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Europ. J. Agronomy 22 (2005) 243–253
European Journal of Agronomy
www.elsevier.com/locate/eja

Evaluating salt tolerance of wheat genotypes using multiple parameters

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Received 13 November 2003; received in revised form 26 February 2004; accepted 15 March 2004

Abstract

Salt tolerance of wheat is known to change with growth stage. Identifying the multiple parameters associated with salt tolerance during different growth stages is important for evaluating wheat genotypes and improving their salt tolerance. Thirteen wheat genotypes from Egypt, Germany, Australia and India were grown in soil and exposed to four salinity levels (control, 50, 100 and 150 mM NaCl). Tiller number, leaf number and leaf area per plant at vegetative stage; dry weight per plant at vegetative, reproductive and maturity stages; and yield components of main spike and total grain yield at maturity were determined. The results showed that tiller number was affected more by salinity than leaf number and leaf area at the vegetative stage. Salinity decreased dry weight per plant significantly at all growth stages. Spikelet number on the main stem decreased much more with salinity than spike length, grain number and 1000-grain weight at maturity. According to cluster analysis with multiple agronomic parameters at all growth stages, the Egyptian genotypes Sakha 8 and Sakha 93 and the Indian genotype Kharchia were ranked as the most tolerant to salinity. A change in salt tolerance with growth stages was observed for Sids 1, Gemmeza 7 and Westonia. Drysdale and Sakha 69 were ranked as moderate tolerant. The remaining genotypes showed the lowest tolerance to salinity at all growth stages. We conclude that an increase in tiller number per plant and spikelet number per spike will improve the salt tolerance of wheat genotypes in breeding programs. Cluster analysis with multiple agronomic parameters simultaneously to evaluate the salt tolerance facilitates the rankings of salt tolerance of wheat genotypes.

Keywords: Cluster analysis; Evaluation; Salt tolerance; Wheat genotypes

1. Introduction

Salinity is one of the major factors reducing plant growth and productivity worldwide, and affects about 7% of the world's total land area (Flowers et al., 1997). The percentage of cultivated land affected by salt is even greater, with 23% of the cultivated land being saline and 20% of the irrigated land suffering from secondary salinization. Furthermore, there is also a dangerous trend of a 10% per year increase in the saline area throughout the world (Ponnamieruma, 1984). Egypt is one of the countries that suffer from

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1161-0301/$ – see front matter
doi:10.1016/j.eja.2004.03.002

severe salinity problems. For example, 33% of the cultivated land, which comprises only 3% of total land area in Egypt, is already salinized due to low precipitation (<25 mM annual rainfall) and irrigation with saline water (Ghassemi et al., 1995). Wheat is the most important and widely adapted food cereal in Egypt. However, Egypt supplies only 40% of its annual domestic demand for wheat (Salam, 2002). Therefore, it is necessary to increase wheat production in Egypt by raising the wheat grain yield. Obviously, the most efficient way to increase wheat yield in Egypt is to improve the salt tolerance of wheat genotypes (Epstein et al., 1980; Shannon, 1997; Pervaiz et al., 2002) because increasing the salt tolerance of wheat is much less expensive for poor farmers in developing countries than using other management practices (e.g. leaching salt from the soil surface etc., Qureshi and Barrett-Lennard, 1998).

Salt tolerance of crops may vary with their growth stage (Mass and Grieve, 1994). In general, cereal plants are the most sensitive to salinity during the vegetative and early reproductive stages, and less sensitive during flowering and during the grain filling stage (Mass and Poss, 1989). However, a difference in the salt tolerance among genotypes may also occur at different growth stages. Zeng et al. (2002) reported that various responses of different rice genotypes to salt tolerance exist at different growth stages. Similarly, Kingsbury and Epstein (1984) found that individual lines from 5000 accessions of spring wheat showed differing tolerance during their life cycle. Therefore, the salt tolerance of different wheat genotypes must be evaluated at different growth stages. Such evaluations may facilitate improvement of salt tolerance of tested genotypes in breeding programs or it may prove feasible to irrigate with saline water during the more tolerant growth stages and with low salinity water only during the sensitive growth stages.

Improving salt tolerance of wheat genotypes has been inhibited by a number of factors, such as the lack of effective evaluation methods for salt tolerance to screen the genotypes in breeding programs, low selection efficiency using overall agronomic parameters, and a complex phenomenon involving morphological, physiological and biochemical parameters among genotypes (Zeng et al., 2002). Compared with conventional techniques that score and rank salt tolerance genotypes based on single parameter, some success has already been realized by using multiple agronomic parameters simultaneously at different growth stages (Shannon, 1997; Zeng et al., 2002). An appropriate statistical method is needed to analyse multiple agronomic parameters simultaneously to facilitate ranking genotypes for salt tolerance (Zeng et al., 2002). Cluster analysis is commonly used multivariate statistic that has been suggested for comparisons of genotypes means by Jollife et al. (1989). However, multivariate analysis in the screening of genotypes for salt tolerance has been applied only in potato (Khrais et al., 1988) and rice (Zeng et al., 2002).

The objectives of this study were to identify the relative importance of agronomic parameters associated with salt tolerance, to screen the different wheat genotypes for their salt tolerance at different growth stages from different regions of Egypt using the most salt tolerant wheat genotypes from India as a reference, and to rank salt tolerance by using multivariate analysis of multiple agronomic parameters at different growth stages.

