

## Growth, ion content, gas exchange, and water relations of wheat genotypes differing in salt tolerances

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**Abstract.** Although the mechanisms of salt tolerance in plants have received much attention for many years, genotypic differences influencing salt tolerance still remain uncertain. To investigate the key physiological factors associated with genotypic differences in salt tolerance of wheat and their relationship to salt stress, 13 wheat genotypes from Egypt, Australia, India, and Germany, that differ in their salt tolerances, were grown in a greenhouse in soils of 4 different salinity levels (control, 50, 100, and 150 mM NaCl). Relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), photosynthesis, chlorophyll content (SPAD value), and leaf water relations were measured at Days 45 and 60 after sowing. Mineral nutrient content in leaves and stems was determined at Day 45 and final harvest. Salinity reduced RGR, NAR, photosynthetic rate, stomatal conductance, water and osmotic potentials, and  $K^+$  and  $Ca^{2+}$  content in stems and leaves at all times, whereas it increased leaf respiration, and  $Na^+$  and  $Cl^-$  content in leaves and stems. LAR was not affected by salinity and the effect of salinity on SPAD value was genotype-dependent. Growth of salt-tolerant genotypes (Sakha 8, Sakha 93, and Kharchia) was affected by salinity primarily due to a decline in photosynthetic capacity rather than a reduction in leaf area, whereas NAR was the more important factor in determining RGR of moderately tolerant and salt-sensitive genotypes. We conclude that  $Na^+$  and  $Cl^-$  exclusion did not always reflect the salt tolerance, whereas  $K^+$  in the leaves and  $Ca^{2+}$  in the leaves and stems were closely associated with genotypic differences in salt tolerance among the 13 genotypes even at Day 45. Calcium content showed a greater difference in salt tolerance among the genotypes than did  $K^+$  content. The genotypic variation in salt tolerance was also observed for the parameters involved in photosynthesis, and water and osmotic potentials, but not for turgor pressure.

**Additional keywords:** mineral elements, photosynthesis, plant growth, salinity, salt tolerance.

### Introduction

Salinity limits plant production in nearly 40% of agricultural lands worldwide (Gorham 1992). However, a rapid increase in demand for food production is inevitable due to the world population rising to 8.5 billion by the year 2025 (Ghassemi *et al.* 1995). Therefore, there is a need to have salt-tolerant crop genotypes in saline lands for proper cultivation to meet this increasing demand. Achieving this goal by breeding requires a better understanding of the role of physiological parameters in the salt tolerance of different genotypes so that the traits leading to salt tolerance can be introduced in the new genotypes.

A key parameter is growth rate. Under saline conditions, the relative growth rate of plants (RGR) has been considered to allow more appropriate comparisons of growth among species or genotypes than absolute growth rate (Cramer *et al.* 1994). The RGR is a function of the net assimilation rate

(NAR), which is an index of the photosynthetic-assimilatory capacity of the plant per unit leaf area, and of the leaf-area ratio (LAR), which is an index of the leafiness of the plant (Hunt 1990). At the level of the whole plant, therefore, these growth parameters may make it possible to clarify whether genotypic variation in salt tolerance can be attributed to morphological changes or photosynthetic response (Ishikawa *et al.* 1991).

Salinity inhibits plant growth mainly by water deficit, ion toxicity, and ion imbalance (Greenway and Munns 1980). In wheat, genotypic variation in salt tolerance has been found to be associated with low rates of  $Na^+$  uptake and transport, and high selectivity for  $K^+$  or  $Ca^{2+}$  over  $Na^+$  (Schachtman and Munns 1992; Marschner 1995), whereas there is little genotypic variation in rates of  $Cl^-$  uptake and transport (Gorham *et al.* 1990). By contrast, a negative correlation between salt tolerance and  $Na^+$  exclusion has been found in

alfalfa (Ashraf *et al.* 1986), maize (Cramer *et al.* 1994), lentil (Ashraf and Waheed 1993), cotton (Leidi and Saiz 1997), and rice (Yeo and Flowers 1983). In saline soils, salinity causes not only high  $\text{Na}^+$  and  $\text{Cl}^-$  accumulation in plants, but it can also influence the uptake of essential nutrients such as  $\text{K}^+$  and  $\text{Ca}^{2+}$  due to the effect of ion selectivity (Marschner 1995). The reduced  $\text{K}^+$  and  $\text{Ca}^{2+}$  in plants, in turn, affect the integrity and functioning of the cell membranes under saline conditions, which has been suggested to be an important selection criterion for salt tolerance (Gorham *et al.* 1987). However, in wheat, variation in ion selectivity (e.g.  $\text{K}^+$ ) among genotypes was only considered to be a secondary result of genetic variation in  $\text{Na}^+$  uptake (Munns and James 2003). Therefore, it is necessary to study the role of tissue ion content in salt tolerance of plants to identify whether exclusion of  $\text{Na}^+$  or  $\text{Cl}^-$ , or selectivity of  $\text{K}^+$  or  $\text{Ca}^{2+}$  is the more important trait for salt tolerance of plants, and of wheat in particular.

The sensitivity of photosynthesis to salinity in different genotypes is also of interest (Heuer and Plaut 1989), given that photosynthesis is a major factor in the determination of growth. A close association was previously found between growth and photosynthetic rate in 6 *Brassica* species that differed in their salt tolerances (Ashraf 2001). Similarly, in wheat, James *et al.* (2002) found that differences in the rate of photosynthesis likely accounted for genotypic variation in dry matter production. By contrast, other studies found little or no association between growth and rate of photosynthesis in species such as *Hibiscus cannabinus* (Curtis and Läuchli 1986), *Trifolium repens* (Rogers and Noble 1992), and *Triticum aestivum* (Hawkins and Lewis 1993). Any reductions in the rate of photosynthesis by salinity could also be due to lower stomatal conductance ( $g_s$ ) (Seemann and Critchley 1985). Therefore, genotypic differences in  $g_s$  under salinity are also of interest. For instance, Rivelli *et al.* (2002) observed that  $g_s$  of a low- $\text{Na}^+$  durum landrace was reduced to a greater extent than that of a high- $\text{Na}^+$  durum landrace when plants were grown in a short-term experiment at 150 mM NaCl. Thus, we hypothesise that genotypic differences in salt tolerance may also be associated with differential responses of the photosynthetic parameters of the plants.

The presence of salt in soil solution decreases the osmotic potential of soil, thereby resulting in water stress and making it difficult for the plant to absorb water necessary for growth. As such, leaf water potential is also decreased (Munns 1993), although this decrease is accompanied by a decrease in leaf osmotic potential so as to maintain the leaf turgor pressure of the salinised plant (Tattini *et al.* 1995). Leaf water potential and leaf osmotic potential were always observed to be less negative in salt-tolerant genotypes of sorghum than in salt-sensitive forms (Serraj and Sinclair 2002). Generally, plants are able to tolerate salinity by reducing leaf osmotic potential via either the synthesis of organic solutes or the

accumulation of inorganic ions (Hasegawa *et al.* 2000). Therefore, genotypic differences in salt tolerance may also reflect the importance of the leaf water relations of the plant.

It is clear from the preceding that much fragmentary information exists with regard to the physiological determinants of salt tolerance in plants, especially with respect to their association with genotypic differences. Therefore, the objective of this study was to provide a more comprehensive understanding of the role of numerous physiological parameters in determining the salt tolerance among 13 wheat genotypes grown in soil under saline conditions within the same experiment.

## Materials and methods

### Plant material

Thirteen varieties of spring wheat (*Triticum aestivum* L.) from different countries were used in this study. Eight varieties (Sakha 8, Sakha 93, Sakha 61, Sakha 69, Giza 168, Sids 1, Sahel 1, and Gemmeza 7) were obtained from the Agricultural Research Centre, Giza, Egypt. Sakha 8 and Sakha 93 are usually cultivated in saline areas in Egypt. Additionally, Thasos and Triso were obtained from Germany, Westonia and Drysdale from Australia, and Kharchia was from India. Kharchia is the most salt-tolerant of all wheat genotypes, and is used as a standard for salt tolerance tests of wheat worldwide.

### Growth conditions

This study was carried out in the greenhouse from the middle of March to the middle of August 2002. The air temperature ranged from 23 to 28°C in the daytime and from 15 to 18°C at night. Relative humidity fluctuated between 45 and 85% between day and night.

