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Effect of Salinity on Morpho-Physiological Characteristics of Spring Wheat Genotypes

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Abstract
Bread wheat germplasm tolerant to salinity with high end-use quality is required to maintain grain production in saline lands. Four spring bread wheat cultivars with different tolerance to salinity and their three F_3 progenies were evaluated at four levels of salt concentrations; 0, 150, 200 and 250 Mm NaCl. Na^+, K^+ and Cl^- concentrations in the wheat penultimate leaf and some of the biomass and yield related traits were measured. The allelic variations of *Glu-1* loci in the crosses were examined. Salinity had a significant positive effect on Na^+ and Cl^- concentrations and the Na^+/K^+ ratio. As salinity level increased, yield and 1000 grain weight and K^+ concentration were declined. Most of the F_3 progenies did not show much improvement in terms of biomass or yield related traits. However, F_3 progenies of the Cajema×Lerma Rojo cross showed an improved quality score.

Keywords: Quality; Salinity; Wheat; Yield

Introduction

With food productivity decreasing due to the deleterious effects of stressful agricultural conditions such as drought, cold and salinity (Mahajan and Tuteja 2005), minimizing yield losses is a major area of concern for all nations to cope with the increasing food requirements. Bread wheat (*Triticum aestivum* L.), along with other important cereal crops, constitutes a major portion of the total calorie requirements to most people worldwide, particularly to those residing in developing countries. Plant-base combat (i.e., developing saline resistant cultivars) with saline conditions seems to be the most promising way in minimizing the yield losses due to salinity (Harati and Noori 2005). However, breeding for adaptation to abiotic stress conditions is challenging because of the complexity of (i) the target environments and (ii) the adaptive mechanisms adopted by plants when exposed to the stressful conditions (Reynolds *et al*. 2005). Plant responses to salinity occur in two phases: a rapid osmotic phase that inhibits growth of young leaves and a slower ionic phase that accelerates senescence of mature leaves (Munns *et al*. 2002). The direct selection of superior salt-tolerant genotypes under field conditions is hindered by the significant influence that environmental factors have on the response of plants to salinity (Yamaguchi and Blumwald 2005).

In plants, increases of salt concentrations affect nearly all the major physiological processes including photosynthesis, protein synthesis, energy metabolism and lipid metabolism (Parida and Das 2005). The sensitivity of rice crop to salinity was attributed to the plant's inability to keep sodium (Na^+) and chloride (Cl^-) ions out of the transpiration stream (Hollington 1998). While increases in leaf Na^+ concentrations may help to maintain plant turgor, Na^+ cannot completely substitute for K^+ which is specifically required for protein synthesis and enzyme activation (Marschner 1988). Maintenance of adequate levels of K^+ is essential for plant survival in saline habitats. High K^+ concentrations in the stroma are necessary for the maintenance of optimum photosynthetic capacity under stress conditions (Chow *et al.* 1990). Under saline-sodic or sodic conditions, high levels of external Na^+ not only interfere with K^+ acquisition by the roots, but also may disrupt the integrity of root membranes and alter their selectivity. Application of external K^+ fertilizer resulted in increases in corn yield grown in sandy soil irrigated with increasing levels of saline water, though not proportional to the salty irrigating waters (Bar-Tal *et al*. 1991). The authors, then, concluded that other than its beneficial effects on increasing Na^+/K^+ ratio in the plant cells, fertilization with K^+ did not reduce the deleterious effects of salinity. Chilling and salinity stresses have severely reduced 1000 grain weight in durum wheat (Katerji *et al.* 2005). They successfully improved 1000-grain weight, ash content and ß-carotene content of their durum wheat germplasm. However, the main parameters explaining gluten indices declined considerably in their durum wheat progenies.

The major class of glutenin polypeptides have been identified in wheat endosperm, designated as HMW-GS and LMW-GS; both classes occur in flour as cross-linked proteins, resulting from inter-polypeptide disulphide linkage. These genes coding for HMW-GS (*Glu-1*) and LMW-GS (*Glu-3*) are located respectively on the long and short arms of 1A, 1B and 1D chromosomes (Payne *et al.* 1980; Gupta *et al*. 1991). It is generally accepted that there are additive effect and epistasis interaction between glutenin subunits for bread making quality (Gupta *et al*. 1989; Nieto-Taladriz *et al*. 1994). The relationship between protein composition and bread making quality showed that the quantities of total flour protein, albumin+ globulin, and high and low molecular weight glutenin subunits in flour were significantly and positively correlated with bread loaf volume. However, the ratio of high to low molecular weight glutenin subunits had little association with loaf volume (Wang *et al*. 2007).

