Australian Journal of Crop Science AJCS
AJCS 5(10):1173-1178 (2011) ISSN:1835-2707

Improving the salt tolerance of Chinese spring wheat through an evaluation of genotype genetic variation

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Abstract

Salinity represents a major environmental constraint to crop production across the world. Therefore, the effects of salinity on plant growth, mechanisms of salt tolerance in plants, and the screening for salt-tolerant crops have gained increasing attention of late. This study evaluated the effects of salinity on the growth of three different genotypes of Chinese spring wheat (Yongliang 4, Ba 9595 and MX 9H-15) compared to the salt-tolerant wheat genotype Sakha 93 from Egypt. All wheat genotypes were grown under greenhouse conditions under four salt levels (0, 50, 100 and 150 mM NaCl). Our results showed that salinity reduced plant height, total leaf area, shoot fresh weight and dry weight in all genotypes, whereas it increased the leaf SPAD value. Nevertheless, the precise impact of salinity on plant growth varied among the different growth stages and were also genotype dependent. This study confirms that Sakha 93 is the most salt tolerant among the tested genotypes in terms of plant growth and suggests that there is the potential to improve the salt tolerance of Chinese spring wheat. Achieving the latter would play an important role in increasing the yield of spring wheat in China.

Keywords: Hetao Plain, genetic variation, salt tolerance, spring wheat.
Abbreviations: DAS-days after sowing, DW-dry weight, FW-fresh weight.

Introduction

Salinity is one of the major constraints to plant growth and leads to lower agricultural production, ecological imbalance and poor human health (Richard et al., 2006; Hu and Schmidhalter, 2004). Therefore, the effect of salt stress on plant growth and development, the mechanisms of salt tolerance in plants, and the breeding of and screening for salt-tolerant crops have received increasing attention in recent years (Singh et al., 2008). Salinity poses a potentially serious problem in Inner Mongolia, which is one of the key growing regions of spring wheat in China, its growing area of about 460,000 ha in 2005 accounting for 27.5% of the total growing area in China and its grain yield of 1.44 million tons being 23.8% of the total production in China. One major base for grain production is in the Hetao Plain of Inner Mongolia, which is characterized by a semi-arid climate and which is intensively irrigated with water from the Yellow River. The resulting combination of high natural salinity in the soil with secondary salinization poses an increasingly critical problem for crop production in this and other such regions. Indeed, Sun et al. (2004) reported that over 50% of cultivated lands in the Hetao Plain suffer from various degrees of salinity. Currently, Yongliang 4, Ba 9595 and MX 9H-15 are major spring wheat genotypes in Hetao Plain, with Yongliang 4 in particular being regarded as the best genotype and providing a good quality of wheat flour. However, despite it being known that genetic variation with respect to salt tolerance exists among several crop species including wheat, which is generally moderately tolerant to salinity (Maas, 1985; El-Hendawy et al., 2009), there is no information available in this regard for the three Chinese wheat genotypes. Therefore, the aim of this study was to evaluate the genetic variation in salt tolerance of Chinese wheat. In so doing, we compared these responses of these genoptypes to that of Sakha 93, which is regarded as a salt resistant spring wheat in Egypt (El-Hendaway et al., 2005a, b). Moreover, we also took into account the responses throughout the lifecycle of the crops, recognizing that the salt tolerance of a given crop can vary with according to its growth stages as well, with cereal plants generally being the most sensitive to salinity during the vegetative stage and less sensitive during the reproductive stages (Maas and Poss, 1989; El-Hendawy et al., 2005a, b). In so doing, we hope to better and more fully characterize the commonly used genotypes of Chinese spring wheat with respect to salt tolerance and thereby assess their suitability as crops in the face of potentially increasing salt levels.

Results

Plant height

The effects of salinity on the height of Sakha 93 were observed starting from the second week (Fig. 1). The final height of the plant subjected to high salt stress was reduced by 23% compared with the control, whereas the reduction in the plant exposed to medium salt levels was only about 7% at the second

Table 1. Salt tolerance indices of the agronomic parameters under study for four wheat genotypes under different salinity levels and at different growth stages.

