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Assessing the Suitability of Various Physiological Traits to Screen Wheat Genotypes for Salt Tolerance

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Abstract

Success of improving the salt tolerance of genotypes requires effective and reliable screening traits in breeding programs. The objective was to assess the suitability of various physiological traits to screen wheat genotypes for salt tolerance. Thirteen wheat genotypes from Egypt, Germany, Australia and India were grown in soil with two salinity levels (control and 150 mmol/L NaCl) in a greenhouse. The physiological traits (ion contents in leaves and stems, i.e. Na^+, Cl^-, K^+, Ca^{2+}), the ratios of K^+/Na^+ and Ca^+/Na^+ in the leaves and stems, net photosynthesis rate, stomatal conductance, transpiration rate, chlorophyll content (SPAD value), and leaf water relations, were measured at different growth stages. The physiological traits except for Na^+ and Cl^- in stems and the leaf transpiration rate at 150 mmol/L NaCl showed a significant genotypic variation, indicating that the traits that have a significant genotypic variation may be possibly used as screening criteria. According to the analysis of linear regression of the scores of the physiological traits against those of grain yield, however, the physiological traits of Ca^{2+} and Ca^{2+}/Na^+ at 45 d and final harvest with the greatest genotypic variation were ranked at the top. From a practical and economic point of view, SPAD value should be considered to be used as screening criteria and/or there is a need to develop a quick and practical approach to determine Ca^{2+} in plant tissues.

Key words: physiological traits; salinity; screening; wheat genotypes.

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Over 6% of the world's total land area and 20% of irrigated land are salt-affected (Munns 2005). Between 35% and 50% of the world's population in about 80 countries are in semiarid areas where salinization is a major problem. To solve the world's food problem, therefore, an increase in the food production in semiarid regions is particularly important. Both leaching salt from the soil surface and genetic improvement of salinity tolerance in current genotypes (Kingsbury and Epstein 1984; Shannon 1997) have been proposed as the most effective strategies to solve salinity problems. Although leaching salt from soil surfaces can ameliorate salt stress, it is not feasible on a large scale in semiarid regions due to the lack of good quality water resources, low soil permeability, and the high cost of amendments (Qureshi et al. 1990). Thus, the improvement of current genotypes to be more salt-tolerant is an alternative solution (Zeng et al. 2002). Unfortunately, improving salt tolerance of genotypes is often inhibited by the lack of effective evaluation methods for salt tolerance among genotypes (Shannon 1997). Therefore, it is very important to develop an effective evaluation approach for screening salt-tolerant genotypes, which should be reliable, quick, easy, practical and economic.

Screening large numbers of genotypes for salinity tolerance by evaluating plant biomass or yield in the field is difficult due to spatial heterogeneity of soil chemical and physical properties, and to seasonal fluctuations in rainfall (Yeo et al. 1990; Munns and James 2003). Significant genetic variation for salt tolerance might exist, but the confounding presence of drought stress makes it difficult to identify genotypes with salt tolerance. Because of the complex nature of salt tolerance, physiological traits have been recommended as selection criteria for screening

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(Yeo et al. 1990; Noble and Rogers 1992), which are considered as more reliable and feasible to screen for specific traits rather than salt tolerance itself in terms of biomass or yield in saline soil (Munns and James 2003).

Salinity causes plant physiological changes mainly due to ion toxicity and nutrient imbalance, water deficit and low photosynthesis in plants. Thus, Na^+ and Cl^- exclusion, K^+/Na^+ or Ca^{2+}/Na^+ discrimination, leaf water relations and photosynthesis should be used for screening germplasms for salinity tolerance. The published reports indicate that the salt tolerance of plants is associated with Na^+ and Cl^- exclusion in rice (Garcia et al. 1995) and wheat (Munns and James 2003), K^+/Na^+ or Ca^{2+}/Na^+ discrimination in rice and wheat (Asch et al. 2000; Zeng et al. 2003; El-Hendawy et al. 2005a), photosynthesis rate, stomatal conductance and transpiration rate in maize (Shabala et al. 1998) and wheat (James et al. 2002); leaf water and osmotic potential, and turgor pressure in tomato (Guerrier 1996) and wheat (Rivelli et al. 2002). However, using a single specific physiological trait in breeding programs has not yet proven as good as first expected (Jackson et al. 1996), because no single process can account for the variation in plant response to salinity. Thus, a combination of physiological traits is logically a desirable objective in screening for salt tolerance of genotypes. Furthermore, physiological parameters change with growth stages. In order to find out what time and which organs should be chosen for physiological traits of ion effects, the physiological traits at different growth stages and in different plant organs should be evaluated as well. Besides the assessment of the reliability of physiological traits, however, it is also necessary to assess if they are quick, easy and economic techniques for screening.