Loamy soil was collected from the soil surface (0–15 cm). The soil was air-dried, ground, passed through a 5-mm mesh screen, and thoroughly mixed. The soil consisted of 23% clay, 48% silt, and 29% sand, and the organic matter content was 1.66%. The air-dried soil, which had a gravimetric water content of 9%, was filled layer-wise in 4 layers in 7-L pots.

Four salt levels (control (no added NaCl), 50, 100, and 150 mM NaCl) in the soil were applied. The final water content (25% on dry soil basis) was achieved by adding tap water or salt solution (50, 100, and 150 mM NaCl) to each layer. To avoid an osmotic shock for seedling emergence, however, the topmost soil layer was not salinised until 10 days after sowing. Twenty-five seeds were sown in each pot. One week after sowing, the seedlings were thinned to 20 per pot.

Nitrogen, and P and K were initially applied as 0.2 g  $\text{NH}_4\text{NO}_3$  and as 0.2 g  $\text{KH}_2\text{PO}_4$  per pot, respectively. The same amounts of N, P, and K were applied another 3 times at 20, 40, and 60 days after sowing. During the experiment, the pots were weighed daily and the water loss was replaced by adding tap water as needed. All treatments were replicated 4 times.

Three plants at 45 days after sowing and 5 plants at grain maturity were randomly sampled from each pot. Plants were harvested and separated into leaves and stems. Samples were dried at 65°C for 48 h. Dried samples were stored for ion analysis.

### Growth analysis

Three plants at 45 and 60 days after sowing were randomly sampled from each pot. Plants were harvested and separated into leaves and stems. Leaf area was measured using a LI-3000 Area Metre (LI-COR, Walz Co., OR, USA). After the leaf area was determined, the samples were dried at 65°C for 48 h and then their dry weights were determined.

RGR (g/g.day), NAR (g/m<sup>2</sup>.day), and LAR (m<sup>2</sup>/g) were derived using the following equations (Hunt 1990):

$$\text{RGR} = \frac{1}{W} \times \frac{\partial W}{\partial T} \quad (1)$$

$$\text{NAR} = \frac{1}{L_A} \times \frac{\partial W}{\partial T} \quad (2)$$

$$\text{LAR} = \frac{L_A}{W} \quad (3)$$

where  $W$ ,  $T$ , and  $L_A$  are plant dry weight (g), time (day), and leaf area (m<sup>2</sup>), respectively.

#### Analysis of ion concentrations

Oven-dried samples of leaves and stems of plants at 45 days after sowing and at final harvest were ground into a fine powder by passing them through a 0.5-mm-diam. sieve. For the determination of Na<sup>+</sup>, K<sup>+</sup>, and Ca<sup>2+</sup> content, 300 mg of ground dry material of the stems or leaves was digested by adding 3 mL concentrated HNO<sub>3</sub> (65%) and 2 mL H<sub>2</sub>O<sub>2</sub> (30%) for 30 min at 2600 kPa (80 psi) in a MDS-2100 microwave oven (CEM Corp., Matthews, NC, USA). After digestion, each sample was brought up to a 50-mL final volume with distilled-deionised water. The concentration of Na<sup>+</sup> was determined with an Inductively Coupled Plasma Emission Spectrometer (ICP model Liberty 200, Varian Australia Pty Ltd, Mulgrave, Vic., Australia). The K<sup>+</sup> and Ca<sup>2+</sup> contents were determined with a flame photometer (ELEX 6361, Eppendorf, Netheler-Hinz GmbH., Germany).

For Cl<sup>-</sup>, 100 mg of ground sample was extracted with 100 mL distilled water and was shaken for 1 h and then filtered. Chloride was determined using an ion chromatography analyser (Model LC20-1, Dionex, Sunnyvale, CA 94086, USA).

#### Photosynthetic parameters

Photosynthetic rate ( $A$ ), stomatal conductance ( $g_s$ ), respiration rate ( $R$ ), and transpiration rate ( $E$ ) were determined on the second-youngest leaf that was fully expanded at 45 and 60 days after sowing. Measurements were made with a LI-COR 6400 portable gas exchange system (Analytical Development Co., England). Because the leaf did not fill the leaf chamber, the leaf area was determined independently and photosynthetic parameters were estimated with a re-computation program (LI-COR, Lincoln, NE, USA). Measurements were conducted in a growth chamber during the light period. Plants were transferred into the growth chamber (with an air temperature of 25°C, a photosynthetic photon flux density of 750 μmol/m<sup>2</sup>.s, and a CO<sub>2</sub> level of 400 μmol/mol) 1 day before the measurements were performed.

#### Leaf chlorophyll measurement

Leaf chlorophyll content was determined using a hand-held SPAD 502 m (Minolta, Osaka, Japan). Average SPAD chlorophyll readings were calculated from 5 measurements from the leaf tip to the leaf base. The measurements were made at 45 and 60 days after sowing.

#### Water relation measurements

Leaf water potential ( $\Psi$ ) and osmotic potential ( $\Psi_\pi$ ) from the middle of the second-youngest leaf with a fully developed blade were measured 2 times each at 45 and 60 days after sowing.  $\Psi$  was measured with a pressure bomb (PMS Instrument Co., model 1002, Corvallis Co., OR, USA) according to the technique of Scholander *et al.* (1965). Immediately after  $\Psi$  was determined, the leaf material was frozen in dry ice. The leaf samples were then thawed at room temperature, placed in a syringe, and the leaf sap was expressed under pressure;  $\Psi_\pi$  was then

determined with a vapour pressure osmometer (Wescor 5100C, Wescor Inc., Logan, USA). Turgor pressure ( $T_p$ ) was estimated as the difference between  $\Psi_\pi$  and  $\Psi$ .

#### Statistical analysis of data

A factorial experimental design with 13 genotypes and 4 salinity levels was arranged in a completely randomised design with 4 replications. Data were analysed by ANOVAs using COSTAT Version 3.03 (software, Berkeley, CA 94701, USA). Relationships between the scores of grain yield and the scores of different physiological parameters were analysed by simple linear regression using JMP (SAS Institute 2000).

## Results

The 13 genotypes used in this study were classified into 3 groups: salt-tolerant (Kharchia, Sakha 93, and Sakha 8), moderately tolerant (Drysdale and Sakha 69), and salt-sensitive genotypes (Westonia, Giza 168, Sakha 61, Gemmeza 7, Sids 1, Thasos, Triso, and Sahel 1) based on the rankings of these genotypes in terms of grain yield and agronomic parameters (El-Hendawy *et al.* 2004). Thus, in Figs 1–5, the salt tolerance of the genotypes increases in going to the right.

#### Genotypic variation in growth

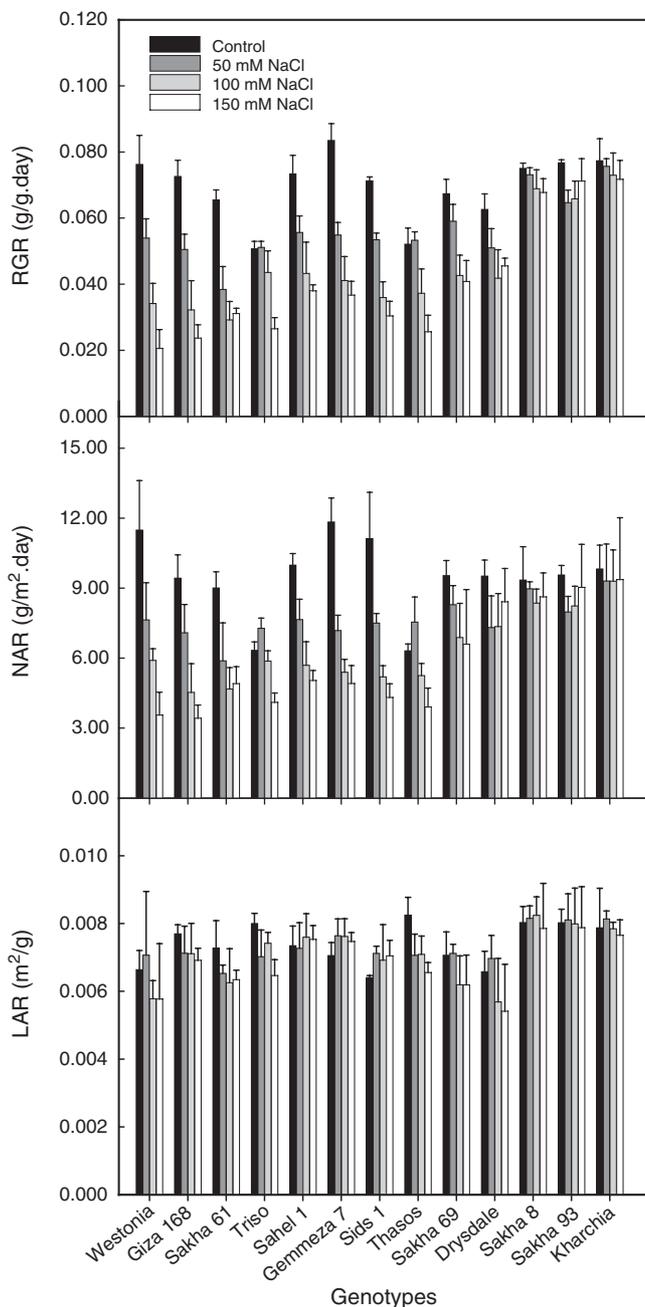
RGR and NAR decreased significantly with increasing salinity (Fig. 1). Both parameters were reduced by about 20% at 50 mM NaCl, 37% at 100 mM NaCl, and 43% at 150 mM NaCl as compared with the control. However, there was no significant effect of salinity on LAR (Fig. 1).

