolerance mechanism against salinity.

Relative growth rate (RGR) is a key parameter for stress studies, making possible the more appropriate comparison of growth among species or genotypes under salinity than absolute growth rate (Cramer et al., 1994). It takes into account both the initial and final plant biomass over a specified time period (Hunt, 1990) and could be rewarding to achieve goals of higher yield from salt affected lands. RGR also affects the rate of accumulation of Na^+ in shoots and genetic differences in rates of Na^+ accumulation in the shoot may be attributed to genetic variation in shoot vigour (example, Yeo et al., 1990 for rice). A fast-growing plant will have a lower concentration in the shoot than a slow growing plant, for the same shoot uptake rate. These differences suggested that understanding the physiological mechanisms controlling salt tolerance at seedling stages is very important for prediction of growth performance of plant under salt stress.

Although, genotypic variation exists for salt tolerance in terms of physiological characters (Asch et al., 2000; Akhtar et al., 2010), the difficulty could be exacerbated when the ranges of genotypic differences are relatively small, especially for some physiological characters such as K^+ and Ca^{2+} contents (Zeng et al., 2003). Moreover, in some plants, other reproductive stages are more sensitive to salinity than tillering stage (Lutts et al., 1995), and even in wheat salinity stress significantly decreased the number of spikelets per spike and total straw yields. This loss of spike-bearing tillers accounts for most of the yield reduction in salt-stressed wheat (Maas et al., 1996). Thus, the specific objective of this study was to assess various physiological parameters at initial growth stage, along with grains weight and other yield components influencing salt tolerance potential of wheat genotypes.

MATERIALS AND METHODS

Plant material and experimental conditions

Four bread wheat (*Triticum aestivum* L.) genotypes having contrasting salt tolerance response; Kharchia-65, SARC-3 and S-9476 as salt tolerant and S-8189 as salt-sensitive was used in this experiment. Kharchia-65 was included as reference salt tolerant variety (Ashraf, 2002; Hollington, 2000), while the other three were ranked in our previous studies (Hollington et al., 2001; Saqib et al., 2011). Seeds of four genotypes were sown into soil filled pots. All the pots were under natural light (sunlight) with air temperature ranging from 12 to 32°C during the day and 10 to 26°C during night, while relative humidity was in range of 40 and 65% at day and night. The glass roof was available on wire-house to avoid the rainfall on the experimental units. Normal sandy loam soil was collected from the soil surface (0 to 15 cm), air-dried, ground and sieved through a 2.0-mm mesh screen. Air-dried soil with 31% saturation was filled in plastic pots having 30 kg capacity (38 cm diameter × 30 cm depth) having no leaching possibility. Commercial grade NaCl was mixed in the soil with a mechanical mixer to achieve artificial salinity levels of 8.0 and 15 dS m^{-1} and the control was set with no NaCl addition, having normal soil (EC 1.29 dS m^{-1}). Twenty healthy seeds treated with proper fungicide of each genotype were sown in each pot. Each pot was considered a replicate and each treatment had four replicates. After germination, plants were thinned and uniform seedlings were kept in each pot. The recommended dose of NPK was added and irrigations were applied on 'when required' basis till maturity.

Plant growth measurements

Initial plant growth in term of plant height, number of tillers and no of leaves and shoot dry weight was recorded along with other physiological parameters. Shoot fresh weight (FW) was also recorded and the samples were put in forced hot air draft oven (Model DHG-9053A, R & M Marketing, Sussex, UK) to dry at 65 ± 5°C to constant weight, and shoot dry weight (DW) was recorded after cooling the samples to room temperature. At maturity, the remaining plants were harvested for grain yield and other yield related parameters along with ionic analysis.

The shoot dry biomass at final harvest was measured after detaching the spikes from the individual plants. RGR (g g^{-1} day^{-1}) was calculated from the following equation according to Husain et al. (2004):