The objective of this study was to evaluate the association of physiological traits of wheat such as ion contents in leaves and stems, photosynthetic parameters, chlorophyll content and water relations at different growth stages with salt tolerance in terms of grain yield. Ranking the genotypes based on the salt-tolerant indices will help to assess the suitability of various physiological traits for screening salt-tolerant wheat genotypes.

Results

In order to compare the salt tolerance of genotypes, the salt tolerance indices were used to score and rank the genotypes according to El-Hendawy et al. (2005b). To evaluate the reliable physiological parameters used for the screening criteria for salt tolerance of wheat genotypes, an objective measure based on the grain yield was considered. Ranking of salt tolerance of 13 wheat genotypes based on the grain yield was reported by El-Hendawy et al. (2005b). Briefly, since Kharchia was used as the standard (i.e. as the salt tolerance reference), the number of scores for Kharchia was one for grain yield regardless of genotypes. Among the Egyptian wheat genotypes, Sakha 8 and Sakha 93 were ranked as number two compared with number one for Kharchia and were the most salt-tolerant genotypes compared with the others. Giza 168 and Sakha 61 were the most salt sensitive genotypes. German genotypes, Thasos and Triso, and Australian genotypes, Drysdale and Westonia, also showed a genotypic difference in salt tolerance. Thasos from Germany and Drysdale from Australia were moderately tolerant to salinity, while Triso from Germany and Westonia from Australian were most sensitive to salinity according to their scores on grain yield.

To evaluate the association of physiological traits with the plant tolerance objective (grain yield), ion contents in leaves and stems at different harvests, and leaf photosynthesis and water relations measured at different harvest times in the different level of salinity were also scored according to the salt tolerance indices (Tables 1–4).

The relationships between the scores of physiological traits and grain yield were further analyzed using linear regression (Tables 5–7). If the regression coefficient is significant, the slope of the equation reflects the degree of genotypic variation for a given physiological trait. The slope with a higher value indicates a greater variation among genotypes than others. In general, the scores on ion contents in leaves, leaf net photosynthesis rate (A), stomatal conductance (g_S), chlorophyll content (SPAD value), leaf water potential (Ψ), and leaf turgor pressure (T_p), regardless of measuring time and leaf osmotic potential (Ψ_π) at day 60 were significantly correlated with the scores on grain yield (Tables 5–7). However, Na^+ and Cl^- in stems and leaf transpiration rate (E) regardless of measuring time were not significantly correlated with grain yield, suggesting that the investigated organs can be important factors limiting the evaluation of salt tolerance. In order to determine whether the physiological parameters can be used as an easy, quick and economic technique, the suitability of various physiological traits were assessed as follows.

Discussion

Traits of Na^+ and Cl^- exclusion

Traits used for screening germplasm have included Na^+ exclusion (Yeo and Flowers 1986) and Cl^- exclusion (Rogers and Noble 1992). In this study, salt tolerance for most salt-tolerant genotypes (Kharchia, Sakha 8 and Sakha 93) were associated with the exclusion of Na^+ and Cl^- in leaves, which is in agreement with the work in the published reports (Kingsbury and Epstein 1984; Schachtman and Munns 1992; Dvorak et al. 1994; Chhipa and Lal 1995; Asch et al. 2000; Munns and James 2003). Several mechanisms may control leaf Na^+ accumulation in salt-tolerant genotypes. The net Na^+ and Cl^- uptake may be controlled by the roots, by the net loading of Na^+ and Cl^- in the

Table 1. Scores for the relative salt tolerance of 13 wheat genotypes on ion contents in leaves at day 45 and final harvest and on grain yield at 150 mmol/L NaCl