At a given salinity level, both RGR and NAR increased with the increasing salt tolerance of the wheat genotype (Fig. 1). For example, RGR and NAR for salt-tolerant, moderately tolerant, and salt-sensitive groups were decreased by an average of 8, 17, and 21% at 50 mM NaCl; 10, 30, and 42% at 100 mM NaCl; and 7, 27, and 55% at 150 mM NaCl, respectively; compared with the control. However, no genotypic variation was observed for LAR (Fig. 1).

#### Genotypic variation in ion content

Sodium and Cl<sup>-</sup> contents in the leaves and stems were increased at both Day 45 and final harvest with increasing salinity (Fig. 2). For example, compared with the control, Na<sup>+</sup> content in the leaves and stems was increased by about 2-, 4-, and 7-fold at Day 45, and by 8-, 18-, and 39-fold at final harvest at 50, 100, and 150 mM NaCl, respectively. The analogous values for Cl<sup>-</sup> were about 9-, 12-, and 14-fold at Day 45, and 17-, 21-, and 28-fold at final harvest, respectively.

Differences among the 3 genotypic groups were greater for Na<sup>+</sup> and Cl<sup>-</sup> content in the leaves than in the stems, and also at final harvest than at Day 45 (Fig. 2). For example, at 150 mM NaCl, Na<sup>+</sup> and Cl<sup>-</sup> contents in the leaves of the salt-tolerant group were increased by an average of 4- and 11-fold at Day 45, and 26- and 16-fold at final harvest, respectively, as compared with the control. However, Na<sup>+</sup> and Cl<sup>-</sup> contents in the leaves of the salt-sensitive group were increased by an



**Fig. 1.** Effect of different salinity levels on RGR, NAR, and LAR of different wheat genotypes 45 and 60 days after sowing. Error bars, which fit within the plot symbol if not shown, represent standard deviations.

average of 9- and 18-fold at Day 45, and 48- and 36-fold at final harvest, respectively. The contents of  $\text{Na}^+$  and  $\text{Cl}^-$  in the stems were increased by an average of 4- and 10-fold at Day 45, and 31- and 29-fold at final harvest for the salt-tolerant group, and by 9- and 13-fold at Day 45, and 47- and 29-fold at final harvest for the salt-sensitive group, respectively, when comparing 150 mM NaCl with the control. The patterns of  $\text{Na}^+$  and  $\text{Cl}^-$  contents in

the leaves and stems for the moderately tolerant group were much more similar to those of the salt-tolerant group than to those of the salt-sensitive group (Fig. 2). Surprisingly, Westonia displayed a low  $\text{Na}^+$  content even though it was the most sensitive genotype examined. In contrast to  $\text{Na}^+$  content, however, Westonia had a higher  $\text{Cl}^-$  content in the leaves than did genotypes in the salt-tolerant group.

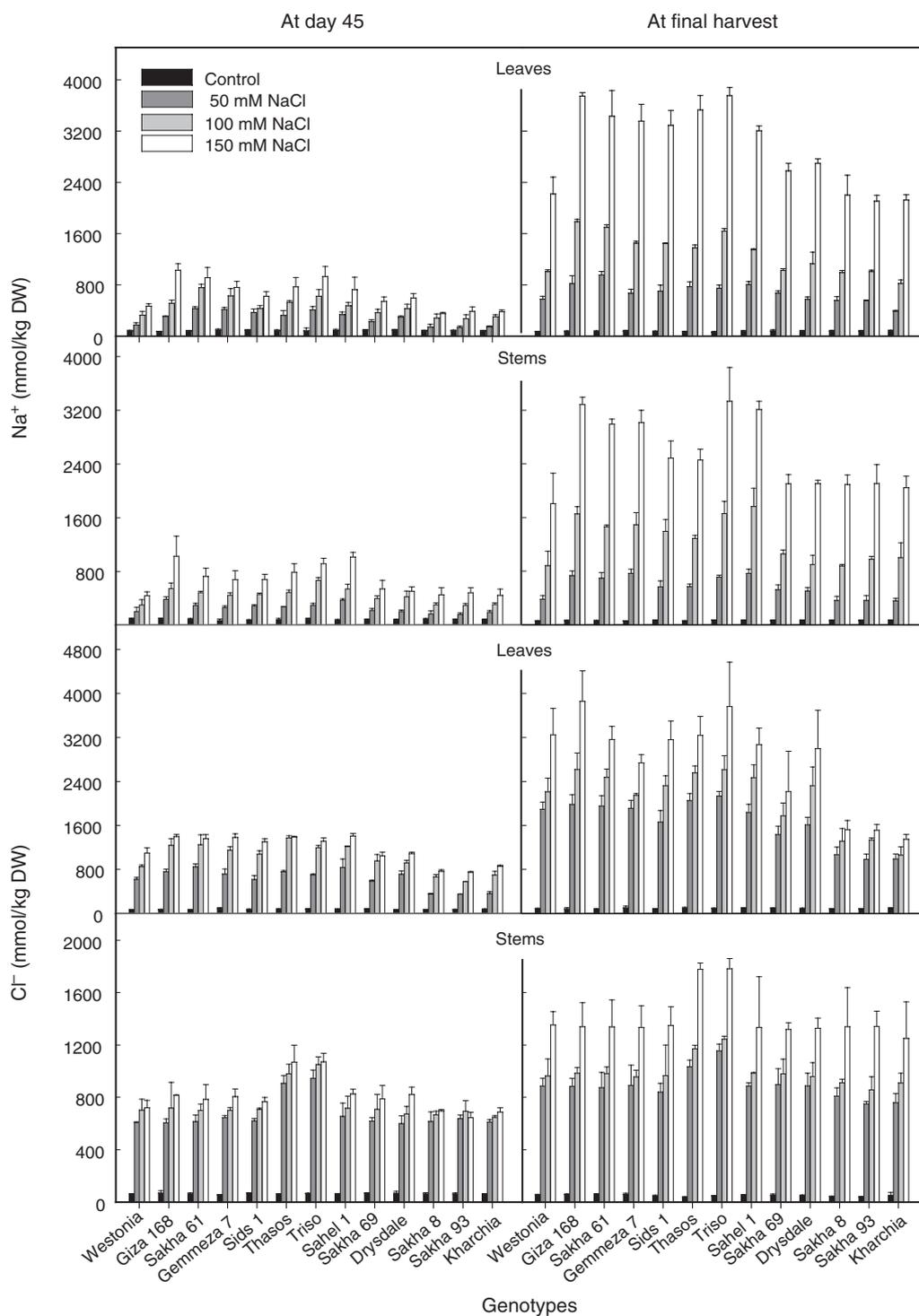
Salinity reduced  $\text{K}^+$  and especially  $\text{Ca}^{2+}$  content in the leaves and stems. Furthermore, the decrease in  $\text{K}^+$  content in the leaves was greater than that in stems, whereas the decrease in  $\text{Ca}^{2+}$  content in the leaves and stems was similar. The decrease in  $\text{K}^+$  and  $\text{Ca}^{2+}$  content in the leaves and stems was similar at both harvest times (Fig. 3).