$$RGR = \frac{(\ln WS_2 - \ln WS_1)}{(t_2 - t_1)}$$

Where, WS_1 and WS_2 are the shoot fresh weights (g) at times t_1 and

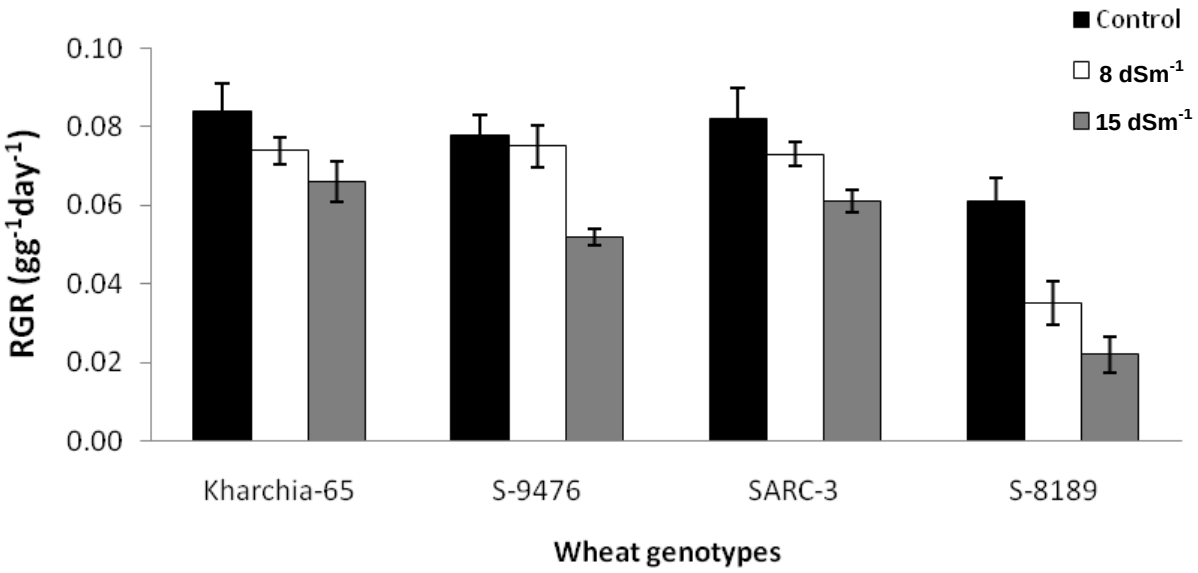

Figure 1. Relative growth rate of four contrasting bread wheat genotypes at different salinity levels (means ± SE; n = 4).

t_2 (days).

Plant chlorophyll contents index (CCi)

Chlorophyll content index (CCi) of leaves in terms of SPAD values was measured by using a hand-held SPAD- 502 meter (Minolta, Osaka, Japan), a cost-effective way to measure photosynthetic capacity than chlorophyll fluorescence (Munns et al., 2006). Leaf chlorophyll content index was measured from the leaf tip to the leaf base and then averaged.

Water relation measurements

Predawn leaf water potential (Ψ) and osmotic potential (Ψ_π) of plants from the middle of the second youngest fully developed leaf blade were measured with a pressure bomb (PMS Instrument Co., Model 1002, Corvalis Co., Oregon, USA) according to the technique followed by Scholander et al. (1965). Immediately after leaf water potential, the same leaf material was stored in freezer at -4°C after wrapping in aluminum foil. The leaf samples were thawed at room temperature and then osmotic potential of sap was determined with a vapor pressure osmometer (Wescor 5100C, Wescor Inc., Logan, USA). Turgor pressure (T_p) was estimated as the difference between Ψ_π and leaf water potential.

For relative water content (RWC) in leaf, fresh leaf samples were washed with distilled water and weighed after drying with paper towel to get fresh weight (FW) and then soaked in distilled water again under dark conditions at 22°C for 6 h for hydration and then turgid weight (TW) was recorded after cleaning the water drops from leaf surface. All the leaf samples were then dried at 65 ± 5°C for constant weight, after which their dry weight (DW) was determined. Leaf RWC was calculated using the formula of Barrs and Weathrley (1962).

Plant ionic analysis

The leaf samples were taken for ionic analysis (Na^+ and K^+) of the leaf tissue. After oven drying, leaf samples were ground and wet digested by overnight soaking in concentrated HNO_3 and $HClO_3$, and then heated at 400°C for 3 h to completed digestion. Diluted samples were used for Na^+ and K^+ analysis using flame photometer (Sherwood Model 410, Sherwood Scientific Ltd, Cambridge, UK).

Plant grain yield (g/plant)

At final harvest after maturity, the remaining four plants from each pot were harvested and data regarding number of spikelets and spike length were recorded alongside dry biomass after detaching spikes were also weighed. The spikes of mature plants were threshed and hundred-grain weight and grain yield per plant were recorded.

Statistical analysis

The experiment was laid out according to completely randomized design in factorial arrangement with four replications. Data were analyzed by ANOVA test using GENSTAT Discovery edition (Pyne et al., 2005). Pearson's correlation test was also carried out for various parameters and traits to study the relationships among others. Differences between treatments and genotypes means were assessed at 5% probability.

RESULTS

Relative growth rate (RGR)

RGR is considered more appropriate for comparing growth among species or genotypes under salt stress conditions than absolute growth values (Cramer et al., 1994). A distinct behavior was observed between two contrasting groups (salt sensitive and tolerant genotypes) when exposed to salinity (Figure 1). The reduction in RGR at 8 dS m^{-1} was 4 to 12% in salt tolerant