Genotypes	Ion content in leaves at day 45						Ion content in leaves at final harvest						Grain yield
	Na^+	Cl^-	K^+	Ca^{2+}	K^+/Na^+ ratio	Ca^{2+}/Na^+ ratio	Na^+	Cl^-	K^+	Ca^{2+}	K^+/Na^+ ratio	Ca^{2+}/Na^+ ratio	
Kharchia	1	1	1	1	1	1	1	1	1	1	1	1	1
Sakha 8	1	1	1	1	1	1	1	1	1	2	1	1	2
Sakha 93	1	1	1	1	1	1	1	1	1	2	1	1	2
Sakha 69	2	3	3	3	4	4	2	2	2	3	3	3	3
Drysdale	2	3	2	3	4	4	2	4	2	2	3	3	3
Sids 1	3	5	5	5	4	5	4	4	4	4	5	4	4
Thassos	4	5	5	5	5	5	5	4	5	4	5	5	4
Gemmeza 7	3	5	4	5	5	5	4	3	5	4	5	4	4
Triso	5	5	5	5	5	5	5	5	5	5	5	5	5
Sahel 1	3	5	4	5	5	5	4	4	4	5	5	5	5
Westonia	1	3	4	4	3	4	1	4	5	5	4	4	5
Giza 168	5	5	5	5	5	5	5	5	5	5	5	5	5
Sakha 61	5	5	5	5	5	5	5	4	5	5	5	5	5

Table 2. Scores among wheat genotypes for their relative salt tolerance on ion contents in stems at day 45 and final harvest and on grain yield at 150 mmol/L NaCl

Genotypes	Ion content in stems at day 45						Ion content in stems at final harvest						Grain yield
	Na^+	Cl^-	K^+	Ca^{2+}	K^+/Na^+ ratio	Ca^{2+}/Na^+ ratio	Na^+	Cl^-	K^+	Ca^{2+}	K^+/Na^+ ratio	Ca^{2+}/Na^+ ratio	
Kharchia	1	1	1	1	1	1	1	1	1	1	1	1	1
Sakha 8	1	1	1	1	1	1	1	1	1	1	1	1	2
Sakha 93	1	1	1	1	1	1	1	1	1	1	1	1	2
Sakha 69	1	2	4	4	3	4	1	1	2	4	2	3	3
Drysdale	1	2	4	4	3	4	1	1	2	4	2	4	3
Sids 1	3	2	4	5	4	5	3	1	4	4	4	4	4
Thassos	3	5	4	5	5	5	3	5	4	5	4	5	4
Gemmeza 7	3	2	5	5	5	5	4	1	4	4	5	5	4
Triso	5	5	4	5	5	5	5	5	5	5	5	5	5
Sahel 1	5	2	4	5	5	5	5	1	4	5	5	5	5
Westonia	1	1	4	5	2	4	1	1	5	5	2	4	5
Giza 168	5	2	5	5	5	5	5	1	4	5	5	5	5
Sakha 61	3	2	5	5	5	5	4	1	5	5	5	5	5

xylem, and/or the removal of Na^+ and Cl^- by the leaf sheath. Since the scores on Na^+ and Cl^- in stems for most of the salt-tolerant genotypes were low (i.e. the stems did not store more Na^+ and Cl^- compared with leaves), the mechanism of control of net Na^+ and Cl^- accumulation in leaves may be only due to the higher selectivity of the roots and/or to low net loading of Na^+ and Cl^- in the xylem. Genotypic variation of Na^+ or Cl^- exclusion in leaves was greater at the final harvest than at 45 d after sowing. For some genotypes, however, the physiological traits of Na^+ or Cl^- exclusion in leaves and stem could not be well associated with salt tolerance (Tables 1 and 2). For instance, Westonia was classified as most sensitive to salinity according to its scores on grain yield, but Na^+ accumulation in plant was scored as the number one in leaves and stems at both sampling times. The scores on Cl^- accumulation in plants were ranked between one and four. The results in Table 5 further show that traits of Na^+ and Cl^- were ranked after the Ca^{2+} and K^+ according to their slope of linear regression.