Genotypes	Salinity levels (mM NaCl)	30 days after sowing				60 days after sowing		
		Total leaf area	Plant height	Shoot FW	Shoot DW	Plant height	Shoot FW	Shoot DW
Sakha 93	50	1.03	1	0.78	0.82	1.07	0.91	0.9
	100	0.91	0.94	0.52	0.6	0.96	0.82	0.84
	150	0.63	0.83	0.4	0.52	0.87	0.52	0.58
Ba 9595	50	0.84	1.06	0.86	0.89	0.98	0.94	0.9
	100	0.59	0.97	0.56	0.61	0.92	0.68	0.72
	150	0.46	0.78	0.43	0.5	0.84	0.54	0.55
Yongliang 4	50	0.87	0.98	0.9	0.92	0.97	0.93	0.88
	100	0.67	0.91	0.62	0.68	0.87	0.79	0.76
	150	0.45	0.8	0.4	0.49	0.78	0.51	0.53
MX 9H-15	50	0.69	1	0.7	0.73	0.92	1.05	0.98
	100	0.59	0.96	0.53	0.61	0.92	0.78	0.79
	150	0.42	0.87	0.37	0.45	0.84	0.5	0.55

Figure 1

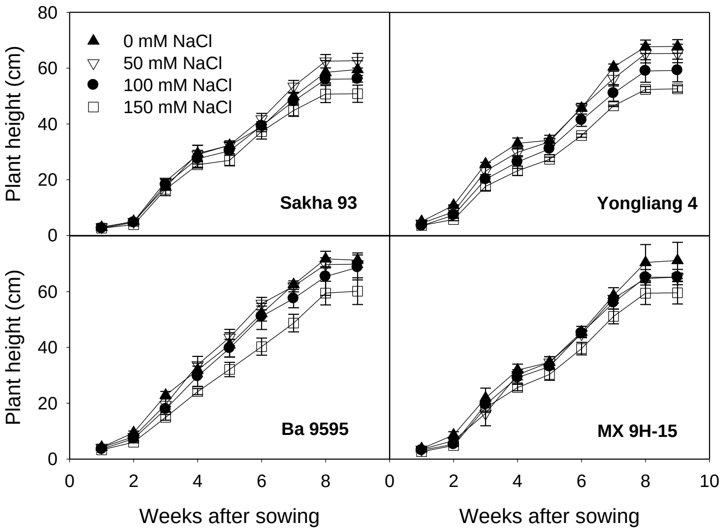

Fig 1. The effect of different levels of salt stress (0, 50, 100 and 150 mM NaCl) on the plant height of four wheat genotypes (Sakha 93, Ba 9595, Yongliang 4 and MX 9H-15) with time. Error bars represent standard deviations and fit within the plot symbol if not shown.

week after sowing. Plant heights for the genotype Ba 9595 (Fig. 1) showed significant differences between control and high salt treatments at all weeks. Significant differences between the control plants and those growing under low salt stress were limited to the fourth week after sowing. Similarly, the height of Yongliang 4 plants grown under low salt stress was significantly lower than that of the control treatment four weeks after sowing (Fig. 1), although no significant difference was observed thereafter. Moderate and high salinity levels reduced the plant heights compared to that of the control treatment throughout the entire experiment. Low and moderate salinity caused a decrease in plant height of MX 9H-15 at early stages (2-3 weeks after sowing). At high levels, the height of the plants was lower than that of the control plants, being reduced by as much as 16% after 9 weeks (Fig. 1).

Total leaf area

In all genotypes, the increased salt levels caused a reduction in leaf area per plant compared to the control at 30 DAS (Fig. 2). For example, the total leaf area of Ba 9595 was reduced by 16% (low), 42% (medium) and 54% (high) compared to control values. Similarly, the total leaf area of Yongliang 4 was reduced by 14% (low), 23% (medium) and 55% (high). The greatest reduction in the total leaf area was experienced by the genotype MX 9H-15 (Fig. 2).