2. Materials and methods

2.1. Plant materials

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 in Giza, Egypt. Sakha 8 and Sakha 93 are usually cultivated in saline areas in Egypt. Of the remaining varieties, Thassos and Triso were from Germany, Westonia and Drysdale were from Australia, and Kharchia was from India.

Kharchia is the most tolerant wheat genotype, and is used as a standard for the salt tolerance test of wheat worldwide (Sharma et al., 1994; Ashraf, 2002).

2.2. Growth conditions

This study was carried out in greenhouse from the middle of March to the middle of August 2002. The air temperature ranged from 23 to 28 °C during the day and 15 to 18 °C during the night. Relative humidity fluctuated between 45 and 85% at day/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 initially air-dried soil with 9% gravimetric water content was filled layer-wise in four layers in 7-l pots without a leaching possibility.

Four salt levels (control (no added NaCl), 50, 100 and 150 mM NaCl) in the soil were applied. The salinity levels of 50, 100 and 150 mM NaCl in soil solution were equivalent to an electrical conductivity of 8, 13, and 17 dS m^{-1}, respectively, which were measured at the beginning of the experiment. During the period of the experiment, the electrical conductivity at each salinity level slightly decreased due to the uptake of salt by plants. At the end of the experiment, an electrical conductivity was changed to 5.2, 10, and 14 dS m^{-1}, respectively. The final water content (25% on dry soil basis) was achieved by adding tap water or salt solution to each layer. To avoid an osmotic shock for seedling emergence, however, the topmost soil layer was not salinized until 10 days after sowing. Twenty-five seeds were sown in each pot. One week after sowing, the seedlings were thinned to twenty per pot.

The N, P and K were initially applied as 0.2 g NH_4NO_3 and as 0.2 g KH_2PO_4 per pot. The same amount of N, P and K was applied another three 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 when total amount of water lost was around 200 g for plants to avoid suffering either drought or flooding. All treatments were replicated four times.

2.3. Sampling strategy

Salt tolerance of crops may vary with their growth stage (Mass and Grieve, 1994). Therefore, the measurements were carried out at vegetative, reproductive and grain maturity stages.

Measurements at the vegetative stage were conducted at 45 days after sowing. Three plants from each pot were harvested and separated into leaves and stems. Vegetative growth of wheat plants is characterized by the tillering and leaf appearance and growth on the tillers. Thus, the number of tillers and leaves were recorded. Leaf area was measured by using an LI-3000 Area Meter (LI-COR, Walz Co., Oregon, USA). After the fresh weight (FW) was determined, the samples were dried at 65 °C for 48 h to determine the dry weight (DW).

At 60 and 75 days after sowing, when plants were in the reproductive stage, three plants from each pot were harvested. Plants were separated into leaves, stems and spikes. FW and DW determined as above.

Grain maturity was visually estimated according to the complete loss of green colour from grumes. At grain maturity, five plants from each pot were harvested. Main spikes were separated from the other spikes of the plants, and the spike length and spikelet number were recorded. Ears were threshed. Plant material was then dried at 65 °C for 48 h for the determination of DW. The grain number and thousand-grain weight (TGW) were also determined.

2.4. Ranking of genotypes for salt tolerance

Following Zeng et al. (2002), all the data were converted to salt tolerance indices before cluster analysis to allow comparisons among genotypes for salt tolerance by using multiple agronomic parameters. A salt tolerance index was defined as the observation at salinity divided by the average of the controls. Cluster group ranking numbers can be assigned to cluster groups based on cluster means, and were used to score genotypes. Cluster analysis followed the methods described by Jollife et al. (1989). Cluster group rankings were obtained based on Ward's minimum variance cluster analysis of the averages of the salt tolerance indices for three parameters at vegetative stage (i.e. tiller number, leaf number and leaf area per plant) and four parameters at maturity stage (i.e. spike length and spikelet number for main spike, grain number and 1000-grain weight). Cluster group rankings were obtained based on Single-Link cluster analysis of the means of the salt tolerance indices for total dry weight per plant. All procedures are described fully in the JMP User's Guide (SAS Institute, 2000). The cluster group rankings were obtained from the average of means of the multiple parameters in each cluster group. A sum was obtained by adding the number of cluster group rankings at each salt level in each genotype. The genotypes were finally ranked based on the sums, such that those with the smallest and largest sums were ranked respectively as the most and least tolerant genotypes in terms of relative salt tolerance.

2.5. Statistical analysis of data

Data were analysed by ANOVA. According to Snedecor and Cochran (1980), LSD ($P = 0.05$) was used to compare genotypes means. Data were analysed using an ANOVA split design, where salinity levels were assigned as whole plot, genotypes as sub plots and replicates as blocks.

3. Results

Tiller number, leaf number and leaf area at vegetative stage decreased with increasing salinity (Fig. 1). The low salinity treatment (50 mM NaCl) reduced these parameters to a lesser degree than moderate (100 mM NaCl) and high salinity treatments (150 mM NaCl). At 50, 100 and 150 mM NaCl, for example, tiller number was reduced by 22, 28 and 37.5%, leaf number was reduced by 6, 19 and 28% and leaf area was reduced by 8, 19 and 28%, respectively, as compared with the control treatment (Fig. 1).