Fig. 3 shows obvious differences among the 3 genotypic groups in  $\text{K}^+$  and  $\text{Ca}^{2+}$  content in the leaves. The salt-tolerant group had higher  $\text{K}^+$  and  $\text{Ca}^{2+}$  contents in the leaves than did the moderately tolerant and salt-sensitive groups. For example, at moderate and high salinity levels,  $\text{K}^+$  and  $\text{Ca}^{2+}$  contents in the leaves for the salt-tolerant genotypes were about 19 and 32% higher at Day 45 and 15 and 19% higher at final harvest than the values in the moderately tolerant genotypes, and about 36 and 49% higher at Day 45 and 42 and 55% higher at final harvest than the values in the salt-sensitive genotypes. Compared with the genotypic variation in  $\text{Na}^+$  and  $\text{K}^+$  content in the stems, the  $\text{Ca}^{2+}$  content in the stems demonstrated a greater genotypic difference. At the moderate and high salinity levels,  $\text{Ca}^{2+}$  content in the stems for the salt-tolerant group was about 54 and 69% higher at Day 45 and 44 and 57% higher at final harvest than the values in the moderately tolerant and salt-sensitive groups, respectively (Fig. 3).

#### Genotypic variation in photosynthetic parameters

Data of photosynthetic rate ( $A$ ), stomatal conductance ( $g_s$ ), and respiration rate ( $R$ ) at Day 45 after sowing are presented in Fig. 4. However, the results at Day 60 were only given in the text. Compared with the control,  $A$  at 50, 100, and 150 mM NaCl was reduced by 15, 23, and 28% at Day 45 and by 19, 37, and 40% at Day 60, respectively, whereas  $g_s$  was reduced by 31, 43, and 49% at Day 45 and by 29, 53, and 56% at Day 60, respectively. At both time points,  $R$  was increased by approximately 1.8, 2.1, and 2.6 times at 50, 100, and 150 mM NaCl, respectively, compared with the control.

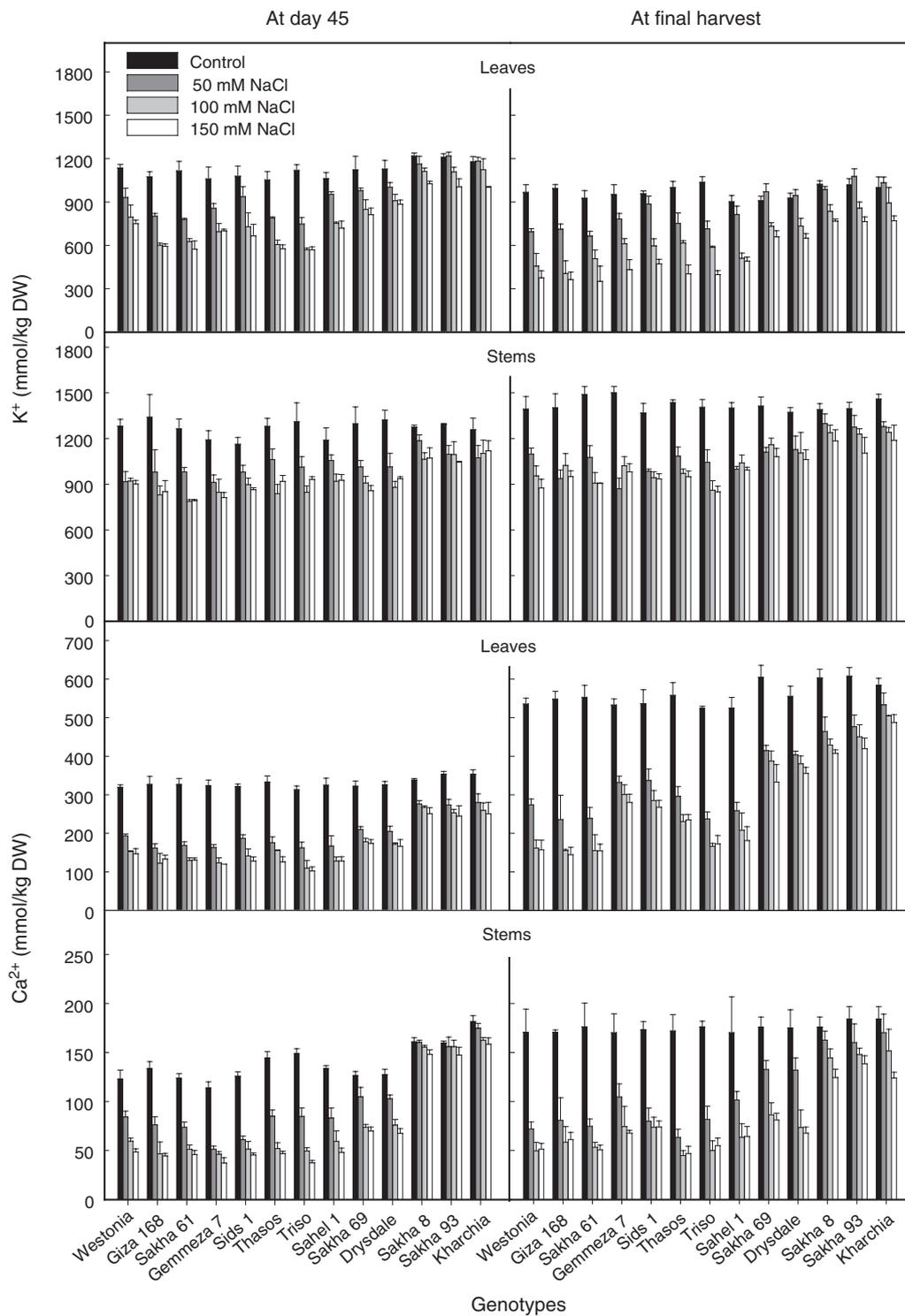
There were also genotypic differences in  $A$  at Day 60, and in  $g_s$  and  $R$  at both measurement times. The photosynthetic rate of the salt-tolerant genotypes at low salinity was increased, but at high salinity it was decreased by about 14% at both times. By contrast,  $A$  of the moderately tolerant and salt-sensitive groups at low salinity decreased by an average of 4 and 28% at Day 45, and by 15 and 28% at Day 60 compared with the control; at high salinity it decreased by an average of 14 and 36% at Day 45 and by 28 and 50% at Day 60, respectively. The reduction in  $g_s$  for all 3 groups



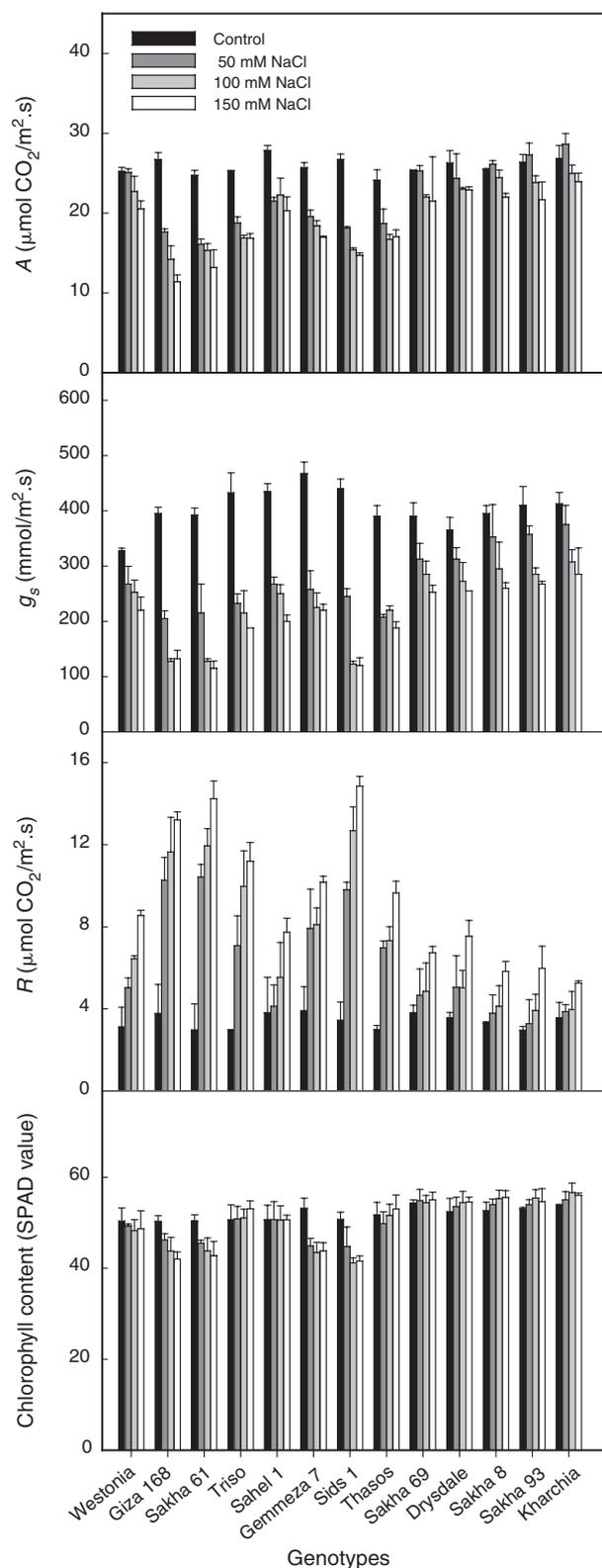
**Fig. 2.** Effect of different salinity levels on sodium and chloride content in the leaves and stems at Day 45 after sowing and at final harvest for different wheat genotypes. Error bars, which fit within the plot symbol if not shown, represent standard deviation.