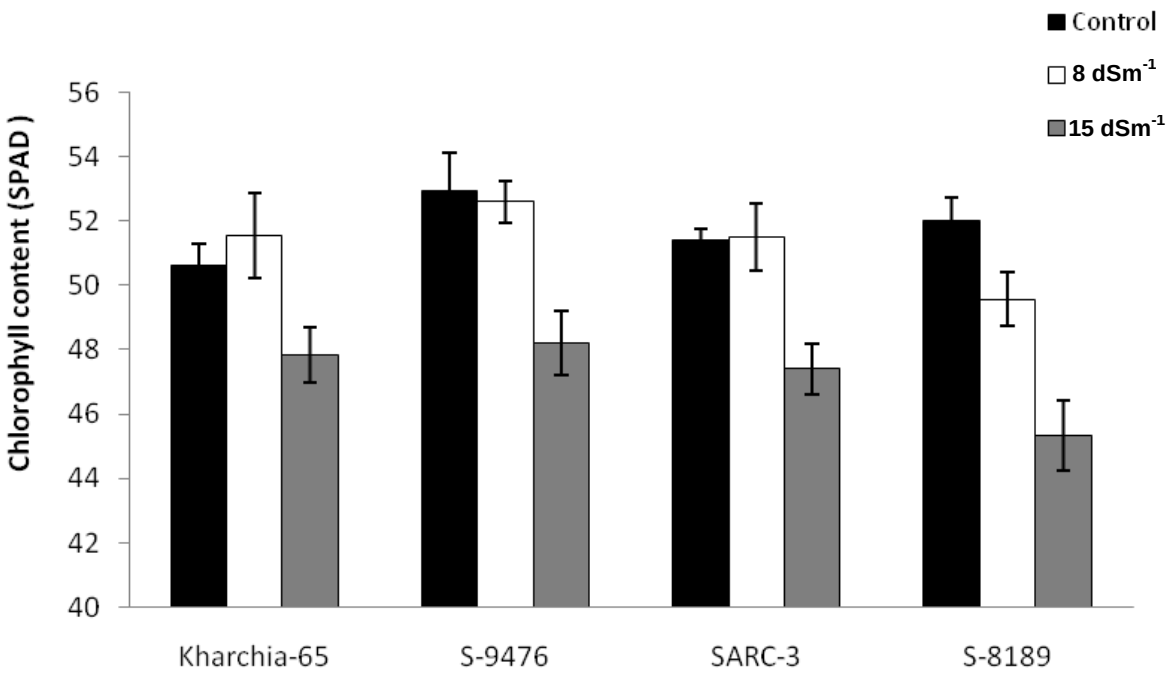

Figure 2. Chlorophyll content index (CCi) measured in term of SPAD value of four contrasting bread wheat genotypes sown at different salinity levels (means ± SE; n = 4).

wheat genotypes (Kharchia-65, SARC-3 and S-9476), whereas it was 43% in salt sensitive genotype (S-8189) when compared with the control treatment. At higher salinity level (15 dS m^{-1}), reduction in RGR was in the range of 22 to 34% of control in salt tolerant genotypes-that is; 22, 26 and 34% in Kharchia-65, SARC-3 and S-9476, respectively while the salt sensitive genotype S-8189 endured a reduction of 64% of the control in RGR at the same level. The differences among genotypes were however not discrete at lower salinity level.

Leaf chlorophyll content index (CCi)

Leaf CCi decreased with salinity (Figure 2), but the intensity was not so much at 8 dS m^{-1} and non-significant differences were found among wheat genotypes at this level except in salt sensitive genotype S-8189. The results also show a slight increase in chlorophyll content of salt-tolerant genotypes (Kharchia-65, SARC-3) and negligible change in S-9476, while the maximum decrease was in the case of salt-sensitive genotype (S-8189) with a magnitude of 13%.

Water relations of stressed plant leaves

Salt stress also drastically decreased the leaf water potential (Ψ) with significant differences among genotypes (Figure 3) with a contrasting response for leaf water potential, especially at higher salinity. The decrease in leaf water potential at 15 dS m^{-1} was -0.87 to -0.93 MPa in salt tolerant genotypes and -1.24 MPa in salt sensitive genotypes compared with the -0.44 to -0.56 and -0.58 MPa in control, respectively. In comparison with water potential, leaf osmotic potential showed a less variation with respect to salinity and among genotypes. The osmotic potential was 2 to 3 folds in salt tolerant genotypes as compared to salt tolerant sensitive genotype (S-8189), which had more than six folds change in osmotic potential at higher salinity level (15 dS m^{-1}) compared to the control. The RWC of all four contrasting wheat genotypes was adversely affected by salinity stress but a rapid decrease appeared at level of 15 dS m^{-1} and the magnitude of reduction in RWC was higher in salt-sensitive as compared to salt-tolerant genotypes at higher salinity level.

Ionic analysis for Na^+ and K^+ in leaf tissue

Salinity caused a significant increase in Na^+, as well as many fold decrease in K^+ contents of leaf sap in wheat (Figure 4). Na^+ in leaf was increased by about 2 and 3 folds of control at 8 and 15 dS m^{-1}, respectively. Kharchia-65 had lower Na^+ content in leaves at both treatments (8 and 15 dS m^{-1}) than the other salt tolerant genotypes, while the salt-sensitive genotype (S-8189) had high Na^+ contents in leaves at both salinity levels.