At vegetative stage, the relative salt tolerance indices for all the measured parameters varied among genotypes (Table 1). The salt tolerance indices of tiller number ranged from 0.42 to 1.00 at low salinity and from 0.25 to 0.88 at high salinity among genotypes. However, the salt tolerance indices ranged from 0.86 to 1.09 for leaf number and from 0.85 to 0.99 for leaf area at low salinity and from 0.44 to 0.88 for leaf number and from 0.57 to 0.90 for leaf area at high salinity. The results at the vegetative stage showed that Kharchia, Sakha 8 and Sakha 93 were affected the least by increasing salinity. For instance, tiller number, leaf number and leaf area at 150 mM NaCl were decreased by 25, 14 and 15% for Kharchia, 12, 18 and 14% for Sakha 8 and 16, 18 and 10% for Sakha 93, respectively, as compared with the control. However, tiller number, leaf number and leaf area per plant for Sakha 61, Giza 168, Sids 1, Sahel 1, Gemmeza 7, Thassos, Triso and Westonia (Fig. 1), which were the most sensitive to salinity, decreased by an average of 50, 34 and 35% at 150 mM NaCl, respectively. To rank the salt tolerance of genotypes at the vegetative stage based on multiple parameters, genotypes

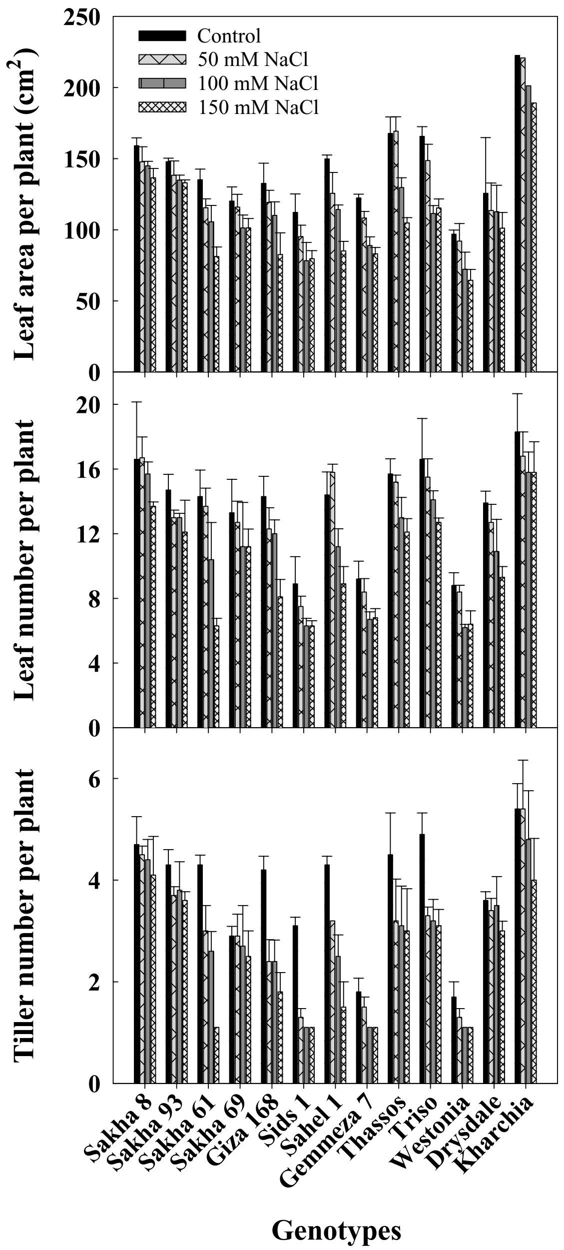

Fig. 1. Effect of different salinity levels on plant growth parameters (tiller number, leaf number and leaf area per plant) at day 45 for different wheat genotypes. Error bars represent standard deviations. Error bars fit within the plot symbol if not shown.

were divided into four cluster groups at low and moderate salinity and five cluster groups at high salinity by using Ward's minimum variance cluster analysis (Table 2). At the vegetative stage, Kharchia, Sakha 8

Table 1
Salt tolerance indices of agronomic parameters in wheat genotypes under different salinity levels at different growth stages