was greater than that for *A*. At both measurement times, *g<sub>s</sub>* at 150 mM NaCl was reduced by an average of 35% for the salt-tolerant, 40% for the moderately tolerant, and 60% for the salt-sensitive genotypes, as compared with the control. The

increase in *R* for the salt-sensitive genotypes was higher than that in the salt-tolerant and moderately tolerant genotypes. At both measurement times, *R* was reduced by about 1.2 times at low salinity and 1.7 times at high salinity for the salt-tolerant



**Fig. 3.** Effect of different salinity levels on potassium and calcium content in the leaves and stems at Day 45 after sowing and at final harvest for different wheat genotypes. Error bars, which fit within the plot symbol if not shown, represent standard deviation.



**Fig. 4.** Effect of different salinity levels on photosynthetic parameters and chlorophyll content (SPAD value) at 45 days after sowing for different wheat genotypes. Error bars, which fit within the plot symbol if not shown, represent standard deviations.

and moderately tolerant groups as compared with the control, whereas it was decreased by about 2.2 times at low salinity and 3.2 times at high salinity for the salt-sensitive group.

#### Genotypic variation in chlorophyll content (SPAD value)

The effect of salinity on SPAD values varied according to the salt tolerance of the genotypes (data at Day 45 only presented in Fig. 4). SPAD values in the salt-tolerant group increased slightly with salinity at Days 45 and 60, whereas the opposite was found in the salt-sensitive group. For instance, SPAD values decreased by about 6 and 8% at Day 45 and by about 19 and 28% at Day 60 at low and high salinity levels, respectively, as compared with the control. The effect of salinity on SPAD values in the moderately tolerant group also differed with measurement time. It was slightly increased with increasing salinity at Day 45, whereas it was decreased by about 7% at high salinity at Day 60.

#### Genotypic variation in leaf water relations

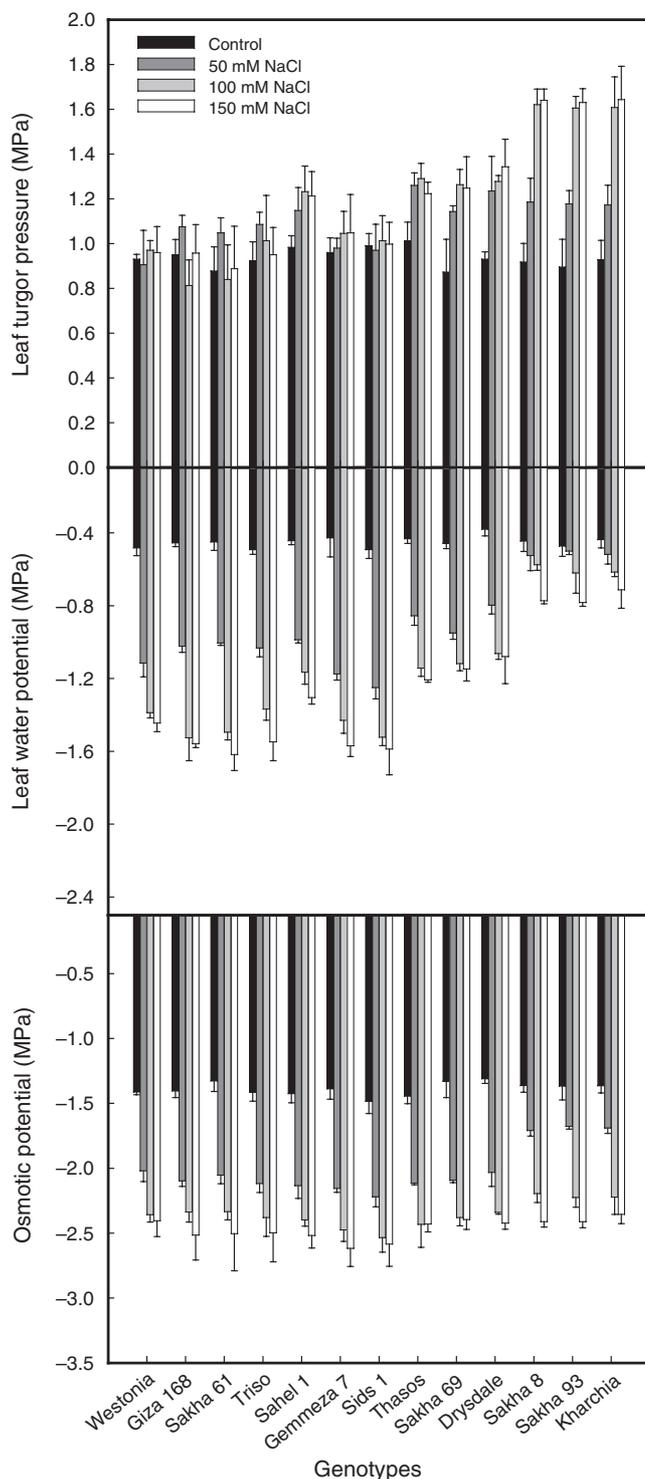
Salinity significantly affected both leaf water potential ( $\Psi$ ) and osmotic potential ( $\Psi_\pi$ ) at Day 45 (Fig. 5). Data at Day 60 are similar to those at Day 45 and are not presented. Leaf  $\Psi$  was decreased by 0.45, 0.71, and 0.81 MPa at Day 45. Leaf  $\Psi_\pi$  was decreased by 0.62, 0.92, and 1.05 MPa at Day 45. Leaf turgor pressure ( $T_p$ ) was increased by 0.20, 0.24, and 0.28 MPa at Day 45 when salinity increased from the control to 50, 100, and 150 mM NaCl, respectively (Fig. 5).

The salt-tolerant genotypes had significantly higher leaf  $\Psi$  compared with other genotypes. A difference in leaf  $\Psi_\pi$  among the 3 groups was observed only at low salinity at Day 45. There was almost no genotypic difference in leaf  $T_p$  regardless of salinity level.

## Discussion

### Role of growth in determining the salt tolerance of wheat genotypes

At the whole-plant level, the decreases observed in RGR could be attributed to a photosynthetic response (NAR) and/or morphological changes (LAR), depending on the genotype (Hunt 1990; Ishikawa *et al.* 1991). The results from the present study demonstrate that the decrease in RGR for the salt-tolerant genotypes Sakha 8, Sakha 93, and Kharchia was related to NAR, but not to LAR (Fig. 1), indicating that the reduced growth in these genotypes under salinity was primarily a result of a decline in the rate of photosynthesis. These results are in agreement with the reports by Cramer *et al.* (1994). A similar trend was found in the salt-sensitive genotypes Sids 1, Sahel 1, and Gemmeza 7. However, the reduction in RGR of both the other salt-sensitive genotypes (Sakha 61, Giza 168, Thasos, Triso, and Westonia) and the moderately tolerant genotypes Sakha 69 and Drysdale was associated with both NAR and



**Fig. 5.** Effect of different salinity levels on leaf water potential ( $\Psi$ ), osmotic potential ( $\Psi_{\pi}$ ) and turgor pressure ( $T_p$ ) at 45 days after sowing for different wheat genotypes. Error bars, which fit within the plot symbol if not shown, represent standard deviations.

LAR (Fig. 1). This suggests that both leaf expansion and photosynthetic rate are the growth-limiting factors in these genotypes (Morales *et al.* 1998).