Trait of ion selectivity (Ca^{2+} and K^+)

Surprisingly, traits of Ca^{2+} and K^+ contents regardless of the tissue ages and organs were always ranked at the top according to the slopes from the linear regression (Table 5). This suggests a greater genetic difference in ion selectivity of K^+ and Ca^{2+} over Na^+ at 150 mmol/L NaCl in wheat genotypes. Similarly, Cramer et al. (1994) also found that for maize genotypes, the

Table 3. Scores among wheat genotypes for their relative salt tolerance on net photosynthesis rate (A), stomatal conductance (g_S), transpiration rate (E), and chlorophyll content (SPAD value) at 45 d and 60 d after sowing and on grain yield at 150 mmol/L NaCl

Genotypes	Photosynthesis parameters and SPAD value at day 45				Photosynthesis parameters and SPAD value at day 60				Grain yield
	A	g_S	E	SPAD	A	g_S	E	SPAD	
Kharchia	1	1	3	1	1	1	3	1	1
Sakha 8	1	1	3	1	2	2	3	2	2
Sakha 93	1	1	3	1	2	2	3	2	2
Sakha 69	1	1	4	1	3	3	3	3	3
Drysdale	1	1	1	1	3	3	1	3	3
Sids 1	2	3	4	3	4	4	3	3	4
Thassos	3	2	5	5	5	5	5	5	4
Gemmeza 7	3	3	3	2	5	4	3	4	4
Sahel 1	3	3	1	2	5	5	1	4	5
Triso	4	5	5	5	5	5	5	5	5
Westonia	2	2	2	3	5	4	3	5	5
Giza 168	5	5	4	5	5	5	3	5	5
Sakha 61	5	5	4	5	5	5	4	5	5

Table 4. Scores among wheat genotypes for their relative salt tolerance on leaf water potential (Ψ), leaf osmotic potential (Ψ_π) and leaf turgor pressure (T_p) at 45 d and 60 d after sowing and on grain yield at 150 mmol/L NaCl

Genotypes	Water relations at day 45			Water relations at day 60			Grain yield
	Ψ	Ψ_π	T_p	Ψ	Ψ_π	T_p	
Kharchia	1	1	1	1	1	2	1
Sakha 8	1	1	1	1	1	1	2
Sakha 93	1	1	2	1	1	1	2
Sakha 69	3	1	3	3	3	2	3
Drysdale	3	2	3	2	3	1	3
Sids 1	4	3	3	4	3	3	4
Thassos	5	5	4	5	5	4	4
Gemmeza 7	5	3	5	4	5	3	4
Sahel 1	3	2	3	4	4	2	5
Triso	5	5	5	5	5	4	5
Westonia	5	1	5	5	4	5	5
Giza 168	5	3	5	5	5	3	5
Sakha 61	5	3	5	5	5	4	5

concentration of Ca^{2+} and K^+ and their ratios over Na^+ were more related with salt tolerance in two hybrids than traits of Na^+ exclusion. Because of the higher selectivity of Ca^{2+} and K^+ in most tolerant genotypes, higher Ca^{2+} and/or K^+ over Na^+ in leaves appears to protect the plant from the effects of toxic ions (Rengel 1992). The published reports (Cuin et al. 2003; Tester and Davenport 2003) suggested that a high K^+/Na^+ or Ca^{2+}/Na^+ ratio is more important for many species than simply maintaining a low concentration of Na^+. Although there was no association of Na^+ accumulation in leaves for the salt sensitive genotype (Westonia) (Tables 1 and 2), its selectivity of Ca^{2+} over Na^+ was strongly associated with its salt tolerance. Interestingly, the results in this study indicate that Ca^{2+} content in plants demonstrated the greatest genotypic variation and was well correlated with the salt tolerance ranked by using grain yield (Table 5).

Traits of photosynthetic parameters and SPAD value

Salinity causes not only ion toxicity and imbalance, but also low photosynthesis in plants. Because photosynthesis, stomatal conductance and chlorophyll content in leaves can be measured by a non-destructive, rapid and easy technique using a porometer and SPAD meter, these physiological traits may be important to be used as screening criteria if they would be closely associated with salt tolerance of genotypes at a given

Table 5. Equations of linear regression, slopes and regression coefficients between the scores on grain yield (X) and the scores on ion contents in leaves and stems (Y) at day 45 and final harvest at 150 mmol/L NaCl