Genotypes	Salinity levels (mM NaCl)	Tiller number at day 45	Leaf number at day 45	Leaf area at day 45	Total biomass at day 45	Total biomass at day 60	Total biomass at day 75	Total biomass at final harvest	Spike length	Spikelet number	Grain number	1000-grain weight	Grain yield
Kharchia	50	1.00	0.92	0.99	0.97	0.89	0.67	0.88	0.91	0.99	1.00	0.95	0.94
	100	0.89	0.86	0.90	0.93	0.88	0.67	0.82	0.88	0.96	0.91	0.94	0.91
	150	0.75	0.86	0.85	0.90	0.81	0.59	0.69	0.86	0.90	0.87	0.94	0.83
Sakha 8	50	0.97	1.01	0.93	0.90	0.89	0.70	0.90	0.90	0.98	1.03	1.00	0.92
	100	0.93	0.95	0.91	0.88	0.81	0.60	0.79	0.84	0.86	0.89	0.99	0.82
	150	0.88	0.82	0.86	0.86	0.74	0.52	0.69	0.81	0.83	0.88	0.97	0.75
Sakha 93	50	0.86	0.88	0.94	0.98	0.92	0.73	0.80	0.90	0.98	1.01	0.94	0.94
	100	0.89	0.88	0.91	0.92	0.83	0.56	0.77	0.87	0.84	0.95	0.96	0.89
	150	0.84	0.82	0.90	0.83	0.80	0.51	0.68	0.84	0.79	0.84	0.92	0.77
Sakha 69	50	0.99	0.95	0.96	0.86	0.79	0.62	0.73	0.87	0.94	0.94	0.88	0.96
	100	0.93	0.84	0.84	0.92	0.62	0.40	0.53	0.80	0.67	0.83	0.92	0.64
	150	0.87	0.84	0.84	0.84	0.51	0.34	0.51	0.75	0.65	0.77	0.80	0.58
Drysdale	50	0.94	0.91	0.90	0.86	0.73	0.66	0.78	0.89	0.93	0.93	0.99	0.84
	100	0.98	0.78	0.90	0.92	0.63	0.45	0.71	0.77	0.70	0.85	0.97	0.61
	150	0.85	0.67	0.80	0.84	0.55	0.40	0.66	0.68	0.70	0.81	0.88	0.57
Sids 1	50	0.42	0.84	0.85	0.86	0.69	0.54	0.84	0.92	0.99	1.02	0.95	0.86
	100	0.36	0.71	0.70	0.86	0.51	0.40	0.59	0.83	0.64	0.75	0.86	0.53
	150	0.36	0.70	0.71	0.79	0.43	0.35	0.57	0.74	0.64	0.76	0.73	0.48
Gemmeza 7	50	0.82	0.92	0.88	1.00	0.66	0.56	0.74	0.93	1.04	1.10	0.91	0.87
	100	0.60	0.73	0.73	0.81	0.48	0.47	0.59	0.80	0.62	0.83	0.83	0.58
	150	0.60	0.74	0.68	0.76	0.43	0.38	0.55	0.71	0.64	0.84	0.69	0.50
Giza 168	50	0.57	0.86	0.90	0.85	0.62	0.57	0.70	0.85	0.89	0.98	0.87	0.75
	100	0.56	0.84	0.83	0.86	0.49	0.37	0.60	0.70	0.57	0.79	0.86	0.51
	150	0.42	0.56	0.62	0.68	0.36	0.29	0.52	0.68	0.51	0.72	0.69	0.37
Sahel 1	50	0.75	1.09	0.84	0.89	0.65	0.50	0.68	0.94	0.94	0.90	0.88	0.67
	100	0.59	0.78	0.76	0.80	0.58	0.36	0.54	0.74	0.62	0.82	0.87	0.49
	150	0.36	0.62	0.57	0.65	0.51	0.31	0.44	0.67	0.52	0.74	0.70	0.33
Thassos	50	0.71	0.97	1.01	0.85	0.65	0.50	0.72	0.91	0.91	0.98	0.92	0.74
	100	0.69	0.83	0.77	0.72	0.51	0.42	0.50	0.74	0.59	0.81	0.82	0.47
	150	0.66	0.77	0.62	0.69	0.40	0.27	0.39	0.71	0.57	0.80	0.71	0.42
Triso	50	0.67	0.94	0.90	0.78	0.66	0.48	0.72	0.86	0.87	0.92	0.94	0.73
	100	0.65	0.85	0.67	0.73	0.59	0.37	0.57	0.74	0.51	0.74	0.81	0.41
	150	0.63	0.76	0.70	0.67	0.41	0.24	0.47	0.59	0.49	0.71	0.65	0.33
Westonia	50	0.78	0.95	0.95	0.85	0.62	0.58	0.73	0.86	0.90	1.07	0.95	0.83
	100	0.67	0.70	0.75	0.80	0.49	0.40	0.42	0.78	0.59	0.78	0.91	0.38
	150	0.67	0.73	0.67	0.70	0.34	0.28	0.40	0.70	0.60	0.74	0.64	0.33
Sakha 61	50	0.68	0.96	0.85	0.86	0.57	0.51	0.59	0.84	0.94	0.91	0.81	0.61
	100	0.59	0.73	0.78	0.78	0.45	0.34	0.40	0.74	0.61	0.69	0.64	0.39
	150	0.25	0.44	0.60	0.70	0.40	0.25	0.37	0.69	0.60	0.67	0.53	0.38

and Sakha 93 were ranked at the top for salt tolerance. By contrast, Sids 1 and Sakha 61 were ranked as the most sensitive.

To determine the variation of salt tolerance at different growth stages, the above-ground biomass per plant was measured at 45, 60 and 75 days after sowing and at final harvest. The average biomass of the 13 genotypes at 150 mM NaCl was decreased by 23, 49, 64 and 47% at 45, 60 and 75 days after sowing and at final harvest, respectively (Fig. 2). The averaged indices of biomass