#### *Role of exclusion of $\text{Na}^+$ and $\text{Cl}^-$ in determining the salt tolerance of wheat genotypes*

The exclusion of harmful ions ( $\text{Na}^+$  and  $\text{Cl}^-$ ) from the shoots has been found to be associated with genotypic variation in salt tolerance (Greenway and Munns 1980). For those genotypes that cannot exclude toxic ions from the shoots, salt builds up to toxic levels in the leaves, becoming the major cause of reduced growth (Munns 1993). The results from the present study found that the salt-tolerant genotypes had among the lowest  $\text{Na}^+$  and  $\text{Cl}^-$  content in the leaves and stems at both Day 45 and final harvest (Fig. 2), suggesting that these genotypes had a better ability to exclude harmful ions from the shoots, which in turn contributed to their salt tolerance. However, this study also showed that the salt-sensitive genotypes did not necessarily have the highest  $\text{Na}^+$  and  $\text{Cl}^-$  content in the plant tissues. For example, Westonia had the lowest  $\text{Na}^+$  content in the leaves and stems at both sampling times regardless of levels of salinity (Fig. 2). However, another mechanism in plants to combat salinity is to sequester the toxic ions  $\text{Na}^+$  or  $\text{Cl}^-$  into vacuoles, which would result in the higher tissue  $\text{Na}^+$  or  $\text{Cl}^-$  content observed in salt-tolerant plants. Munns and James (2003) reported that several salt-tolerant wheat genotypes do indeed demonstrate very high leaf  $\text{Na}^+$  levels. Similarly, other studies have demonstrated that salt tolerance is not necessarily correlated with the content of leaf  $\text{Na}^+$  in several plant species, including rice (Yeo and Flowers 1983), maize (Cramer *et al.* 1994), and cotton (Leidi and Saiz 1997).

The results in Fig. 2 show that there was no difference in  $\text{Cl}^-$  content in the stem between salt-tolerant and salt-sensitive genotypes for both sampling times, which is consistent with the finding of little genotypic variation within the wheat genus *Triticum* in  $\text{Cl}^-$  accumulation by Gorham *et al.* (1990). A review by Ashraf (2004) summarised that glycophytes can use both ion exclusion and inclusion mechanisms in response to saline substrates. The mechanism that is used depends on the pattern of ion distribution between the leaves and on ion compartmentation within the cell (Greenway and Munns 1980; Cheeseman 1988; Munns *et al.* 2002). Given this complexity, it is only with a full understanding of the ion response mechanisms of a particular species that ion content measurements *per se* would serve as selection indicators for salt tolerance (Ashraf 2004).

#### *Role of $\text{K}^+$ and $\text{Ca}^{2+}$ in determining the salt tolerance of wheat genotypes*

In saline soils, salinity not only causes high  $\text{Na}^+$  and  $\text{Cl}^-$  accumulation in plants, it also influences the uptake of essential nutrients such as  $\text{K}^+$  and  $\text{Ca}^{2+}$  through the effects of ion selectivity (Marschner 1995). Therefore, the maintenance of higher  $\text{K}^+$  and  $\text{Ca}^{2+}$  contents in salt-tolerant genotypes may be one of the mechanisms underlying their superior salt tolerance. However, Munns and James (2003) suggested that

variation in ion selectivity (e.g.  $K^+$ ) among genotypes of wheat is probably a secondary result of genetic variation in  $Na^+$  uptake. In the present study, significant genotypic variation in  $K^+$  and  $Ca^{2+}$  contents in the leaves and stems existed compared with the exclusion of  $Na^+$  and  $Cl^-$  (Fig. 2). Both  $K^+$  and  $Ca^{2+}$  contents were highest in the salt-tolerant genotypes and lowest in the salt-sensitive genotypes. For  $K^+$ , the variation among genotypes was greater in the leaves than in the stems for all salinised treatments. Calcium content showed a greater variation among the genotypes than that of  $K^+$ .

We can speculate that the lower  $K^+$  and  $Ca^{2+}$  contents observed in salt-sensitive genotypes may explain their higher sensitivity to salinity, given that under saline conditions, both ions play an important role in essential physiological processes. At the cellular and whole-plant level,  $K^+$  is involved in the maintenance of tissue rigidity, leaf stomatal movement, turgor maintenance, and osmoregulation, and is one of the most prominent inorganic solutes in charge balance, protein synthesis, and homeostasis (Chow *et al.* 1990). Maathuis and Amtmann (1999) emphasised that one of the key elements in salinity tolerance is capacity to maintain a high cytosolic  $K^+/Na^+$  ratio because cytoplasmic  $Na^+$  competes for  $K^+$  binding sites and hence inhibits metabolic processes that crucially depend on  $K^+$ .

Calcium is important in preserving the integrity of the cell membrane during salt stress (Rengel 1992), influencing  $K^+/Na^+$  selectivity (Cramer 2002), and is also used as a secondary messenger in many signal transduction pathways within the cell (Knight 2000). Shabala *et al.* (2003) reported that high  $Ca^{2+}$  caused almost complete recovery of membrane potential in root cells, which may be able to prevent  $K^+$  leakage from the cell. As a result, the  $K^+/Na^+$  ratio will be restored and cell metabolic functions will be preserved. Because high  $Ca^{2+}$  prevented net  $K^+$  efflux and activity of the plasma membrane  $H^+$ -pump, plasma membrane  $K^+$  and  $H^+$  transporters play the key role in the amelioration of negative salt effects by  $Ca^{2+}$  (Shabala 2000). Non-selective cation channels are generally considered to constitute the major pathway for  $Na^+$  influx, which may be inhibited by  $Ca^{2+}$  (Demidchik and Tester 2002; Essah *et al.* 2003). The ameliorative effects of  $Ca^{2+}$  on  $Na^+$  toxicity in plants have been reported since as far back as 1902 (LaHaye and Epstein 1971). In the past 2 decades, however, there has been a very large number of papers published on  $Na^+-Ca^{2+}$  interactions in plants (Cramer 2002). However, because  $Ca^{2+}$  does not always completely ameliorate the inhibition of growth by  $Na^+$  for most plants, and because salinity can disturb normal functions without disturbing overall  $Ca^{2+}$  tissue concentrations, especially in the early growth stages (Cramer 2002),  $Ca^{2+}$  content in the plant has not typically been proposed as a useful trait for the screening of salt tolerance of wheat genotypes. Compared with the traits of  $Na^+$  and  $Cl^-$  exclusion, however, the far greater genotypic

variation that we observed in the  $Ca^{2+}$  content of wheat plants (even at 45 days) in the present study strongly suggests the potential utility of  $Ca^{2+}$  content for the screening of salt tolerance of wheat genotypes. Similarly, it has been found that plant  $Ca^{2+}$  content was highly correlated with relative salt-tolerance of 6 *Brassica* species (He and Cramer 1993a) and also in *Cicer arietinum* (Soussi *et al.* 2001). However, He and Cramer (1993a, 1993b) reported that the salt tolerance of *Brassica napus* was associated with a reduction in  $Ca^{2+}$  content at a cellular level, but not in whole plants.

#### Role of leaf photosynthesis in determining the salt tolerance of wheat genotypes

The reduction in leaf photosynthesis for salinised treatments derives from the integrated effects of salinity on NAR (Hunt 1990; Ishikawa *et al.* 1991) and on the  $Na^+$  and  $Cl^-$  contents in the leaves (James *et al.* 2002), with the  $Na^+$  and  $Cl^-$  contents in the leaves being negatively correlated with the photosynthesis rate (*A*) (Table 1). *A* of salt-tolerant genotypes was slightly affected by increasing salinity, whereas in salt-sensitive genotypes, *A* was lower by about 1.4 times at low salinity and by about 1.6 times at moderate and high salinity (Fig. 4). Tattini *et al.* (1995) reported that, under saline conditions, decreases in *A* and increases in *R* may slow down growth or stop it entirely. In the salt-sensitive genotypes, *R* was about 2.1 times higher at low salinity and about 2.3 times higher at moderate and high salinities than in the salt-tolerant genotypes. In their study of *Xanthium*, Schwarz and Gale (1981) found that 80% of the reduced carbon assimilation could be accounted for by a reduction in *A*, with 20–25% being the result of an increase in *R*. However, the distributions contributing to reduced carbon assimilations probably vary among species and their salt tolerances. Additionally,

**Table 1. Correlation coefficients between photosynthesis rate (*A*) and  $Na^+$  and  $Cl^-$  contents in leaves, and between *A* and stomatal conductance ( $g_s$ ) and chlorophyll content (SPAD value), respectively**

Correlation analysis was performed using the replicates of each treatment with data combined across salt levels