Sampling time	Organs	Parameters	Regression equations	Slope	r^2
Day 45	Leaves	Ca^{2+}/Na^+	$Y = -0.38 + 1.05X$	1.05	0.78***
Day 45	Leaves	Ca^{2+}	$Y = -0.28 + 1.03X$	1.03	0.84***
Day 45	Leaves	K^+	$Y = -0.37 + 0.94X$	0.94	0.84***
Day 45	Leaves	K^+/Na^+	$Y = -0.24 + 0.94X$	0.94	0.71***
Day 45	Leaves	Cl^-	$Y = -0.17 + 0.94X$	0.94	0.75***
Day 45	Leaves	Na^+	$Y = 0.67 + 0.23X$	0.23	0.42**
Day 45	Stems	Ca^{2+}/Na^+	$Y = -0.35 + 1.05X$	1.05	0.78***
Day 45	Stems	K^+	$Y = 0.21 + 0.95X$	0.95	0.50***
Day 45	Stems	Ca^{2+}	$Y = -0.44 + 0.95X$	0.95	0.96***
Day 45	Stems	K^+/Na^+	$Y = 0.06 + 0.71X$	0.71	0.65***
Day 45	Stems	Na^+	$Y = 0.72 + 0.21X$	0.21	0.14*
Day 45	Stems	Cl^-	$Y = 0.75 + 0.18X$	0.18	0.18*
Harvest	Leaves	Ca^{2+}	$Y = -0.22 + 1.10X$	1.10	0.83***
Harvest	Leaves	K^+/Na^+	$Y = -0.34 + 1.03X$	1.03	0.84***
Harvest	Leaves	Ca^{2+}/Na^+	$Y = -0.34 + 1.00X$	1.00	0.89***
Harvest	Leaves	Na^+	$Y = 0.69 + 0.95X$	0.95	0.65***
Harvest	Leaves	K^+	$Y = -0.69 + 0.94X$	0.94	0.78***
Harvest	Leaves	Cl^-	$Y = -0.12 + 0.87X$	0.87	0.66***
Harvest	Stems	Ca^{2+}	$Y = -0.03 + 1.07X$	1.07	0.96***
Harvest	Stems	Ca^{2+}/Na^+	$Y = -0.33 + 1.03X$	1.03	0.82***
Harvest	Stems	K^+	$Y = 0.47 + 0.89X$	0.89	0.45***
Harvest	Stems	K^+/Na^+	$Y = 0.12 + 0.67X$	0.67	0.69***
Harvest	Stems	Na^+	$Y = 0.90 + 0.21X$	0.21	0.12*
Harvest	Stems	Cl^-	$Y = 1.14 + 0.01X$	0.01	0.03*

*not significant at $P \leq 0.05$; ** Significant at the 0.01 probability level;*** Significant at the 0.001 probability level.

Table 6. Equations of linear regression, slopes and regression coefficients between the scores on grain yield (X) and the scores on net photosynthesis rate (A), stomatal conductance (g_S), transpiration rate (E), and chlorophyll content (SPAD value) (Y) at 45 d and 60 d after sowing at 150 mmol/L NaCl

Sampling time	Parameters	Regression equations	Slope	r^2
Day 45	A	$Y = 0.46 + 0.45X$	0.45	0.47**
Day 45	g_S	$Y = 0.33 + 0.45X$	0.45	0.46**
Day 45	SPAD	$Y = 0.35 + 0.45X$	0.45	0.47**
Day 45	E	$Y = 2.2 + 0.15X$	0.15	0.002*
Day 60	A	$Y = -0.22 + 1.04X$	1.04	0.98***
Day 60	g_S	$Y = -0.02 + 0.97X$	0.97	0.97***
Day 60	SPAD	$Y = -0.22 + 0.92X$	0.92	0.95***
Day 60	E	$Y = 1.66 + 0.13X$	0.13	0.001*

*not significant at $P \leq 0.05$; ** Significant at the 0.01 probability level;*** Significant at the 0.001 probability level.

level of salinity. In this study, significant genotypic variation in net photosynthesis rate, stomatal conductance, and SPAD values were observed for both sampling times (Tables 3 and 6). However, the genotypic variation was greater at 60 d after sowing than at 45 d. From an economic point of view, the earlier sampling time is better.