Shoot fresh weight (FW) and dry weight (DW)

At 30 DAS, the shoot FW of all 4 genotypes was obviously reduced by salinity (Fig. 3). At 60 DAS, however, there was no

Table 2 Rankings of genotyes for their relative salt tolernce in terms of plant growth parameters (total leaf area, plant height, shoot fresh weight (FW) and dry weight (DW)) at days 30 and (plant height, shoot fresh weight (FW) and dry weight (DW)) at 60 days after sowing in a cluster analysis (Ward´s minium variance anylsis).

Genotypes	Salinity levels (mM NaCl)			Sum	Genotype ranking
	50	100	150		
			30 days after sowing		
Sakha 93	1	1	1	3	1 (most tolerant)
Ba 9595	2	2	2	6	2
Yongliang 4	1	3	2	6	2
MX 9H-15	3	2	3	8	3
			60 days after sowing		
Sakha 93	1	1	1	3	1 (most tolerant)
Ba 9595	2	2	1	5	2
Yongliang 4	2	2	3	7	3
MX 9H-15	3	3	1	7	3

Figure 2

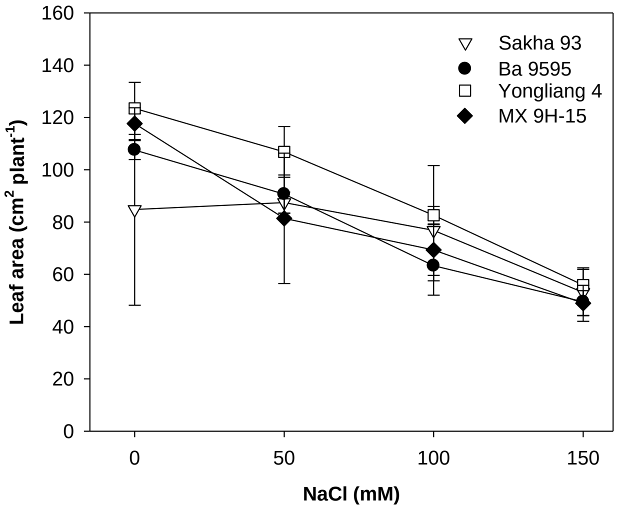

Fig 2. The effect of different levels of salt stress (0, 50, 100 and 150 mM NaCl) on the total leaf area per plant of four wheat genotypes (Sakha 93, Ba 9595, Yongliang 4 and MX 9H-15) at day 30 after sowing. Error bars represent standard deviations and fit within the plot symbol if not shown.

difference in the shoot FW between the low-salt and control treatments for any genotype. At medium to high levels, the shoot FW was reduced by 18% and 48% for Sakha 93, by 32% and 45% for Ba 9595, by 21% and 50% for Yongliang 4, and by 26% and 50% for MX 9H-15, respectively (Fig. 3). Similar to the shoot FW, the shoot DW was also decreased by salinity at 30 DAS (Fig. 3). As compared with the control treatment, for example, the DW per plant for Sakha 93 was reduced by 19% (low), 40% (medium) NaCl and 47% (high), compared with analogous values of 12%, 39% and 49% for Ba 9595; 9%, 32% and 52% for Yongliang 4; and 27%, 40% and 52% for MX 9H-15. The effect of salinity on the shoot DW changed at 60 DAS, however (Fig. 3). Except for Yongliang 4, no difference in the shoot DW existed between the control and low salt treatments. Moreover, the moderate and high salinity treatments caused comparatively higher reductions in the DW per plant for Sakha 93, Ba 9595 and Yongliang 4. An exception here was MX 9H-15, for which the moderate treatment did not cause a reduction in the shoot DW compared to the control plants.

Leaf SPAD value

SPAD values at DAS were generally increased by salinity regardless of genotype, although there was no significant difference between the low-salt and the control treatments for Yongliang 4 (Fig. 4). The SPAD values were around 8% (low), 12% (medium), and 10% (high) higher than those of the control for Sakha 93, with analogous values of 11%, 10% and 16% for Ba 9595; 5%, 10% and 14% for Yongliang 4; and 13%, 10% and 17% for MX 9H-15. A similar trend for the SPAD value of the flag leaf was observed in all genotypes at DAS (Fig. 4). However, there were no significant differences in the SPAD values between the moderate salt and the control treatments for Sakha 93, Yongliang 4 and MX 9H-15.