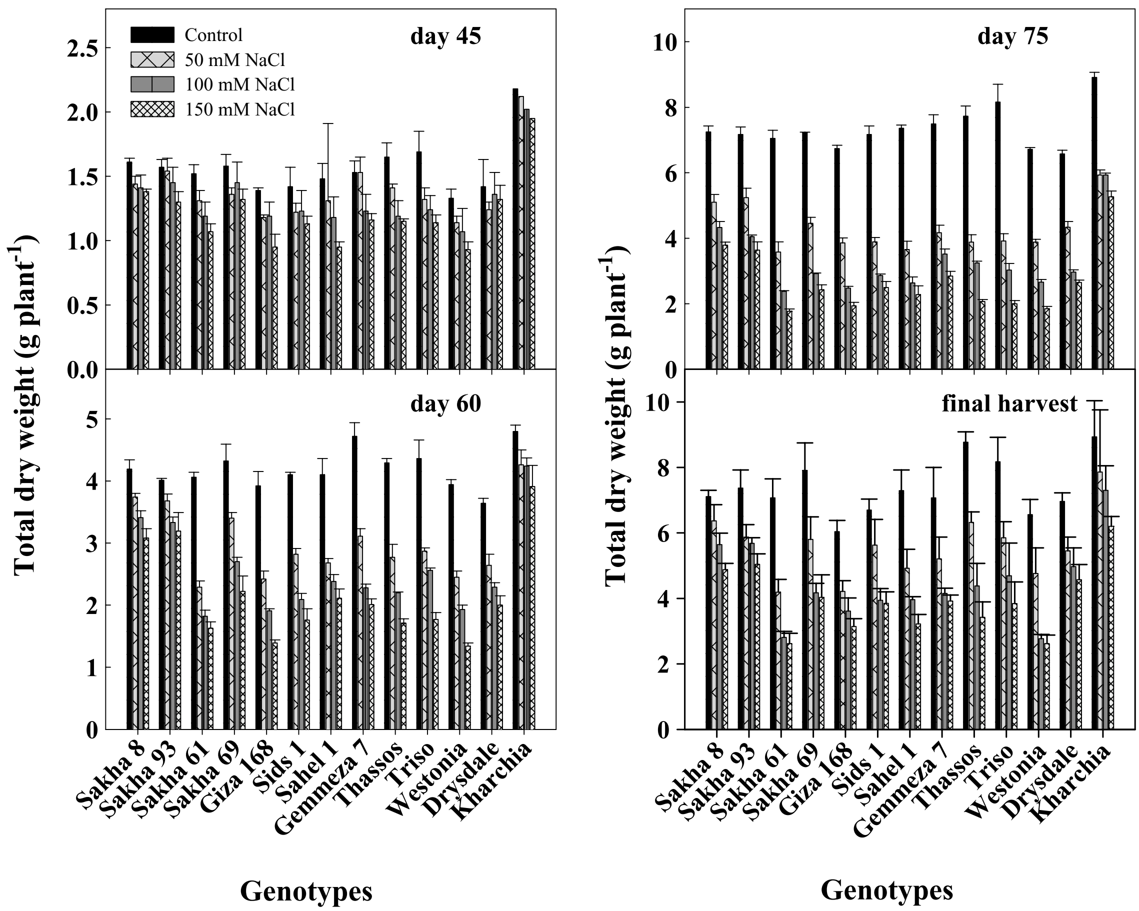

Fig. 2. Effect of different salinity levels on total dry weight per plant at 45, 60 and 75 days after sowing and at final harvest for different wheat genotypes. Error bars represent standard deviations. Error bars fit within the plot symbol if not shown.

from all varieties at 50 mM NaCl ranged from 0.78 to 1.00, from 0.57 to 0.92, from 0.48 to 0.73 and from 0.59 to 0.90 at 45, 60 and 75 days after sowing and at final harvest, respectively. At high salinity, however, they ranged from 0.65 to 0.90 at day 45, from 0.40 to 0.81 at day 60, from 0.24 to 0.59 at day 75 and from 0.37 to 0.69 at final harvest (Table 1). The results also show a wide variation among genotypes. For instance, the biomass at 150 mM NaCl was decreased by an average of 14, 22, 46 and 31% for Sakha 8, Sakha 93 and Kharchia (the most salt tolerant genotypes), whereas it was decreased by an average of 29, 59, 70 and 54% for Sakha 61, Giza 168, Sids 1, Sahel 1, Gemmeza 7, Thassos, Triso and Westonia at 45, 60 and 75 days after sowing and at final harvest, respectively (Fig. 2). The biomass indices for Kharchia, Sakha 8 and Sakha 93 were about two times greater than those for Sakha 61, Westonia, Thassos and Triso. Based on single-link cluster analysis, Kharchia was ranked as the most salt tolerant genotype, followed by Sakha 8 and Sakha 93. Sakha 61, Sids 1, Sahel 1 and Giza 168 were ranked as the most salt sensitive genotypes (Table 3).

At final harvest, grain yield per plant at 150 mM NaCl was reduced by an average 22% for the most tolerant genotypes, whereas it was reduced by an average 61% for the least tolerant genotypes (Fig. 3). On average, spike length and spikelet number on the main spike, grain number and TGW at 150 mM NaCl were reduced by 16, 16, 14 and 6%, respectively, in the three most tolerant genotypes (Kharchia, Sakha 8