The significant differences in Na^+ contents of leaf

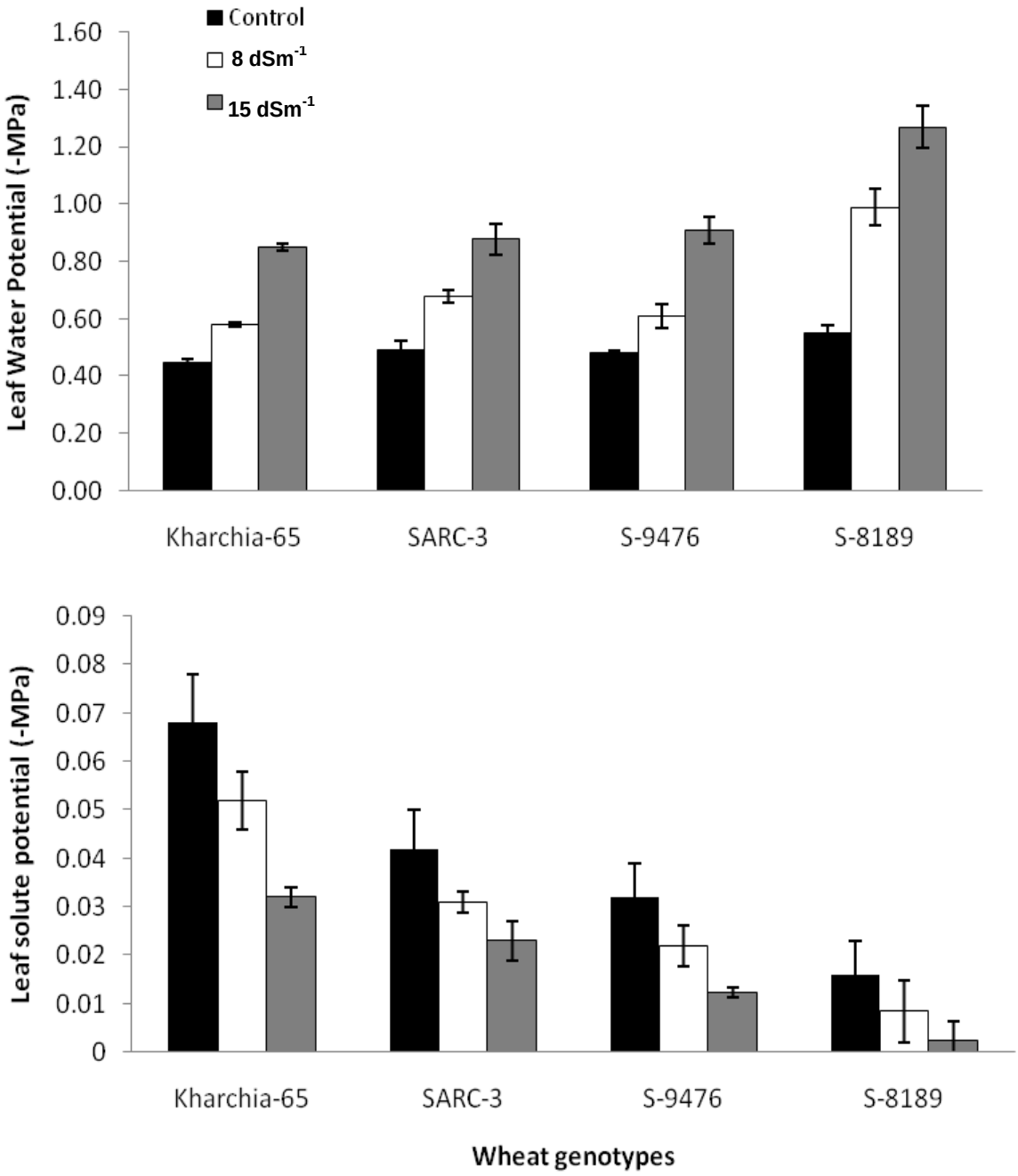

Figure 3. Effect of different levels of salinity on leaf water and osmotic potential in four contrasting bread wheat genotypes (means ± SE; n = 4).

showed the genotypic variation in their ability to exclude Na^+ from the leaves.

Moreover, potassium contents in leaves decreased with increase in salinity and reduction at 8 and 15 dS m^{-1} in salt tolerant genotypes was 16 to 34%, while the magnitude of this reduction in the salt sensitive genotype (S-8189) was 52% compared to the control plants. The salt tolerant genotypes S-9476 showed the highest K content at higher salinity level along with Kharchia-65. It was interesting to find that S-9476 also had higher Na^+ contents in its leaves.

Initial growth response to salinity

Plant height, number of tillers and other yield related traits decreased in all wheat genotypes with increasing salinity (Figure 5). The reduction in the parameters was