In this work, the morpho-physiological characteristics of the progenies of three wheat crosses along with their parental lines were examined. We studied the effect of three levels of salinity along with an untreated control and reported the ionic concentrations (Na^+, K^+ and Cl) in the prependecular leaf of the bread wheat. Of particular interest, we also examined the transgressive segregation of alleles responsible for wheat quality within these crosses and interestingly, observed transgressive segregant progenies of one of the crosses with improved quality score (Payne *et al*. 1981).

Materials and Methods

Germplasm development and experimental design

In this study, we examined the ionic concentrations effects on yield components of the parental lines and the progenies of the test crosses obtained from a common moderate salt-tolerant spring wheat cultivar from CIMMYT- Mexico "Cajema" as the common parent and three other parents including "Sette Cerros", Lermaroja (both moderate salt tolerant cultivars from CIMMYT-Mexico), and Ho_2 (a moderate salt tolerant cultivar from Libya). We also screened the parental lines and the progenies to investigate the transgressive segregations in allelic variations in the germplasm studied. The crosses were conducted at Abouraihan Campus, University of Tehran. For morphological and quality scoring determinations, F_3 generation of these test crosses and the parental lines were used along with one check cultivar (a British salt-sensitive) - "Axona". For assessing morphological and mineral concentrations, a factorial experiment with two factors, i.e. germplasm and salinity, was employed. The germplasm factor included (i) the four parental lines, (ii) the three test crosses at F_3 generation, and (iii) the salt-sensitive check cultivar (eight in total). The salinity factor consisted of four levels of salty water, i.e. 0 mM, 150 mM, 200 mM and 250 mM. We conducted the experiment in a randomized complete block design with three replications. After normalizing the data by square root transformation, the GLM procedure was used for statistical tests of significance. Dancan Multiple Range Test (DMRT) was used for mean comparisons.

Growth condition and salinity stress imposition

The experiment was conducted in a glasshouse with a day/night temperature of 22±2 °C/16±2°C. The plants were grown in natural daylight supplemented with 400 watt mercury vapor lamps for 16 h per day. We used river sand as potting medium. The sand was washed with tap water for one week and oven dried. Plastic pots of 18 cm diameter / 19 cm deep were filled with 4.40 kg oven-dried sand. The potting medium prior to the experiment was equilibrated with full strength nutrient solution (Hewitt *et al.* 1966). For salt stress imposition, we designated four irrigation regimes of full strength nutrient solution (Hewitt *et al*. 1966) each accompanied by NaCl at final concentrations of 0 mM (for control), 150, 200 and 250 mM. Seeds were germinated during six days and thereafter, five seedlings of each breeding material were transplanted in each pot (10×10cm). Salt imposition was started 18 days after the experiment commencement and the concentrations were increased gradually by increasing increments of 25 mM every other day until the final treatment concentration was met. Additionally, twice per week, 400 ml of deionized water was added to each pot to maintain sand moisture and to prevent salt accumulation.

Plant morphological and physiochemical characteristics

For plant characters we measured plant height (PH), spike length (SL), spike weight (SW), straw weight (STW), number of grains per spike (NGPE), grain yield per plant (GYPP) and 1000 kernel weight (1000 KW). For assessing sodium, potassium and chloride ionic contents in the leaves, the perpendicular leaf from 2-3 plants of each replication were removed 30 days after the beginning of the salt treatment (Chow *et al*. 1990; Harati and Sadat Noori 2005)

Protein extraction and assessing banding patterns

For protein extraction, we employed the sequential extraction procedure described by Singh *et al*. (1991), with some modifications (Izadi-Darbandi *et al*. 2010). Identification of banding patterns of HMW-GS and giving quality scores were conducted according to Payne and Lawrence (cited in Payne *et al*. 1981).