Genotypes	$Na^+$	$Cl^-$	$g_s$	Chl.
Westonia	-0.79***	-0.76***	0.64**	0.06 n.s.
Giza 168	-0.88***	-0.98***	0.96***	0.84***
Sakha 61	-0.88***	-0.94***	0.93***	0.84***
Gemmeza 7	-0.93***	-0.93***	0.96***	-0.19 n.s.
Sids 1	-0.90***	-0.81***	0.90***	0.13 n.s.
Thassos	-0.87***	-0.94***	0.97***	0.89***
Triso	-0.88***	-0.97***	0.98***	0.87***
Sahel 1	-0.77***	-0.93***	0.89***	-0.07 n.s.
Sakha 69	-0.44 n.s.	-0.50*	0.80***	-0.04 n.s.
Drysdale	-0.63**	-0.68**	0.66**	-0.37 n.s.
Sakha 8	-0.40 n.s.	-0.49 n.s.	0.72***	-0.46 n.s.
Sakha 93	-0.48 n.s.	-0.47 n.s.	0.72***	-0.36 n.s.
Kharchia	-0.43 n.s.	-0.42 n.s.	0.69**	-0.33 n.s.

n.s., Not significant; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

Semikhatova *et al.* (1993) found that increased  $R$  derives mainly from the additional energy cost for the salt economy of the cell (i.e. pumping ions from the cytoplasm into the vacuole). Therefore, increases in  $R$  in salt-sensitive genotypes may be related to accumulation of harmful ions in cytoplasm that could reduce the efficiency of RuBP carboxylase and other enzymes that are related to photosynthetic capacity (Seemann and Critchley 1985).

The reduction in  $A$  by salinity can be due to either stomatal or non-stomatal factors (Heuer and Plaut 1989). The data presented here show that salinity significantly decreased  $g_s$  for all genotypes. Furthermore,  $A$  was significantly correlated with  $g_s$  in all genotypes (Table 1). It is also noteworthy that the reduction in  $g_s$  for the salt-tolerant genotypes was greater than that for  $A$ . This indicates that the reduction in  $A$  for these genotypes is largely due to the reduction in  $g_s$ . Similarly, Robinson *et al.* (1983) found that although  $g_s$  of salt-tolerant spinach genotypes was decreased by 350 mM NaCl, the significant decrease in  $A$  was observed. The reduction in  $A$  of the moderately tolerant and salt-sensitive genotypes was associated with a combination of stomatal and non-stomatal factors (Heuer and Plaut 1989). This conclusion is supported by the significant correlation between  $A$  and  $g_s$  and  $\text{Na}^+$  and  $\text{Cl}^-$  contents in the leaves at Day 45 in both genotypes (Table 1).

The reduction in photosynthesis under salinity can also be attributed to a decrease in chlorophyll content (Delfine *et al.* 1999). Here, the results of analyses of chlorophyll content (SPAD value) showed a varying pattern among genotypes. In the salt-tolerant genotypes, chlorophyll content increased with salinity and was not correlated with  $A$  (Fig. 4, Table 1). However, in the salt-sensitive genotypes Sakha 61, Giza 168, Thasos, and Triso, chlorophyll content was decreased by an average of 15% at Day 45 and 33% at Day 60 at moderate and high salinities. In the moderately tolerant genotypes and the salt-sensitive genotypes Gemmeza 7, Sids 1, and Sahel 1, chlorophyll content was decreased by salinity and was significantly associated with  $A$  at Day 60, but not at Day 45. Altogether, this indicates that the responses of chlorophyll content to salt stress depended on differences in salt tolerance among the wheat genotypes. Similar to findings in alfalfa (Winicov and Seemann 1990), sunflower (Ashraf 1999), and cowpea (Murillo-Amador *et al.* 2002), therefore, responses of chlorophyll content in wheat to salinity depended on both salinity level and the degree of salt tolerance of genotypes. In cowpea, for example, Murillo-Amador *et al.* (2002) found that the chlorophyll content of the salt-tolerant genotypes was increased under salinity, whereas in salt-sensitive genotypes, it was different.

#### *Roles of leaf water relations in determining the salt tolerance of wheat genotypes*

Salinity in the growth medium causes a reduction in leaf water potential ( $\Psi$ ), leading to a decline in leaf turgor pressure ( $T_p$ )

of the salinised plant (Tattini *et al.* 1995). A review by Ashraf (2004) summarised that salt-sensitive cultivars had higher leaf turgor than salt-tolerant ones, and also a greater increase in leaf turgor pressure in response to salinity. However, results from the present study did not show genotypic variation in leaf turgor at either of the 2 harvesting times. Genotypic variation was only observed for leaf water potential and for osmotic potential at Day 60, which is in agreement with the findings for sorghum by Serraj and Sinclair (2002).

In conclusion, growth of the salt-tolerant genotypes (Sakha 8, Sakha 93, and Kharchia) was reduced by salinity primarily due to a decline in photosynthetic capacity rather than a reduction in leaf area, whereas NAR was the important factor in determining RGR of the moderately tolerant and salt-sensitive genotypes. Sodium and  $\text{Cl}^-$  exclusion did not always reflect salt tolerance, whereas levels of  $\text{K}^+$  in the leaves and  $\text{Ca}^{2+}$  in the leaves and stems were closely associated with differences in salt tolerance among 13 genotypes even at Day 45. Calcium content showed a greater difference among genotypes than  $\text{K}^+$  content. The genotypic variation in salt tolerance was also observed for the parameters of photosynthesis, and water and osmotic potentials, but not for turgor pressure.

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#### **References**

- Ashraf M (1999) Interactive effect of salt (NaCl) and nitrogen form on growth, water relations and photosynthetic capacity of sunflower (*Helianthus annuus* L.). *Annals of Applied Biology* **135**, 509–513.
- Ashraf M (2001) Relationships between growth and gas exchange characteristics in some salt-tolerant amphidiploid *Brassica* species in relation to their diploid parents. *Environmental and Experimental Botany* **45**, 155–163. doi: 10.1016/S0098-8472(00)00090-3
- Ashraf M (2004) Some important physiological selection criteria for salt tolerance in plants. *Flora* **199**, 361–376.
- Ashraf M, Mcneilly T, Bradshaw AD (1986) The response to NaCl and ionic content of selected salt-tolerant and normal lines of 3 legume forage species in sand culture. *New Phytologist* **104**, 463–471.
- Ashraf M, Waheed A (1993) Responses of some local exotic accessions of lentil (*Lens culinaris* Medic) to salt stress. *Journal of Agronomy and Crop Science – Zeitschrift für Acker Und Pflanzenbau* **170**, 103–112.
- Cheeseman JM (1988) Mechanisms of salinity tolerance in plants. *Plant Physiology* **87**, 547–550.
- Chow WS, Ball MC, Anderson JM (1990) Growth and photosynthetic responses of spinach to salinity—implications of  $\text{K}^+$  nutrition for salt tolerance. *Australian Journal of Plant Physiology* **17**, 563–578.
- Cramer GR (2002) Sodium–calcium interactions under salinity stress. In 'Salinity: environment–plants–molecules'. (Eds A Läubli, U Lüttge) pp. 205–227. (Kluwer: Dordrecht, The Netherlands)