Practically, evaluating the genotypes for salt tolerance should be directly made in the field. Compared with using a porometer

Table 7. Equations of linear regression, slopes and regression coefficients between the scores on grain yield (X) and the scores on leaf water potential (Ψ), leaf osmotic potential (Ψ_π) and leaf turgor pressure (T_p) (Y) at 45 d and 60 d after sowing at 150 mmol/L NaCl

Sampling time	Parameters	Regression equations	Slope	r^2
Day 45	Ψ	$Y = -0.21 + 0.92X$	0.92	0.77***
Day 45	T_p	$Y = -0.18 + 0.86X$	0.86	0.77***
Day 45	Ψ_π	$Y = 0.74 + 0.21X$	0.21	0.15*
Day 60	Ψ	$Y = -0.26 + 0.97X$	0.97	0.88***
Day 60	Ψ_π	$Y = -0.22 + 0.94X$	0.94	0.88***
Day 60	T_p	$Y = 0.25 + 0.54X$	0.54	0.44**

*not significant at $P \leq 0.05$; **Significant at the 0.01 probability level;*** Significant at the 0.001 probability level.

to measure the photosynthesis rate and stomatal conductance, the SPAD meter is much more handy and practical for large scale screening when there are a large number of test genotypes to be evaluated through breeding programs. The effectiveness of the SPAD meter as a screening method has been examined in a number of studies as an index for response of chlorophyll content to stress. For instance, this technique was used for screening groundnut genotypes for tolerance to iron-deficiency chlorosis (Samdur et al. 2000) and it is also used to estimate tissue tolerance for high Na^+ accumulation (Munns and James 2003). In both studies, a closer relationship between SPAD value and tolerance to iron-deficiency chlorosis and high Na^+ accumulation were observed. The previous studies also showed, the SPAD value was linearly correlated with maximum net photosynthesis rate in soybean (Ma et al. 1995), in rice (Laza et al. 1996), and in wheat (Gutierrez-Rodriguez et al. 2000).

Although the transpiration rate is important for controlling the accumulation of salt ions in shoots (Walker et al. 1990; Storey 1995; Moya et al. 1999), the correlation between the scores on leaf transpiration rate and on grain yield among the tested wheat genotypes was not significant for both sampling times (Tables 3 and 6). It seems that the transpiration rate as a screening criterion may be more important for drought stress than for salt stress, since two genotypes, Drysdale and Sahel 1, with drought tolerance character were ranked as number one according to their scores on the transpiration rate compared with other genotypes (Table 3). Because one of the stresses caused by salinity is osmotic stress or water deficit, the trait of leaf transpiration in the salt tolerance of genotypes should be improved in order to further increase their salt tolerance.

Traits of leaf water relations

Under saline conditions, low osmotic potentials of the soil solution induce water deficit in plant tissue. As a consequence, the turgor in plants may decrease. Leaf water potential was significantly correlated with grain yield at both sampling times, but it was greater at 45 d after sowing than at 60 d (Table 7). There are also some disadvantages (for example, leaf water potential is sensitive to environmental conditions, such as light intensity). Surprisingly, the scores of Westonia on leaf water potential were closely associated with those of its grain yield, suggesting that successful strategies to establish screening criteria must include physiological traits of leaf water potential along with other traits. Data in Tables 4 and 7 also show that leaf turgor pressure at 45 d after sowing was similar to leaf water potential at 45 d. However, osmotic potential at 45 d was not suitable to be used as a criterion since there was no correlation between osmotic potential and grain yield.

In conclusion, the tested physiological traits except for the traits of Na^+ and Cl^- in stems and the leaf transpiration rate showed a significant genotypic variation, indicating that the traits that have a significant genotypic variation may possibly be used as screening criteria. According to the analysis of linear regression of the scores of the physiological traits against those of grain yield, however, the physiological traits of Ca^{2+} and Ca^{2+}/Na^+ at 45 d and final harvest with the greatest genotypic variation were ranked at the top. From a practical and economic point of view, SPAD value should be considered to be used as screening criteria and/or there is a need to develop a quick and practical approach to determine Ca^{2+} in plant tissues.

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 °C to 28 °C in the daytime and 15 °C 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 four layers in 7-L pots.