Results and Discussion

Salt effects on yield related traits

Salinity treatments decreased biomass and yield related traits in wheat germplasm, invariably. There was significant differences among genotypes for plant height, spike length and number of grains per spike. We observed considerable decreases in the biomass and yield associated traits in wheat parental lines and F_3 progenies. Analysis of variance (Table 1) revealed statistically significant (p-value<0.0001) difference amongst the four levels of salinity for plant height, straw weight, spike length, spike weight, number of grains per spike, grain yield per plant and 1000 grain weight. Decreases of biomass and yield components are hallmarks of growing crops in saline conditions and were repeatedly reported in the literature (Allakhverdiev *et al*. 2000; Reddy *et al*. 2003; Mahajan and Tuteja 2005; Farooq and Azam 2006; Soltani *et al*. 2006; Kara and Altindal 2011). Despite the prominent and significant differences observed in the levels of salinity for biomass and yield related traits, the germplasm revealed to have significant effect only on plant height and spike length (Table 1) (p-value<0.0001) and on the number of grains per spike (p-value<0.05). Comparison of means indicated that NaCl concentration of 150 mM imposed considerable adverse effect on the

Table 1. Analysis of variance of some morpho-physiological traits of wheat genotypes under salinity stress.

Sources of Variation	Salinity	Genotype	Salinity × Genotype	Error	R-Square (%)
df	3	7	21	64	
Biomass associated traits					
Plant height	6667.82***	215.94***	116.32**	36.29	91
Straw weight	5.13***	0.10	0.04	0.10	73
Yield related traits					
Spike length	43.14***	5.87***	1.17**	32.04	85
Spike weight	23.16***	0.06	0.08	14.42	83
Number of grains per spike	4316.24***	51.08*	24.59	21.34	91
Grain yield per plant	15.88***	0.05	0.03	0.08	91
1000 grain weight	5255.18***	34.55	31.84	2190.01	88
Physiochemical traits					
Chloride [Cl -]	23.017***	1.38	0.87	0.79	66
Sodium [Na+]	13.62***	1.47**	0.44	0.33	74
Potassium [K+]	4.26***	0.06	0.02	0.04	85
Na+ / K+ ratio	120.47***	7.68*	3.26	2.99	72