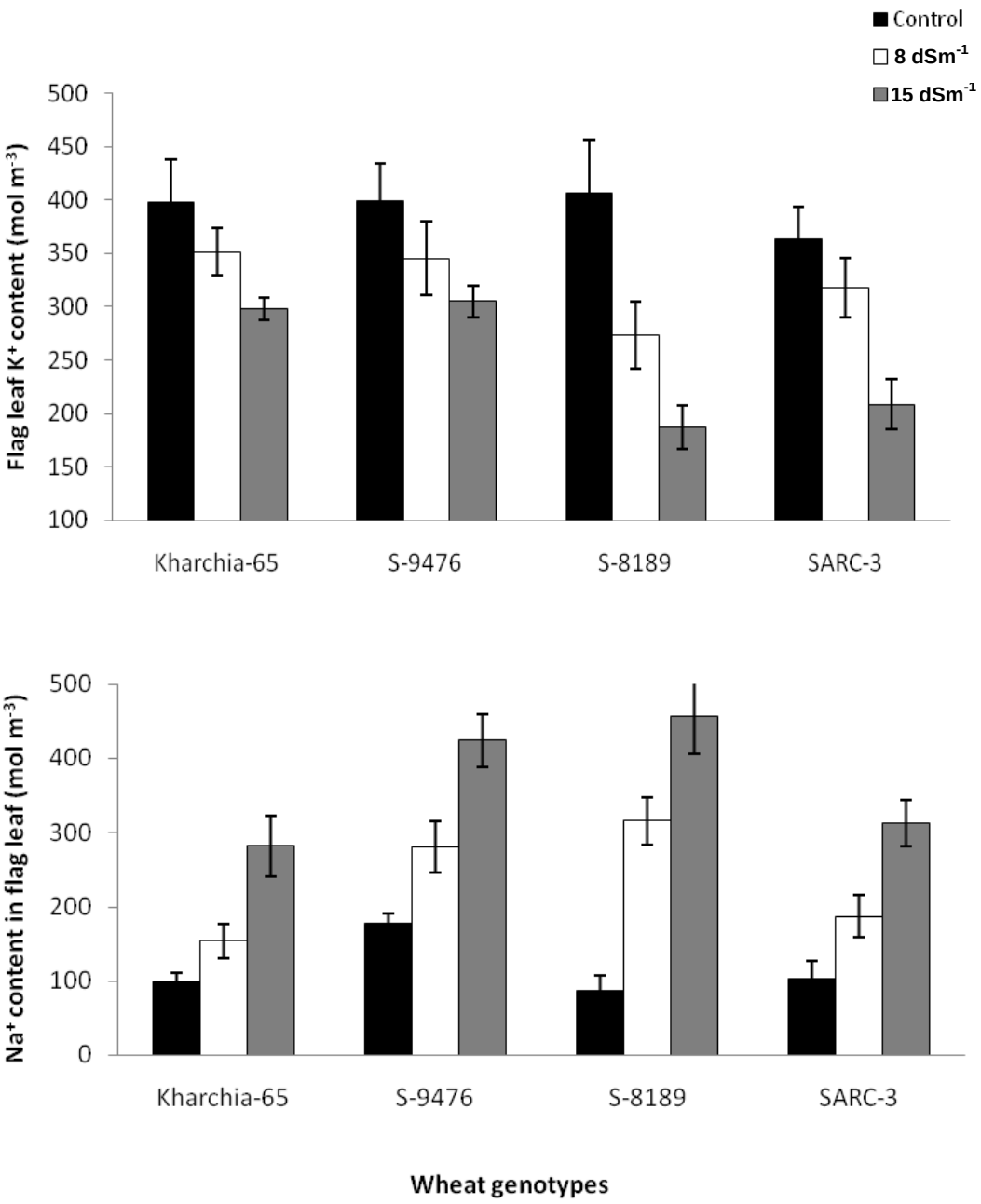

Figure 4. Effect of salinity on leaf Na^+ and K^+ concentration in four contrasting bread wheat genotypes (means ± SE; n = 4).

less at 8 dS m^{-1} salinity level, but increased significantly at higher salinity level (15 dS m^{-1}). The results show that salt tolerant genotypes were least affected at vegetative stage with increasing salinity as the decrease in plant height was 9% in Kharchia-65, 8% in SARC-3 and 9% in S-9476 at 15 dS m^{-1} compared with control. While the reduction in the number of tillers and number of leaves was 17 and 59% for Kharchia-65, 16 and 61% for SARC-3 and 12 and 67% for S-9476, respectively at higher salinity (15 dS m^{-1}). However, the decrease in salt sensitive genotype (S-8189) in terms of number of tillers and number of leaves was 67 and 70%, respectively at same salinity level.

Effect on plant biomass and grain yield

The decrease in biomass increased with salinity treatments and variations were found among genotypes (Figure 6). The reduction in biomass at 15 dS m^{-1} in salt

