INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
13–1109/2014/16–6–1059–1066
http://www.fspublishers.org

Full Length Article

Generation Mean Effects, Heterosis and Heritabilities for Seedling, Adult and Physiological Salinity Tolerance in Spring Wheat (*Triticum aestivum*)

Zulfiqar Ali[1,*], Abdus Salam Khan[1], Ihsan Karim[1], Muhammad Uzair[1], Tariq Mahmood[1], Tariq Saeed[2], Sehrish Sarwar[1], Nida Ghori[1], Zunaira Nisar[1], Syeda Samara Sarwat[1] Abdul Qayyum[3] and Asif Ali Khan[1]
[1]*Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad-38040, Pakistan*
[2]*University of Gujrat, Pakistan*
[3]*Department of Plant Breeding and Genetics, University College of Agriculture, BZU, Multan, Pakistan*
*For correspondence: zulfiqar_ali@uaf.edu.pk; zulfiqarpbg@hotmail.com

Abstract

Soil salinity among other abiotic stresses is a major threat to cultivated land. Breeding salt tolerant cultivars has always been thought to be an effective and cheaper way to overcome salinity problem. Salt tolerant and salt sensitive wheat accessions were hybridized to develop genetic material to investigate inheritance of salt tolerance based on early seedling, adult plant and physiological responses. Salt tolerance in low salinity appeared to be a complex polygenic trait. However, genetic models for most responses were poor fit in high salinity and suggested further investigations. Differences in gene expressions in different NaCl concentrations appeared to be due to different gene regulation or interaction and/or involvement of additional or hidden genes. Both additive and non-additive gene effects required to be considered while designing of breeding programme for improving salt tolerance in wheat and in the statistical model used to find QTL for the salt tolerance. This QTL couple with recurrent selection for specific combining ability of the 4WLRG/1-8 with an excellent cultivar of wheat appears to be a good salt tolerant breeding strategy. © 2014 Friends Science Publishers

Keywords: Additive; Non-additive; Gene effects; NaCl stress tolerance; Generation means

Introduction

Increasing food crisis are forcing agriculture sector and stake-holders to bring more land under cultivation. Global food production will need to be enhanced by about 50% by 2050 to meet the expected population growth (Flowers, 2004; Rengasamy, 2006). At the same time, the most appropriate land has already been cultivated, implying a need for either expansion into new areas to get the projected target or a dramatic increase in production of crop on existing cultivated lands. Salinity is a major threat to this land and above 800 million hectares of land globally is salt-affected (Munns, 2005), consisting of nearly 7% of the total land area of the world. Various chemical, engineering, biological, genetic etc. approaches were advocated to overcome the problem of soil salinity. So, soil salinity is receiving much attention from plant breeders due to its considerable and increasing potential threat to agricultural production. The development of salt tolerant cultivars has always been thought to be one of the effective and cheaper means of tackling the problem of salinity (Shannon, 1984; Qureshi *et al.*, 1990; Hollington, 2000; Ma *et al.,* 2012).

Availability of genetic variability for salinity tolerance in the species, reliable method of screening the germplasm for salt tolerance and a suitable breeding methodology are prerequisites for improving salt tolerance. Previously existence of variation for salt tolerance in bread wheat (Munns *et al.*, 2000; Khan *et al.*, 2003; Ali *et al.*, 2002, 2005, 2007, 2012), methods of screening (Rawson *et al.*, 1988; Jafari-Shabestari *et al.*, 1995; Munns and James, 2003) and breeding methodology (Richards, 1983; Royo and Aragüés, 1999) have been reported. Marker-assisted selection has also been suggested to be the most important for screening of germplasm (Landry and Michelmore, 1987). Recent molecular genetics and plant electrophysiological studies imply that the abilities of a plant to sustain a high cytosolic K^+/Na^+ ratio appear to be critical towards salt tolerance. So far, plant breeders have extensively targeted their efforts towards improvement in K^+/Na^+ ratio by reducing Na^+ uptake and its transport to shoot. The attempts being made by traditional breeding, marker-assisted selection and genetic engineering have so far contributed in the improvement of salt tolerance by (1) breeding for better Na^+ exclusion and (2) breeding for better osmotic adjustment (Shabala and Cuin, 2008). The first approach was particularly advocated for wheat breeding (Munns, 2006). The work on plant genetic improvement in wheat has made some progress and a few salt tolerant wheat