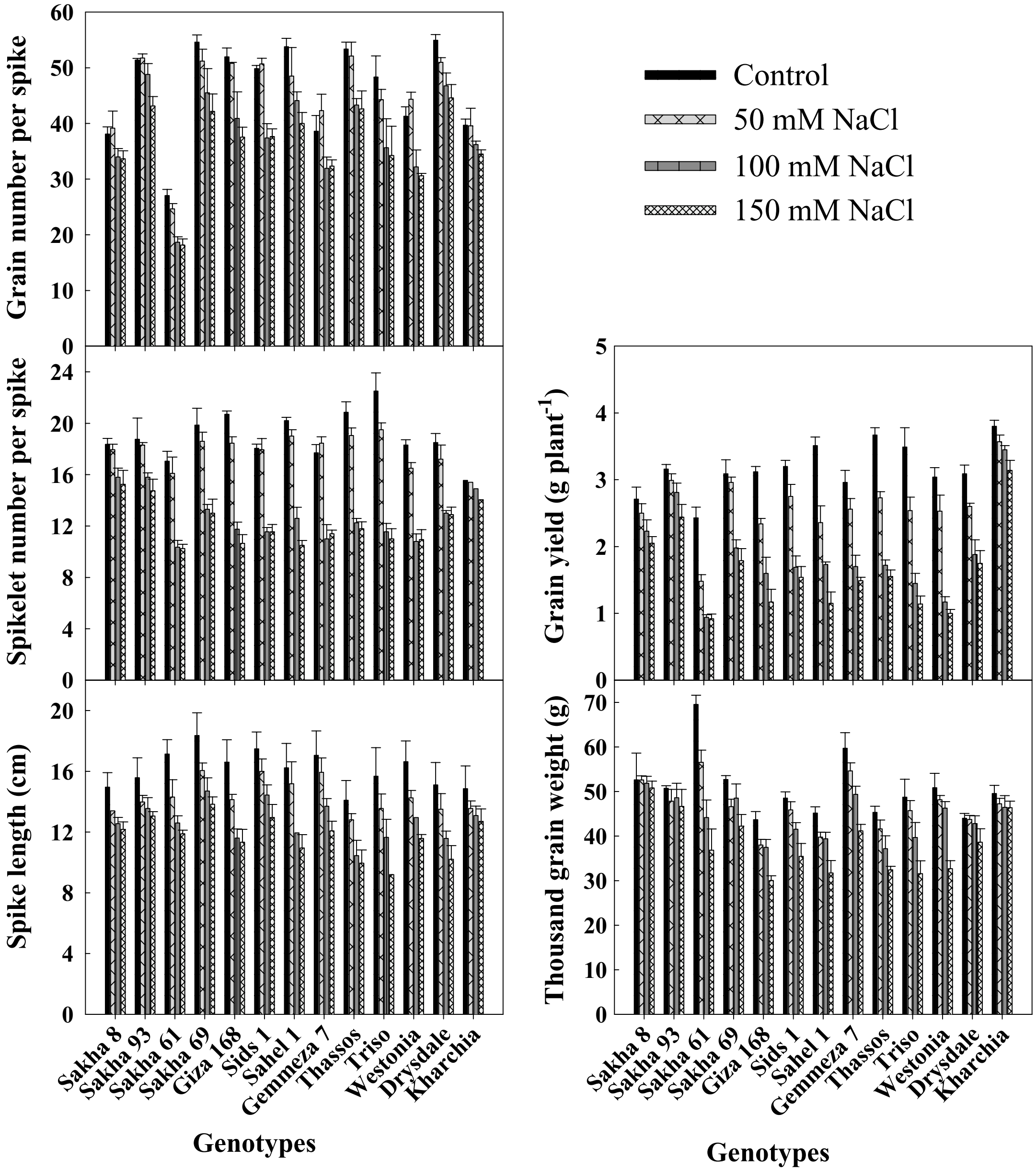

Fig. 3. Effect of different salinity levels on yield of main spike (spike length, spikelet number, grain number and 1000-grain weight) and total grain yield per plant for different wheat genotypes. Error bars represent standard deviations. Error bars fit within the plot symbol if not shown.

and Sakha 93), by 31, 43, 25 and 33%, respectively, in the least tolerant genotypes (Fig. 3).

The salt tolerance indices of yield components of the main spike (i.e. spike length, spikelet number, grain number and thousand grain weight (TGW)) were decreased with increasing salinity (Table 1). The variation of the indices among genotypes increased from low to high salinity. For instance, salt tolerance indices for spikelet number and TGW ranged from 0.89 to 1.04 and from 0.81 to 1.00 at low salinity among

Table 2
Rankings of genotypes for their relative salt tolerance in terms of plant growth parameters (i.e. number of tiller, number of leaf and leaf area per plant) at day 45 in a cluster analysis (Ward's minimum variance analysis)

Genotypes	Salinity levels (mM NaCl)			Sum	Genotype ranking	Tolerance degree
	50	100	150			
Kharchia	1	1	1	3	1	Tolerant
Sakha 8	1	1	1	3	1	Tolerant
Sakha 93	1	1	1	3	1	Tolerant
Sakha 69	1	2	1	4	2	Tolerant
Drysdale	2	2	2	6	3	Moderate
Thassos	2	3	3	8	4	Moderate
Westonia	2	3	3	8	4	Moderate
Triso	3	3	3	9	5	Moderate
Gemmeza 7	3	4	3	10	6	Sensitive
Giza 168	3	3	5	11	7	Sensitive
Sahel 1	4	3	5	12	8	Sensitive
Sids 1	4	5	4	13	9	Sensitive
Sakha 61	4	4	5	13	9	Sensitive

genotypes, respectively, whereas the indices ranged from 0.49 to 0.90 for spikelet number and from 0.53 to 0.97 for TGW at high salinity (Table 1). The salt tolerance indices at high salinity ranged from 0.59 to 0.86 for spike length and from 0.67 to 0.88 for total grain number among genotypes. According to the cluster analysis, the genotypes were divided into four cluster groups at low salinity and five cluster groups at moderate and high salinity (Table 4). The results show that Kharchia, Sakha 8 and Sakha 93 were ranked as the most tolerant genotypes, whereas Giza 168, Triso, Westonia and Sakha 61 were ranked as the least tolerant among all genotypes. Sakha 69 and Drysdale

Table 3
Rankings of genotypes for their relative salt tolerance in terms of total biomass per plant at different growth stages (at day 45, 60, 70 and at final harvest) in a cluster analysis (Single-link cluster analysis)