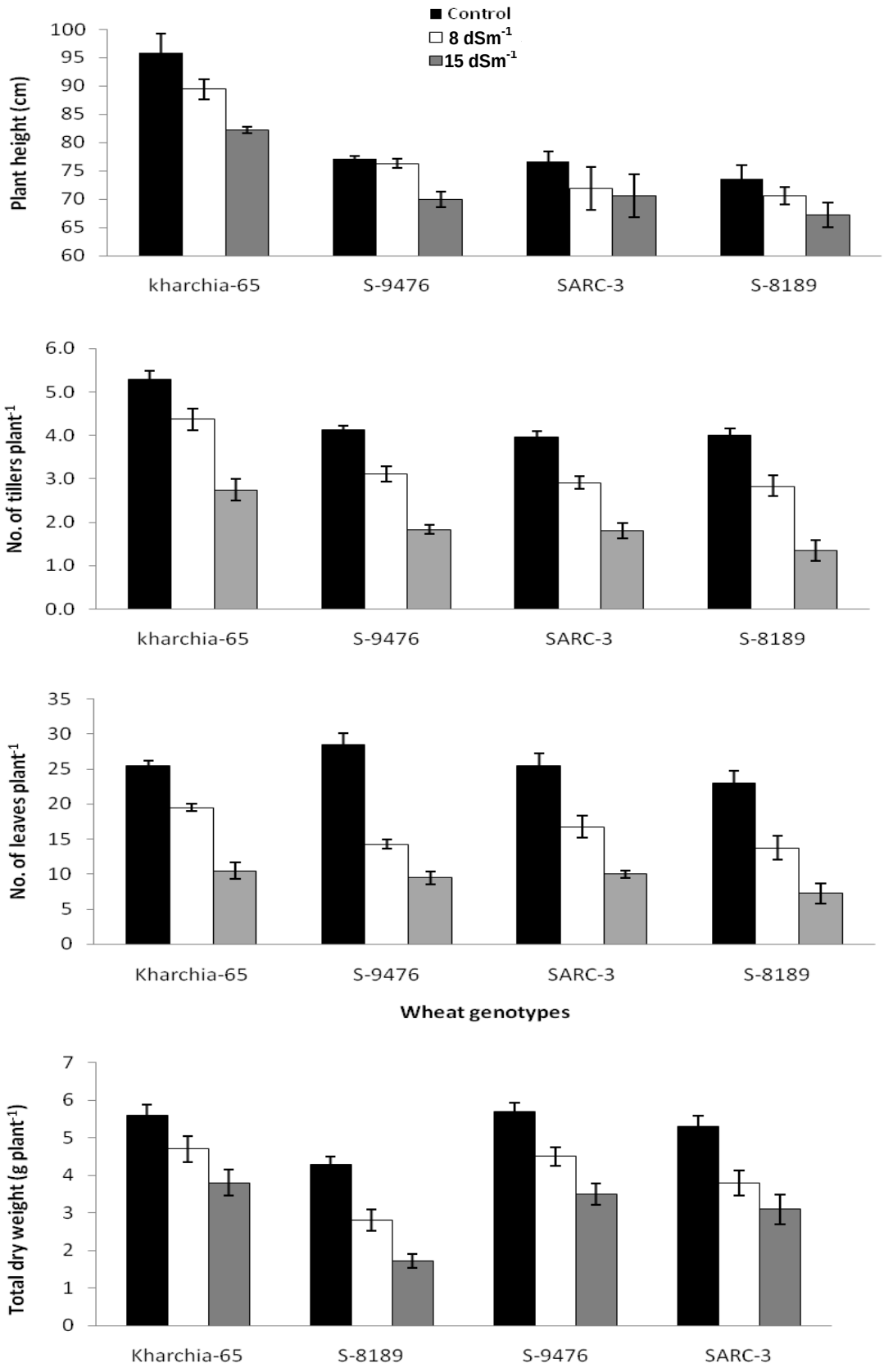

Figure 5. Effect of different salinity treatments on plant height, number of tillers, no. of leaves plant^{-1} and dry weight of four contrasting bread wheat genotypes (means ± SE; n = 4).