*: P< 0.05, **: P< 0.01, ***: P< 0.001

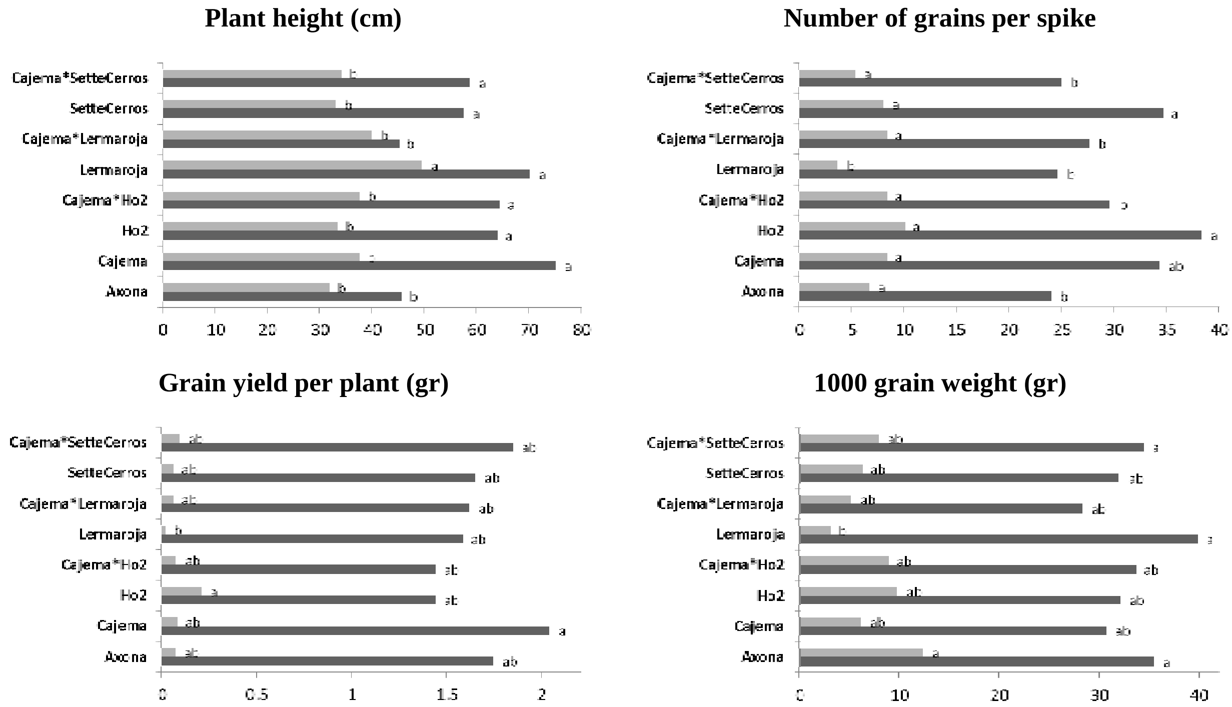

Figure 1. The mean reduction in plant height, number of grains per plant, grain yield per plant and 1000 grain weight in wheat genotypes.

germplasm growth and for concentrations beyond 150 mM there was no considerable decrease. Figure 1 shows decreases observed in plant height, number of grains per plant, grain yield per plant and 1000 grain weight upon imposition of 150 mM NaCl on wheat germplasm. Accordance with other research reports (Francois *et al.* 1986; Katerji *et al*. 2005; Farooq and Azam 2006), salinity has shown a highly significant (p-value<0.01) effect on yield components and increases in salt concentration reduced grain yield per plant and 1000 grain weight significantly.

Salt effects on ion concentration

Analysis of variance showed significant effect of salinity on concentrations of chloride, sodium and potassium and Na^+/K^+ ratio whereas, the germplasm showed only significant effect on sodium concentrations and Na^+/K^+ ratio (Table 1). Upon imposition of salt stress by increasing concentration of NaCl from 0 mM to 150, 200 and 250 mM, we observed the sharpest changes of ionic concentrations occurred from 0 to 150 mM beyond which little or no significant change was detected in the ionic concentrations of chloride, sodium and potassium. In the tested germplasm, increasing concentrations of NaCl caused increases in Na^+ and Cl^- accumulation and decreases in K^+ concentrations in the prependecular leaf of wheat (Figure 2). To present the ionic concentrations in the normally grown wheat and salt stressed germplasm, we averaged the concentrations in all NaCl treated groups and showed them in a two groups of normal versus salt stressed groups (Figure 3). The results were in concordence with the report by Khatun and Flowers (1995) who showed the increase in Na^+

and Cl^- concentrations and decrease in K^+ concentration upon NaCl stress. Negative correlation between Na^+ and K^+ was also postulated before (Marschner 1988; Harati and Noori 2005; Farooq and Azam 2006). Maintenance of low Na/K ratios is thought as one favorable index for salt tolerance (Zhu *et al*. 1998). Analysis of variance showed availability of genetic variation for this character among the germplasm tested. Cajema as a common parent showed the lowest amount of Na/K ratio and F_1 generation between Cajema*Ho2 got the highest related ratio. It seems that the salt tolrenace of Cajema was higher than its cross with Ho2.

Allelic variations in *Glu-1* loci

Apart from the trends observed in yield related traits and changes reported in ionic concentrations in agreement with other previously reported studies, we also noticed a critical observation when profiled the allelic variations of *Glu-1* loci in the parental lines and their progenies. We observed a transgressive segregation, occurred likely due to a crossing over, for improved quality score among F_3 progenies of one of the wheat crosses (Cajema×Lerma Roja). Table 2 summarizes the allelic variations within the parental lines as well as the progenies in *Glu-1* loci. Among evaluated germplasms, the Payne quality scores were 6 (low), 8 (moderate) and 10 (good). Glutenin profile of the parental lines Cajema and Lerma Roja and their hybrid (Cajema×Lerma Roja) is shown in Figure 3. The *Glu-D1* allele in the progenies of Cajema×Lerma Roja was “5+10” alleles instead of their parental “2+12” and 17 instead of 7+8 and 17+18 alleles in *Glu-B1*. This observation can be explained by a crossing over occurred in this cross that adds up the quality score.