Genotypes	Salinity levels (mM NaCl)			Sum	Genotype ranking	Tolerance degree
	50	100	150			
Kharchia	1	1	1	3	1	Tolerant
Sakha 8	1	2	2	5	2	Tolerant
Sakha 93	1	2	2	5	2	Tolerant
Sakha 69	2	3	3	8	3	Moderate
Drysdale	2	3	3	8	3	Moderate
Thassos	3	4	3	10	4	Moderate
Westonia	3	4	4	11	5	Moderate
Triso	4	4	4	12	6	Sensitive
Gemmeza 7	3	4	5	12	6	Sensitive
Giza 168	3	5	5	13	7	Sensitive
Sahel 1	3	5	5	13	7	Sensitive
Sids 1	3	5	5	13	7	Sensitive
Sakha 61	4	5	5	14	8	Sensitive

Table 4
Rankings of genotypes for their relative salt tolerance in terms of yield components of main spike (i.e. spike length, spikelet and grain numbers and 1000-grain weight) in a cluster analysis (Ward's minimum variance analysis)

Genotypes	Salinity levels (mM NaCl)			Sum	Genotype ranking	Tolerance degree
	50	100	150			
Kharchia	1	1	1	3	1	Tolerant
Sakha 8	1	1	1	3	1	Tolerant
Sakha 93	1	1	1	3	1	Tolerant
Drysdale	2	2	2	6	2	Tolerant
Sakha 69	2	2	2	6	2	Tolerant
Sids 1	1	3	3	7	3	Moderate
Gemmeza 7	1	3	3	7	3	Moderate
Thassos	2	3	4	9	4	Moderate
Sahel 1	3	4	4	11	5	Moderate
Giza 168	3	5	4	12	6	Sensitive
Triso	3	4	5	12	6	Sensitive
Westonia	3	4	5	12	6	Sensitive
Sakha 61	3	5	5	13	7	Sensitive

Table 5
Rankings of genotypes for their relative salt tolerance in terms of grain yield per plant in a cluster analysis (Single-link cluster analysis)

Genotypes	Salinity levels (mM NaCl)			Sum	Genotype ranking	Tolerance degree
	50	100	150			
Kharchia	1	1	1	3	1	Tolerant
Sakha 8	1	2	2	5	2	Tolerant
Sakha 93	1	2	2	5	2	Tolerant
Sakha 69	2	3	3	8	3	Moderate
Drysdale	2	3	3	8	3	Moderate
Gemmeza 7	2	4	4	10	4	Moderate
Sids 1	2	4	4	10	4	Moderate
Giza 168	3	4	5	12	5	Moderate
Thassos	3	5	5	13	6	Sensitive
Triso	3	5	5	13	6	Sensitive
Sahel 1	4	5	5	14	7	Sensitive
Westonia	4	5	5	14	7	Sensitive
Sakha 61	4	5	5	14	7	Sensitive

were intermediate between the most and least tolerant genotypes.

The salt tolerance indices of grain yield per plant for Kharchia, Sakha 8 and Sakha 93 were at least two times greater than for Sids 1, Giza 168, Sahel 1, Thassos, Triso and Sakha 61 (the most salt sensitive genotypes at high salinity) (Table 1). Based on simultaneous analysis of the means of salt tolerance indices in grain yield per plant using single-linked cluster analysis, the genotypes were divided into four cluster groups at low salinity and five cluster groups at moderate and high salinity. Kharchia, Sakha 8 and Sakha 93 were ranked as the most tolerant genotypes and Giza 168, Thassos, Triso, Sahel 1, Westonia and Sakha 61 as the least tolerant among all genotypes (Table 5).

4. Discussion

Salt tolerance among wheat genotypes was evaluated in this study using cluster analysis. As pointed out by Khrais et al. (1988) and Zeng et al. (2002), the advantages of using a multivariate analysis in the evaluation of salt tolerance are that it allows: (a) a simultaneous analysis of multiple parameters to increase the accuracy of the genotype ranking; (b) the ranking of genotypes even when plants are evaluated at different salt levels and salt tolerance varies with salinity levels, especially when the salt tolerance indices are averaged across salt levels; and (c) a more convenient and accurate estimation of salt tolerance among genotypes by simply adding the numbers in cluster group ranking at different salt levels. Because there is variation of salt tolerance among the agronomical parameters and also among the different growth stages for wheat plants, the sensitive parameters, which can be single or multiple parameters, must be identified at different growth stages before using the cluster analysis.

Improving the grain yield of wheat is always the main target in plant breeding. Therefore, the evaluation of final grain yield and growth parameters determining grain yield is a critical aspect of breeding programs. The final yield of wheat is determined by the number of spikes per plant and yield components, such as spikelet number, grain number and grain weight. The number of spikes is highly correlated with the the number of tillers. The effect of salinity on tiller number and spikelet number, which both initiate during early growth stages, has a greater influence on final grain yield than on yield components in the later stages (Mass et al., 1983; Mass and Poss, 1989). Among wheat genotypes, however, the salt tolerance also changes at different growth stages (Kingsbury and Epstein, 1984; Ashraf and Waheed, 1993; Zeng et al., 2002).

Vegetative growth of wheat plants is characterized by the tillering and leaf appearance and growth on the tillers. At the vegetative growth stage, therefore, the three agronomic parameters (i.e. tiller number, leaf number and leaf area per plant) were used to evaluate genotypes for salt tolerance. Generally, the values of the three agronomic parameters decreased with increasing salinity (Fig. 1). However, salt sensitive genotypes showed a greater reduction in tiller number (e.g. by about 41%) than tolerant ones (e.g. by about 11%). This may indicate that tiller number and their behaviour under salinity can be used as simple and non-destructive measurement to evaluate wheat genotypes in breeding programs. Nicolas et al. (1994) found that salt stress during tiller emergence can inhibit their formation and can cause their abortion at later stages. When salinity levels are greater than $7.5\,\mathrm{dS\,m^{-1}}$ or 50 mM NaCl, most of the secondary tillers of moderately tolerant genotypes were eliminated, and the number of primary tillers for salt sensitive wheat genotypes was greatly reduced (Eugene et al., 1994). Paradkis (1940) found that high-tillering varieties of wheat had greater grain yield on poor soil than low-tillering ones, whereas low-tillering varieties on rich soil produced as much as or more than the high-tillering ones. Therefore, increasing the salinity tolerance in wheat may require an increase in the capacity of tillering (Islam and Sedgley, 1981).