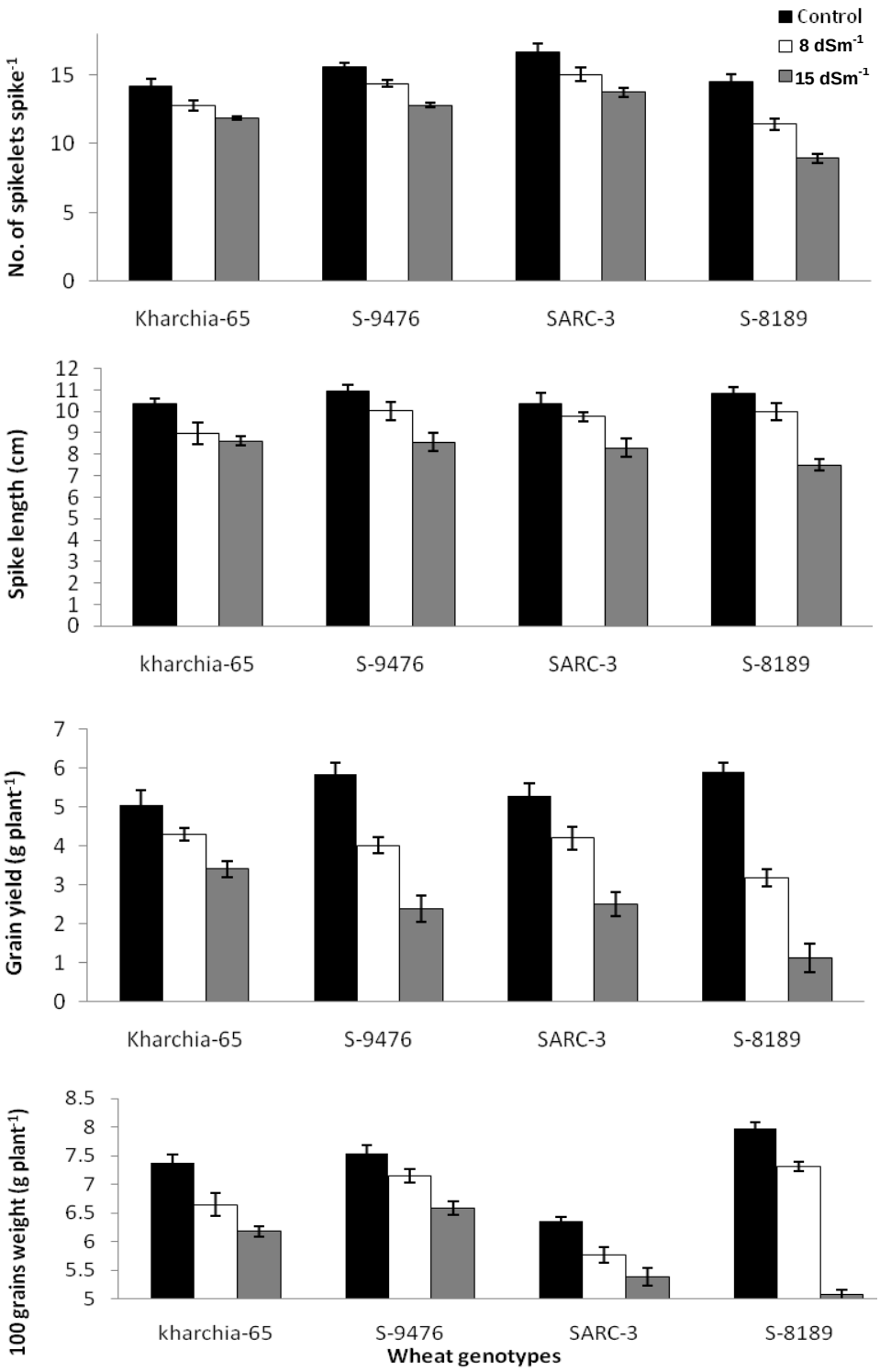

Figure 6. Effect of different salinity treatments on number of spikelets per spike, spike length, grain yield plant^{-1} and 100 grain weight of four contrasting bread wheat genotypes (means ± SE; n = 4).

tolerant genotypes Kharchia-65, S-9476 and SARC-3 was 33, 40 and 42%, respectively when compared with the control, while magnitude of this reduction was 60% for salt sensitive genotype (S-8189).

Furthermore, the grain yield recorded at maturity also reduced significantly with increase in salinity and it was more conspicuous at 15 dS m^{-1} with average of 48% on overall basis in salt-tolerant genotypes, while it was 81%

in the salt sensitive genotype. Similarly, numbers of spikelets per plant and spike length were reduced up to 18 and 16% in salt tolerant genotypes (Kharchia-65, SARC-3 and S-9476) and 19 and 18% in salt sensitive wheat genotype (S-8189), respectively at 15 dS m^{-1} salinity.

DISCUSSION

The results regarding chlorophyll index (SPAD value) did not show any consistent differences due to salinity and among genotypes. For instance, at lower salinity, chlorophyll increased with salinity in salt-tolerant genotypes. However, at higher salinity significant reduction was found among genotypes and contrasting behavior was observed between salt sensitive and tolerant genotypes. Salinity stress also affected the initial growth, plant biomass and other physiological determinants resulting to a significant reduction in grain yield. It is well known that dry mass of plants is reduced in proportion to the increase in salinity (Romero-Aranda et al., 2001). Significant reduction in fresh and dry biomass and varied response of genotypes and growth parameters with increasing salts was observed in *Brassica* (Ashraf, 2001). In addition, genotypic differences in dry matter production and partitioning under stress was also found in wheat (Houshmand et al., 2005) and has been suggested as an indicator of tolerance to salinity stress. The lower shoot dry mass was observed in salt sensitive genotype at both levels of salinity. The reduction in total biomass in the sensitive genotype was probably due to the extra energy utilization for osmotic accumulation, which is much more ATP consuming for osmotic adjustment (Wyn Jones and Gorham, 1983). The reduction in shoot fresh biomass and other physiological processes could cause ultimate reduction in grain yield (Asch et al., 2000) and enhanced accumulation of toxic ions into the shoot (Munns, 2002) as high shoot biomass in plants may be functionally associated with the need of salt stressed plants to restrict the uptake of toxic ions to the shoot, while still maintaining high turgor and a positive growth rate (De Pascale et al., 2003).

Salt increase to toxic levels in the leaves has been a major cause of growth reduction in crops (Munns, 1993), especially in the genotypes that cannot exclude toxic ions from the shoots. The genotypic variations for salt tolerance have been found to be associated with exclusion of Na^+ ion from the shoots in the study as described by Greenway and Munns (1980). The results show that the salt-tolerant genotypes had the lowest Na^+ contents in leaves and this indicates that these genotypes had the ability to exclude toxic ions from the shoots, thus contributing to their salt tolerance (Schachtman and Munns, 1992; Munns et al., 2006).