Table 2. Allelic variation in *Glu-1* loci of wheat genotypes profiled on a SDS-PAGE pattern.

Germplasm	*Glu-A1*	*Glu-B1*	*Glu-D1*	Payne Scores
Cajema×Sette Cerros	2*	7+8	2+12	8
Cajema×Ho2	1	7+8	2+12	8
Cajema×Lermaroja	2*	17	5+10	10
Axona	Null	7+8	2+12	6
Sette Cerros	Null	7+8	2+12	6
Ho2	2*	17+18	2+12	8
Lermaroja	2*	17+18	2+12	8
Cajema	2*	7+8	2+12	8

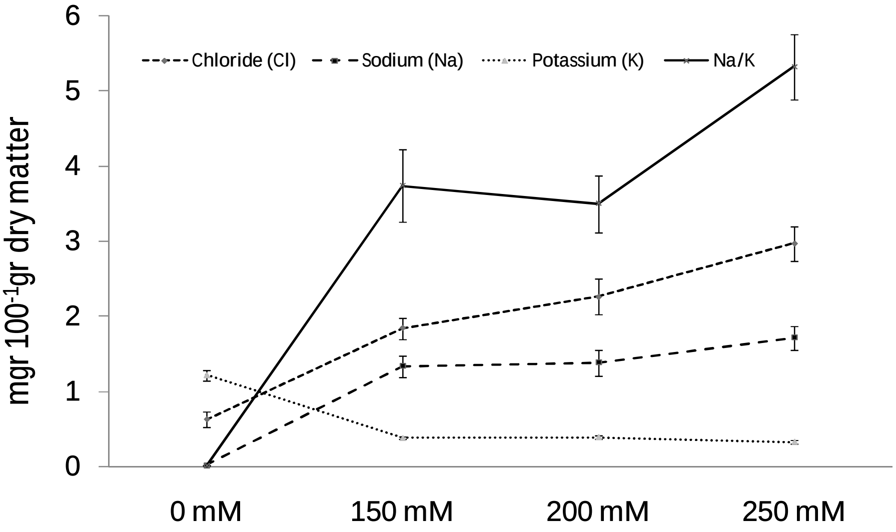

Figure 2. Changes in ionic concentration of Cl^-, Na^+, K^+ and Na^+ / K^+ ratio averaged over all wheat genotypes.