- Cramer GR, Alberico GJ, Schmidt C (1994) Salt tolerance is not associated with the sodium accumulation of 2 maize hybrids. *Australian Journal of Plant Physiology* **21**, 675–692.
- Curtis PS, Läuchli A (1986) The role of leaf-area development and photosynthetic capacity in determining growth of kenaf under moderate salt stress. *Australian Journal of Plant Physiology* **13**, 553–565.
- Delfine S, Alvino A, Villani MC, Loreto F (1999) Restrictions to carbon dioxide conductance and photosynthesis in spinach leaves recovering from salt stress. *Plant Physiology* **119**, 1101–1106. doi: 10.1104/PP.119.3.1101
- Demidchik V, Tester M (2002) Sodium fluxes through nonselective cation channels in the plasma membrane of protoplasts from *Arabidopsis* roots. *Plant Physiology* **128**, 379–387. doi: 10.1104/PP.128.2.379
- El-Hendawy SE, Hu Y, Yakout GM, Awad AM, Hafiz SE, Schmidhalter U (2004) Evaluating salt tolerance of wheat genotypes using multiple parameters. *European Journal of Agronomy* (in press).
- Essah PA, Davenport R, Tester M (2003) Sodium influx and accumulation in *Arabidopsis*. *Plant Physiology* **133**, 307–318. doi: 10.1104/PP.103.022178
- Ghassemi F, Jakeman AJ, Nix HA (1995) 'Salinization of land and water resources.' (University of New South Wales Press Ltd: Canberra, ACT)
- Gorham J (1992) Salt tolerance of plants. *Science in Progress* **76**, 273–285.
- Gorham J, Hardy C, Wyn Jones RG, Joppa LR, Law CN (1987) Chromosomal location of a K/Na discrimination character in the D genome of wheat. *Theoretical and Applied Genetics* **74**, 584–588. doi: 10.1007/BF00288856
- Gorham J, Wyn Jones RG, Bristol A (1990) Partial characterization of the trait for enhanced K<sup>+</sup>–Na<sup>+</sup> discrimination in the D-genome of wheat. *Planta* **180**, 590–597.
- Greenway H, Munns R (1980) Mechanisms of salt tolerance in non-halophytes. *Annual Review of Plant Physiology and Plant Molecular Biology* **31**, 149–190.
- Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. *Annual Review of Plant Physiology and Plant Molecular Biology* **51**, 463–499. doi: 10.1146/ANNUREV.PLANT.51.1.463
- Hawkins HJ, Lewis OAM (1993) Combination effect of NaCl salinity, nitrogen form and calcium-concentration on the growth, ionic content and gaseous exchange properties of *Triticum aestivum* L. cv. gamtoos. *New Phytologist* **124**, 161–170.
- He T, Cramer GR (1993a) Salt tolerance of rapid-cycling *Brassica* species in relation to potassium–sodium ratio and selectivity at the whole plant and Callus levels. *Journal of Plant Nutrition* **16**, 1263–1277.
- He T, Cramer GR (1993b) Cellular-responses of 2 rapid-cycling *Brassica* species, *Brassica napus* and *B. carinata*, to seawater salinity. *Physiologia Plantarum* **87**, 54–60. doi: 10.1034/J.1399-3054.1993.870109.X
- Heuer B, Plaut Z (1989) Photosynthesis and osmotic adjustment of 2 sugarbeet cultivars grown under saline conditions. *Journal of Experimental Botany* **40**, 437–440.
- Hunt R (1990) 'Basic growth analysis: plant growth analysis for beginners.' (Academic Press: London, UK)
- Ishikawa S, Oikawa T, Furukawa A (1991) Responses of photosynthesis, leaf conductance and growth to different salinities in 3 coastal dune plants. *Ecological Research* **6**, 217–226.
- James RA, Rivelli AR, Munns R, Von Caemmerer S (2002) Factors affecting CO<sub>2</sub> assimilation, leaf injury and growth in salt-stressed durum wheat. *Functional Plant Biology* **29**, 1393–1403. doi: 10.1071/FP02069
- Knight H (2000) Calcium signaling during abiotic stress in plants. *International Review of Cytology, A Survey of Cell Biology* **195**, 269–324.
- LaHaye PA, Epstein E (1971) Calcium and salt toleration by bean plants. *Physiologia Plantarum* **25**, 213–218.
- Leidi EO, Saiz JF (1997) Is salinity tolerance related to Na accumulation in upland cotton (*Gossypium hirsutum*) seedlings? *Plant and Soil* **190**, 67–75. doi: 10.1023/A:1004214825946
- Maathuis FJM, Amtmann A (1999) K<sup>+</sup> Nutrition and Na<sup>+</sup> toxicity: the basis of cellular K<sup>+</sup>/Na<sup>+</sup> ratios. *Annals of Botany* **84**, 123–133. doi: 10.1006/ANBO.1999.0912
- Marschner H (1995) 'Mineral nutrition of higher plants.' (Academic Press: London, UK)
- Morales MA, Sanchez-Blanco MJ, Olmos E, Torrecillas A, Alarcon JJ (1998) Changes in the growth, leaf water relations and cell ultrastructure in *Argyranthemum coronopifolium* plants under saline conditions. *Journal of Plant Physiology* **153**, 174–180.
- Munns R (1993) Physiological processes limiting plant-growth in saline soils—some dogmas and hypotheses. *Plant, Cell and Environment* **16**, 15–24.
- Munns R, Husain S, Rivelli AR, James RA, Condon AG, Lindsay MP, Lagudah ES, Schachtman DP, Hare RA (2002) Avenues for increasing salt tolerance of crops, and the role of physiologically based selection traits. *Plant and Soil* **247**, 93–105. doi: 10.1023/A:1021119414799
- Munns R, James RA (2003) Screening methods for salinity tolerance: a case study with tetraploid wheat. *Plant and Soil* **253**, 201–218. doi: 10.1023/A:1024553303144
- Murillo-Amador B, Troyo-Dieguez E, Lopezaguilar R, Lopez-Cortes A, Tinoco-Ojanguren CL, Jones HG, Kaya C (2002) Matching physiological traits and ion concentrations associated with salt stress in cowpea genotypes. *Australian Journal of Agricultural Research* **53**, 1243–1255. doi: 10.1071/AR01133
- Rengel Z (1992) The role of calcium in salt toxicity. *Plant, Cell and Environment* **15**, 625–632.
- Rivelli AR, James RA, Munns R, Condon AG (2002) Effect of salinity on water relations and growth of wheat genotypes with contrasting sodium uptake. *Functional Plant Biology* **29**, 1065–1074. doi: 10.1071/PP01154
- Robinson SP, Downton WJS, Millhouse JA (1983) Photosynthesis and ion content of leaves and isolated-chloroplasts of salt-stressed spinach. *Plant Physiology* **73**, 238–242.
- Rogers ME, Noble CL (1992) Variation in growth and ion accumulation between 2 selected populations of *Trifolium repens* L. differing in salt tolerance. *Plant and Soil* **146**, 131–136. doi: 10.1007/BF00012005
- SAS Institute (2000) 'SAS User's guide (Version 4.0.2).' (SAS Inst.: Cary, NC)
- Schachtman DP, Munns R (1992) Sodium accumulation in leaves of *Triticum* species that differ in salt tolerance. *Australian Journal of Plant Physiology* **19**, 331–340.
- Scholander PF, Hamme HT, Bradstreet ED, Hemmingsen EA (1965) Sap pressure in vascular plants. *Science* **148**, 339–346.
- Schwarz M, Gale J (1981) Maintenance respiration and carbon balance of plants at low-levels of sodium-chloride salinity. *Journal of Experimental Botany* **32**, 933–941.
- Seemann JR, Critchley C (1985) Effects of salt stress on the growth, ion content, stomatal behavior and photosynthetic capacity of a salt-sensitive species, *Phaseolus vulgaris* L. *Planta* **164**, 151–162. doi: 10.1007/BF00396077
- Semikhatova OA, Ivanova TI, Yudina OS (1993) Respiratory cost of plant-growth under conditions of salinity. *Russian Plant Physiology* **40**, 490–497.

- Serraj R, Sinclair TR (2002) Osmolyte accumulation: Can it really help increase crop yield under drought conditions? *Plant, Cell and Environment* **25**, 333–341. doi: 10.1046/J.1365-3040.2002.00754.X
- Shabala S (2000) Ionic and osmotic components of salt stress specifically modulate net ion fluxes from bean leaf mesophyll. *Plant, Cell and Environment* **23**, 825–837. doi: 10.1046/J.1365-3040.2000.00606.X
- Shabala S, Shabala L, Van Volkenburgh E (2003) Effect of calcium on root development and root ion fluxes in salinised barley seedlings. *Functional Plant Biology* **30**, 507–514. doi: 10.1071/FP03016
- Soussi M, Ocana A, Liuch C (2001) Growth, nitrogen fixation and ion accumulation in two chickpea cultivars under salt stress. *Agricoltura Mediterranea* **131**, 1–8.
- Tattini M, Gucci R, Coradeschi MA, Ponzio C, Everard JD (1995) Growth, gas-exchange and ion content in *Olea europaea* plants during salinity stress and subsequent relief. *Physiologia Plantarum* **95**, 203–210. doi: 10.1034/J.1399-3054.1995.950205.X
- Winicov I, Seemann JR (1990) Expression of genes for photosynthesis and the relationship to salt tolerance of alfalfa (*Medicago sativa*) cells. *Plant and Cell Physiology* **31**, 1155–1161.
- Yeo AR, Flowers TJ (1983) Varietal differences in the toxicity of sodium-ions in rice leaves. *Physiologia Plantarum* **59**, 189–195.

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