The various yield components showed different responses to salinity. The TGW was least sensitive to salinity, whereas spikelet number was the most sensitive yield component, which is in agreement with observation in rice (Zeng and Shannon, 2000). Although final yield is directly determined after anthesis, the grain yield can be described in terms of components that are determined sequentially in the course of phenological development (Evans et al., 1975). Grain number is determined during the period of spike emergence to anthesis and grain weight is determined between anthesis and maturity (the least sensitive stage in wheat) (Kirby, 1988; Mass and Grieve, 1990; Frank et al., 1997). Because spikelets initiate at the vegetative stage, the negative effect of salinity on spikelet number indicates that the number of spikelets per spike together with the number of tillers per plant are sensitive parameters at the vegetative stage. This suggests that evaluation for salt tolerance among genotypes can be based on the genetic diversity in tiller and spikelet number. Another advantage is that the tiller number, together with spikelet number, can again be used as a simple and non-destructive measurement to evaluate large number of wheat genotypes in breeding programs; especially, because the two parameters can be determined at early growth stages.

When the developmental pattern of genotypes is so different between growth stages, assessment of the actual salt tolerance of the genotypes may be determined by comparisons of their biomass production over a long growth period (Leland et al., 1994; Munns et al., 2000), which therefore serve as another criterion to evaluate the salt tolerance. The results in this study indicate that the ranking among genotypes for salt tolerance based on the DW per plant at different growth stages was close to that based on agronomic parameters at the vegetative stage (Tables 2 and 3). This indicates that the reduction in DW was closely related to those in tiller and leaf number and leaf area (Hu et al., 1997). The reduction in total biomass in the sensitive genotypes was probably due to the extra energy utilization for osmotic accumulation, which is much more ATP-consuming for osmotic adjustment (Wyn Jones and Gorham, 1993).

A similar salt tolerance at different growth stages was observed in Kharchia, Sakha 8 and Sakha 93. The characteristics of these genotypes are more tillers, higher leaf number and greater leaf area compared with other genotypes, less effect of salinity on final grain yield and the yield components of the main spike, and the salt tolerance of these genotypes remained almost unchanged at different growth stages. Therefore, these characters of salt tolerant genotypes should be introduced in a cross breeding programs as an elite salt tolerance germplasm to incorporate different desirable agronomic traits. In addition, although the Indian wheat genotype, Kharchia, is the most salt tolerant one, Sakha 8 and Sakha 93 have the same salt tolerant characters. Compared to Sakha 8 and Sakha 93, however, Kharchia shows higher yield under non-saline conditions. Working with appropriate breeding programs, which aim to increase the yield in Sakha 8 and Sakha 93, may be more meaningful than working with Kharchia. A change in salt tolerance with growth stages was observed for Sids 1, Gemmeza 7 and Westonia. Sids 1 and Gemmeza 7 were ranked as having intermediate salt tolerance based on the parameters of yield components of the main spike and final grain yield (Tables 4 and 5), which may also result from a slight decrease in the values of EC with growing time due to the salt uptake of plant, whereas they were ranked as having poor salt tolerance based on the parameters at the vegetative stage and total DW (Tables 2 and 3). The opposite trend was observed in Westonia. Therefore, the results suggest that it might be possible to improve the salt tolerance of Sids 1 and Gemmeza 7 for salt tolerance by increasing tillering ability and/or by irrigating more frequently to alleviate soil salt stress at early growth stages. Giza 168, Triso, Thassos, Sahel 1 and Sakha 61 were more sensitive at all growth stages.

Furthermore, Drysdale and Sakha 69 were more sensitive at moderate and high salinity levels and, to become more tolerant at low salinity levels, it is suggested that maintaining the salinity at low levels is an important strategy for improving the growth of these two varieties. Because the Australian genotype Drysdale is also drought-tolerant, Sakha 69 may possibly be used for drought tolerant genotypes in Egypt because both showed similar characters in this study.

In conclusion, because Kharchia, Sakha 8 and Sakha 93 were identified as the most salt tolerant genotypes in the cluster analysis, they can be utilized through appropriate selection and breeding programs for further improvement in salt tolerance of Egyptian wheat genotypes. Because Sids 1 and Gemmeza 7 were more sensitive to salinity at early growth stages and more tolerant at the later stages, their salt tolerance can be improved by developing strategies for agronomic management according to the different growth stages, indicating that the degree of salt tolerance of wheat genotypes to salinity must be evaluated according to different growth stages. When a large number of genotypes have to be evaluated in salt tolerance breeding by using multiple agronomic parameters, cluster analysis can be used to facilitate the ranking of the genotypes for salt tolerance.

Acknowledgements

The authors thank Dr. Munns (CSIRO, Australia) for providing seeds of Westonia, Drysdale and Kharchia and Dr. Bininda-Emonds (Technical University of Munich, Germany) for his corrections of English to this manuscript. This work was supported by a scholarship from Government of Egypt to S.E. El-Hendawy.

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