Salt tolerance indices and ranking of genotypes for salt tolerance

The salt tolerance indices of plant height ranged from 0.98 to 1.06 for the four genotypes at low salt levels and generally

Figure 3

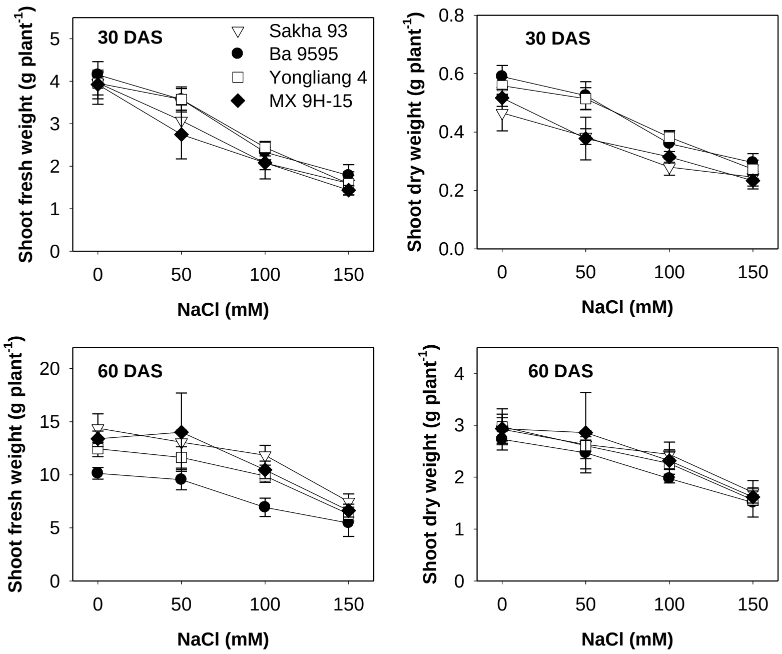

Fig 3. The effect of different levels of salt stress (0, 50, 100 and 150 mM NaCl) on the shoot fresh and dry weight per plant of four wheat genotypes (Sakha 93, Ba 9595, Yongliang 4 and MX 9H-15) at days 30 and 60 after sowing (DAS). Error bars represent standard deviations and fit within the plot symbol if not shown.

Figure 4

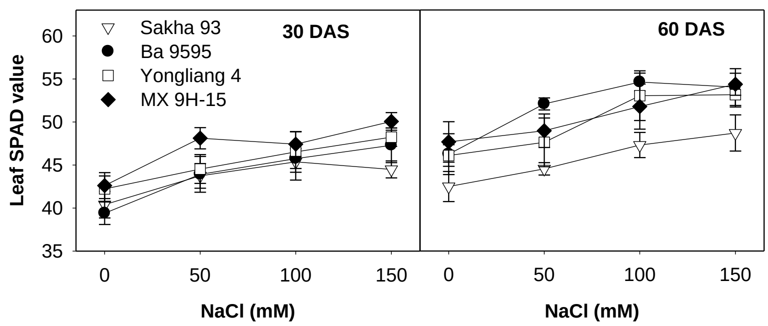

Fig. 4. The effect of different levels of salt stress (0, 50, 100 and 150 mM NaCl) on the leaf SPAD value of four genotypes (Sakha 93, Ba 9595, Yongliang 4 and MX 9H-15) at days 30 and 60 after sowing (DAS). Error bars represent standard deviations and fit within the plot symbol if not shown.