Control (no added NaCl) and 150 mmol/L NaCl in the soil were applied. The final water content (25% on dry soil basis) was achieved by adding tap water or salt solution (150 mmol/L NaCl) to each layer. To avoid an osmotic shock for seedling emergence, however, the topmost soil layer was not salinized until 10 d after sowing. Twenty-five seeds were sown in each pot. One week after sowing, the seedlings were thinned to twenty per pot.

Nitrogen, and P and K were initially applied as 0.2 g NH_4NO_3 and as 0.2 g KH_2PO_4 per pot, respectively. The same amounts of N, P and K were applied another three times at 20, 40 and 60 d 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 four times.

Three plants at 45 d after sowing and five 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.

Analysis of ion concentrations

Oven-dried samples of leaves and stems of plants at 45 d after sowing and at final harvest were ground into a fine powder by passing them through a 0.5-mm diameter sieve. For the determination of Na^+, K^+ and Ca^{2+} content, 300 mg of ground dry material of the stems or leaves was digested by adding 3 mL concentrated HNO_3 (65%) and 2 mL H_2O_2 (30%) for 30 min at 2600 kPa (80 psi) in a MDS-2100 microwave oven (CEM Corporation, Matthews NC, USA). After digestion, each sample was brought up to a 50 mL final volume with distilled-deionized water. The concentration of Na^+ was determined with an inductively coupled plasma emission spectrometer (ICP model Liberty 200, Varian Australia, Mulgrave, Australia). The K^+ and Ca^{2+} contents were determined with a flame photometer (ELEX 6361, Eppendorf, Netheler-Hinz GmbH., Germany).

For Cl^-, 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 analyzer (Model LC20-1, Dionex, Sunnyvale CA, USA).

Photosynthetic parameters

Photosynthesis rate (A), stomatal conductance (g_s) and transpiration rate (E) were determined on the second youngest leaf that was fully expanded at 45 d and 60 d after sowing. Measurements were made with a LI-COR 6400 portable gas exchange system (LI-COR Biosciences Inc., Lincoln, NE, USA). 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 Biosciences Inc.). 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 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and a CO_2 level of 400 $\mu mol \cdot mol^{-1}$) one day before the measurements were carried out.

Leaf chlorophyll measurement

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

Water relation measurements

Leaf water potential (Ψ) and osmotic potential (Ψ_π) from the middle of the second youngest leaf with a fully developed blade were measured twice each at 45 d and 60 d after sowing. Ψ was measured with a pressure bomb (PMS Instrument Company, model 1002, Corvalis OR, USA) according to the technique of Scholander et al. (1965). Immediately after Ψ 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; Ψ_π was then determined with a vapour pressure osmometer (Wescor 5100C, Wescor Inc, Logan UT, USA). Turgor pressure (T_p) was estimated as the difference between Ψ_π and Ψ.

Ranking and scoring of genotypes for salt tolerance

In order to allow comparisons among genotypes, a salt-tolerant genotype, Kharchia, was chosen as a reference (i.e. a standard against which all the other genotypes were compared) (El-Hendawy et al. 2005b). Thus, the measurements of plants from the other genotypes at 150 mmol/L NaCl were divided by their means to convert to relative values (i.e. the salt

tolerance indices). The indices were then used to score and rank the genotypes. Genotypes were classified into five classes according to the formula: number of classes $= 1.0 + 3.3 \log_{10} n$, where n is the number of tested genotypes (Josef 1985). The class intervals of indices were defined as the difference between high and low salt indices divided by the number of classes. Scores were assigned to the class intervals from the highest to the lowest in grain yield, K^+ and Ca^{2+}contents in leaves and stems, the ratios of K^+/Na^+ and Ca^{2+}/Na^+ in leaves and stems, A, g_S, SPAD value and T_p, while scores were assigned to the class intervals from the lowest to the highest for the parameters such as Na^+, Cl^-, E, Ψ, and Ψ_π.

Statistical analysis of data

The factorial experimental design with 13 genotypes and two salinity levels was arranged in a completely randomized design with four replications. Data were analyzed through ANOVA tests, using COSTAT Version 3.03 (software, Berkeley CA, USA). Relationships between the scores of grain yield and the scores of different physiological parameters were analyzed by simple linear regression by using JMP user's Guide (SAS institute 2000).

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