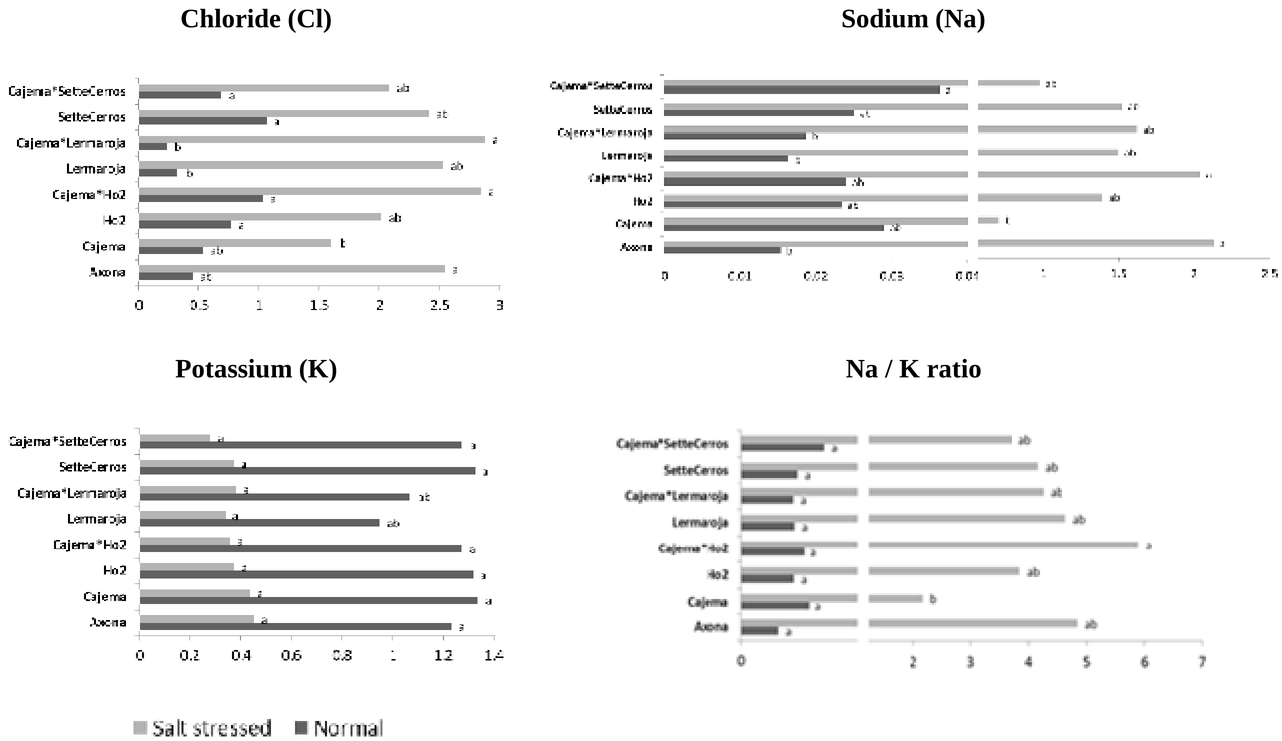

Figure 3. The means of Cl^- , Na^+ and K+ concentrations as well as Na^+ / K^+ ratio in wheat genotypes. The concentrations are given in mgr 100^{-1}gr dry matter.

Salinity had significant effect on concentrations of chloride, sodium and potassium and Na^+ / K^+ ratio. The mean values of F_3 generations in terms of yield and biomass was not reduced compared to their respective parents. It is expected that we will, hopefully, find more potential of salt tolerant lines in the next generations by transgressive segregation. It could be due to separation of the effective genes of salinity tolerance in F_3 progenies. We found transgressive segregation in case of quality scores in F_3 progenies in one of the wheat crosses (Cajema×Lerma Roja).

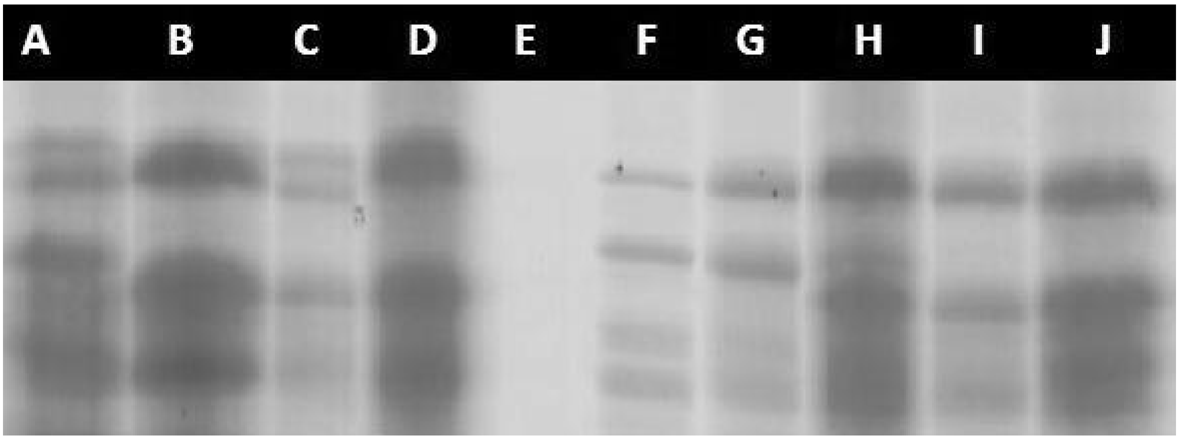

Figure 4. SDS-PAGE banding patterns of *Glu-1D* loci in the wheat genotypes (from left to right; A: Cajema×Ho2, B: Ho2, C: Cajema×Lerma Rojo, D: Lerma Rojo, E: No sample (control), F: Chines Spring, G: Cajema×Sette Cerros, H: Cajema, I: Sette Cerros and J: Axona).

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