decreased with increasing salt concentrations (Table 1). This effect was even more pronounced for total leaf area with the indices decreasing from a range of 0.69 and 1.03 at low salt levels to 0.59 and 0.91 at medium salt levels, and 0.42 and 0.63 at high salt levels. The salt tolerance index of Sakha 93 was the highest of all genotypes, whereas that of MX 9H-15 was the lowest (Table 1). The salt tolerance indices determined on the basis of shoot fresh weight also decreased dramatically with increasing salinity for all genotypes (Table 1). However, regardless of the level of salinity and genotype (the only exception being Yongliang 4 in the low salt treatment), the salt tolerance indices for shoot FW were higher at 60 DAS compared to 30 DAS. For example, the indices rose from a value of 0.78 at DAS to 0.91 at 60 DAS at low salt concentrations and from 0.5 to 0.55 at high salt concentrations for Ba 9595. Similarly, the salt tolerance indices of the shoot DW decreased with increasing salt levels for all genotypes as well as increased over time (Table 1). For example, the indices for Sakha 93 increased to 0.90 from 0.82 between 30 and 60 DAS under the low salt treatment, with analogous values for Ba 9595 under the high salt treatment bring 0.54 and 0.43. The results of the ranking of genotypes for salt tolerance by cluster analysis in Table 2 confirm that Sakha 93 is more salt tolerant than other genotypes, whereas MX 9H-15 is the least tolerant. Among three Chinese genotypes, Ba 9595 is the most tolerant one to salinity.

Discussion

The salt tolerance indices measured on the basis of total leaf area are commonly used to evaluate the salt tolerance of wheat genotypes (El-Hendawy et al. 2005a). Genotypes that maintain a high index under salinity stress are considered tolerant. On this basis, this study confirms that Sakha 93 is more salt tolerant than other genotypes, whereas MX 9H-15 is the least tolerant (Table 2). Our results in Figure 1 and Table 1 suggest that the effect of salinity on plant height was both genotype dependent and also varied with the total time of salt exposure. In particular, the difference in plant height between the control and salt treatments for all genotypes increased after 3-4 weeks growth. In addition, all genotypes were more sensitive to salt stress during the vegetative stage, which agrees with the study by Neumann (1995). The reduction in plant growth through salt stress may be related to several physiological responses, including the effects of salinity on any or all of water status, ion imbalance and ionic toxicity, or carbon allocation and utilization (El-Hendawy et al., 2005a; 2007; Tammam et al., 2008). Benderradji et al. (2011) observed that Na^+ fluxes from roots to leaves is regulated with two members of the HKT tranpsorters family (HKT1;5 and HKT2;2). Of all the growth parameters examined, total leaf area per plant was affected the most, with the drastic reduction in leaf area indicating the high sensitivity of leaf growth to salinity (see also Hu and Schmidhalter, 2004). The effects arose mainly through a reduction in leaf growth and expansion (Asish et al., 2005; Hu and Schmidhalter, 2005; Hu et al., 2006), with the latter possibly obtaining from a loss in cell turgor pressure or a change in hormonal signaling from the roots to the leaves (Ball,1988). As for plant height, our results of the effect of salt stress on total fresh weight suggest that the impact of salt stress differed among the four genotypes examined and varied over time. In the latter case, the influence of salinity was greater at the earlier growth stage (30 DAS) than at the later one (60 DAS). Similar decreases in the FW and DW of leaves, stems and roots due to salt stress have been reported in wheat, rice, pepper and guava (AliDinar et al., 1999; Chartzoulakis and Klapaki, 2000; Zeng et al., 2002; El-Hendawy et al., 2005a, Hu et al., 2006). Finally, measurements of the SPAD value showed an increase under saline conditions, both in the 4th leaf at day 30 and in the flag leaf at day 60 after sowing. Similar results were obtained by Wang and Nil (2000) in *Amaranthus*. Because the SPAD value generally correlates with chlorophyll content (which was not specifically measured here), our results suggest an increase in leaf chlorophyll content under saline conditions, possibly due to the reduction in the leaf area. However, other studies show the opposite trend, with Agastian et al. (2000) reporting a general decrease in chlorophyll content of leaves under salt stress and Del Zoppo (1999) finding that levels of 50-150 mM NaCl caused a relatively small, but non-significant decrease of the chlorophyll α and chlorophyll β content in wheat plants. Nevertheless, the SPAD value represents an important physiological trait that has been used to evaluate the salt tolerance of wheat plants (El-Hendawy et al., 2005a). It is also especially appropriate for use in field studies, given that measuring it using an SPAD meter is quick, easy and non-destructive (El-Hendawy et al., 2009).