The salt-sensitive genotype (S-8189) had high Na^+ contents in leaves as compared to Kharchia-65 and SARC-3, but no significant difference was found between salt tolerant genotype S-9476 and the salt genotype S-8189. Furthermore, the salt tolerant genotype S-9476 was characterized by its grain yield, number of tillers and other physiological traits including K contents. This indicates that Na^+ exclusion did not always confer salt tolerance to all wheat genotypes and only high Na^+ contents could not be proper criteria for screening salt tolerance potential in wheat. Similarly, in rice (Yeo et al., 1990), maize (Cramer et al., 1994) and cotton (Leidi and Saiz, 1997), salt tolerance of some genotypes does not correlate with the leaf Na^+ content. This phenomenon was discussed in detail by Shabala and Cuin (2007) and Chen et al. (2008).

Furthermore, Na^+ contents in leaves correlated significantly with RGR in salt-sensitive genotypes (r^2 = 0.84; Figure not shown), while in salt-tolerant genotypes (r^2 = 0.52), it did not. Therefore, it can be interpreted that the reduction in growth can be attributed to high accumulation of Na^+ ion, although ion contents in plant do not always have a close association with salt tolerance of wheat genotypes. However, when looking into contents of both inorganic ions (K^+ & Na^+) in these two genotypes, we found a difference; high K contents in S-9476, which showed that it had the ability to single out K^+ against Na^+ as compared to very low K contents in leaf of salt sensitive genotype (S-8189). For example, at high salinity treatment, K^+ contents in leaves were less by 24% in S-9476 as compared to 59% in the salt-sensitive genotype (S-8189). This ability of retaining higher contents of K^+ in salt-tolerant genotypes may have been one of the factors for their superiority under salinity stress and might play an imperative role for difference among genotypes as described by Shabala and Cuin (2007). It not only directly influence K^+: Na^+ ratio (Cramer, 2002), but also essential physiological processes could be affected by reduction in K^+ the plant cell (Marshner, 1995). For example, at the cellular and whole plant level, K^+ is involved in the maintenance of tissue rigidity, leaf stomatal movement, turgor maintenance and osmoregulation and in the conservation of membrane integrity (Rengel, 1992). It is also an important factor in influencing K^+: Na^+ ratio (Cramer, 2002). Thus, Na^+ contents in leaves could not be enough for accurate prediction of salt tolerance among genotypes. The differences in salt tolerance among genotypes were therefore significantly associated with both Na^+ and K^+ ions in plant.

Salt stress affected the yield and yield related traits drastically and caused decline in plant height, number of leaves and tillers, spike numbers and length, shoot biomass and grain yield in all four wheat genotypes. The effect of salinity at vegetative state was significant among genotypes in term of plant height, number of tillers and leaves. The differences in salt tolerance among genotypes was observed and the higher salt tolerance was due to the production of more tillers, higher number of

leaves, number of spikelets per plant and grain yield. On the other hand, spike length, plant height and 100 grain weight were less affected by salinity. The salt tolerant genotypes had less reduction in number of tiller (19 to 23 and 45 to 58%) than salt sensitive one (30 and 67%) at 8 and 15 dS m^{-1} salinity treatments, respectively when compared with the control. Such differences in salt tolerance among species or cultivars were previously described by plant scientists Romero-Aranda et al. (2001) and El-Hendawy et al. (2005). Tillers formation is inhibited by salt stress during tillering emergence and can cause their abortion at later stages (Nicolas et al., 1994). The results showed that number of tillers was the sensitive yield component in wheat plant. The reduction in primary tillers at higher salinity was found higher for salt sensitive wheat genotypes. The present study therefore confirmed that two yield related traits; the number of spikelets per spike and number of tillers per plant are the most responsive and sensitive determinants to confer salt tolerance in wheat. Zeng and Shannon (2000) also indicated that yield traits like spikelets per panicle and grain weight are sensitive to salt stress and growth stage in rice. Our results suggest that these two parameters can be used in breeding for salt tolerant wheat programs and can also be used as simple and non-destructive criterion of screening and identification of salt tolerance among wheat genotypes, especially in field, as evaluation can be done at vegetative stage.

Thus, it might be possible to improve the salt tolerance of genotypes by increasing tillering ability and/or by using other management practices to alleviate salts stress at early growth stages. Therefore, these characters of salt tolerance could be selected as desirable traits for a cross breeding programs. In addition, locally selected salt tolerant genotypes through screening (SARC-3 and S-9476) exhibited almost similar salt tolerance characters when compared with internationally established Indian salt tolerant wheat genotype (Kharchia-65).

Conclusion

Soil salinity can greatly decrease the number and productivity spike-hearing tillers. However, grain yield in wheat is highly dependent upon the number of spike-hearing tillers produced by each plant. Hence, knowing the contribution of number of tillers and spikes are essential for breeding and developing salt-tolerant wheat. It was identified that wheat genotypes (SARC-3 and S-9476) are salt tolerant genotypes when tested with Kharchia-65 (reference salt tolerant). Therefore, these could be very valuable breeding stuff, if utilized for appropriate selection and breeding programs for further improvement in salt tolerance of Pakistani wheat genotypes. Also, S-9476 could be a trend setting example as originally it was bred for higher yield, but identified as salt tolerant through a screening programme. The variation for salt sensitivity at different growth stages among genotypes could also be used for developing agronomic strategies according to the plant salt tolerance at different growth stages for better production on saline soils.

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