Materials and methods

Growth conditions

Four wheat genotypes, Sakha 93 from Egypt, and Ba 9595, Yongliang 4 and MX 9H-15 from Hetao Plain (40º10´-41º10´N, 106º25´-112ºE; Temperature: 5.6-7.4ºC (annual average); 22-24ºC (July); Annual precipitation: 150-400 mm), were grown under greenhouse conditions in the research station of the Technische Universität München (TUM), Dürnast (48º24´N, 11º41´E; Annual temperature: 7.5 º C; Annual precipitation: ca 800 m), from the middle of August to the end of October 2008. The greenhouse temperatures at Dürnast ranged between 24 to 30℃ during the daytime to 16 to 19℃ during the night. Uniform wheat seeds of each genotype were surface-sterilized and allowed to pre-germinate in Petri dishes under room temperature for 24 h; thereafter, 25 wheat seeds were sown into pots (diameter 20 cm) designed to prevent leaching possibility. The pots were filled with 6.5 kg of air-dried and thoroughly mixed loamy soil in five layers. The final water content of 25% of dry soil basis was achieved by adding tap water or salt solution as appropriate. In all cases, the topmost layer was kept salt-free to prevent osmotic shock during seed germination and filled with 1.1 kg soil plus 225 mL tap water. Otherwise, each of the four subsurface layers was filled with 1.35 kg soil plus 200 mL of the appropriate salt solution. In total, four salt treatments were examined: 0, 50, 100 and 150 mM NaCl (= control, low, medium, and high, respectively). After one week, the pots were thinned to 18 plants per pot. To facilitate plant growth, mineral fertilizers (0.85 g NH_4NO_3 per pot) were applied three times during the growth period according to our previous experiments (Ruan et al., 2008).

Determination of plant growth

Seven days after sowing (DAS), plant height was recorded weekly using a ruler until growth had ceased. SPAD values of the 4th leaf were measured using the chlorophyll meter SPAD 502 (Minolta, Osaka, Japan) starting four weeks after sowing. Eight weeks after sowing, the SPAD of the flag leaf was also determined. The plants were harvested either 30 or 60 DAS. At the first harvest, the plants were separated into stems and leaves. The total leaf area per pot was measured using a LI-3000A leaf area meter (Li-COR, USA). Both the fresh weight (DW) of the plant shoots as well as the dry weight (DW) after drying at 65℃ for 48 h were determined. At the second harvest, the shoot FW and DW were determined.

Salt tolerance indices and ranking of genotypes for salt tolerance

Following Zeng et al. (2002) and El-Hendawy et al. (2005a), all the data about growth parameters were converted to salt tolerance indices before cluster analysis to allow comparisons mong genotypes for salt tolerance by using multiple agronomic parameters. Briefly, salt tolerance indices were calculated as the observed value of a target trait under a given salinity level divided by the mean value for that trait under the control treatment. Cluster group rankings were obtained based on Ward´s minimum variance cluster anaylsis of the averages of the salt tolerance indices for the several growth parameters at 30 and 60 DAS. All procedures of cluster analysis are described fully in the JMP User´s guide (SAS Institute, 2000) and by El-Hendawy et al. (2005a).

Statistical analysis

The experiment was arranged as a completely randomized design with 4 replications per salt treatment (n=4). All growth parameters and SPAD values were analyzed using individual analyses of variance (ANOVA) in SPSS 13.0. Terms were considered significant at $P \leq 0.05$.

Conclusion

Our results show that salinity reduced all of plant height, total leaf area, and shoot FW and DW in all genotypes, whereas the leaf SPAD values were increased. However, the precise impact was genotype dependent and also varied among the different growth stages. Of the genotypes examined, Sakha 93 was found to be more tolerant against salinity in terms of total leaf area per plant than were the three Chinese genotypes (Ba 9595, Yongliang 4 and MX 9H-15). Thus, there would appear to be the potential to improve the salt tolerance of Chinese spring wheat, a result that would achieve significant agricultural and economic benefits with it.

Acknowledgements

The authors thank the DAAD and CSC for supporting PPP research project. Many thanks to Dr. Shihua Guo (IMAU, China) for providing seeds of Yongliang 4, Ba 9595 and MX 9H-15.

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