To cite this paper: Ali, Z., A.S. Khan, I. Karim, M. Uzair, T. Mahmood, T. Saeed, S. Sarwar, N. Ghori, Z. Nisar, S.S. Sarwat, A.Qayyum and A.A. Khan,, 2014. Generation mean effects, heterosis and heritabilities for seedling, adult and physiological salinity tolerance in spring wheat (*Triticum aestivum*). *Int. J. Agric. Biol.*, 16: 1059–1066

varieties like durum wheat line 149 (in Australia) (Huang *et al*., 2006; James *et al*., 2011), KLR1-4 and KLR 19 (in India), LU-26S and SARC-1 (in Pakistan) and Sakha 8 (in Egypt) (Munns, 2006) have been released but these are not commercially popular among farmers on various accounts. The slow progress in improvement of salinity tolerance in wheat might be due to difficulty in screening huge numbers of accessions on naturally saline soils due to spatial and temporal heterogeneity in soil salinity, lack of proper selection trait truly representing salinity tolerance and grain yield and/or lack of understanding of the genetic basis of salinity tolerance (Akhtar *et al.,* 2012). The identification of genes and better understanding of the genetic basis of salinity tolerance is essential to launch an efficient breeding program. Information from various species studied for salinity tolerance suggests that different genetic architectures may be controlling the character, from a particular major dominant/recessive genes to polygenic control with mainly additive effects, but with some degree of dominance toward tolerance (Gregorio and Senadhira, 1993; Ortiz *et al*., 2008). But yet little is known about the genetic mechanisms for salt tolerance in wheat except Nax genes reported in Australian durum and hexaploid wheat (James *et al*., 2011).

This study evaluates several characters mostly related to early seedling, physiological and adult wheat plant for their relation with salinity tolerance in terms of grain yield, extent of their inheritance and the types of gene actions involved in the phenotype/expression of these traits.

Materials and Methods

Genetic Material

Two wheat inbreds differing in their NaCl tolerance, 4WLRG/1-8 and 4WLRG/1-12, the former salt tolerant and later salt sensitive (Ali *et al*., 2007; Ali *et al*., 2012), were hybridized to develop F_1, F_2, BC_1 and BC_2 generations. Necessary precautionary measures were adopted during the development of genetic material.

Seedling Response to NaCl in Culture Solutions

To develop nursery, seeds of six generations were grown in separate rows in polythene lined iron trays filled with acid washed gravel and placed in a glasshouse maintained at 10-15^{0}C for 10 h photoperiod. The young seedlings at two-leaf stage were transferred into three large iron tubs, (118 × 88 × 30) cm, containing 200 L aerated half strength Hoagland solution (Hoagland and Arnon, 1950). Thirty seedlings (replicas) per treatment of each of parents and F_1, 300 of F_2, and 150 of each of BC_1 and BC_2 were held in foam-plugged holes, holding two seedlings per hole, in thermopore sheets floating on culture solution. Three salinity levels i.e., control, 10 and 15 dS m^{-1} NaCl, and six generations were studied in saline treatment × generation factorial (two-way ANOVA). Salinity in containers was developed using commercial salt (NaCl) in four equal steps across four days, one step/day, starting on 3rd day of seedling transplantation in the containers. The culture solutions were adjusted to 6.0-6.5 pH with 1N HCl and/or NaOH. The culture solutions were changed fortnightly. Root length and fresh biomass of seedlings was measured after their four weeks growth in solution culture.

Whole Plant Response to NaCl in Soil Beds

This experiment was conducted in three soil beds with size of (2100 × 105 × 25) cm. One soil bed represented one NaCl treatment. The salinity in the beds was developed by adding commercial grade NaCl salt to obtain the required salinity levels of control, 10 and 15 dS m^{-1} measuring electrical conductivity (EC) of soil extract as explained elsewhere (Rayment and Higginson, 1992). The NaCl salt was mixed well with the soil which was used to fill 25 cm upper layer of the soil beds. Each of P_1, P_2, F_1, F_2, BC_1 and BC_2 generations were space-planted using completely randomised design. Inter-plant and inter-row distances were kept at 15 and 22.5 cm, respectively. All other agronomic and cultural practices were kept uniform to reduce the experimental error. Twenty four guarded plants for each of P_1, P_2, F_1, 240 for F_2, and 120 for each of BC_1 and BC_2 generations were used to record data at appropriate time for fertile tillers, grains per spike, 100 grain weight, grain yield per plant, Na^+, K^+, K^+/Na^+ ratio and Cl^-.

Determination of Na^+, K^+ and Cl^- Concentration

Fully expanded leaves (2nd to flag leaf) of guarded plants of every generation grown in control, 10 and 15 dS m^{-1} were taken and stored separately in micro-tubes at 5^{o}C in refrigerator. The cell sap was extracted using the standard technique of centrifugation (Gorham *et al*., 1984). The cell sap was diluted by adding de-ionized water. The concentrations of Na^+ and K^+ ions in the samples were measured with the help of flame photometer (Buck Scientific, Model: PFP-7). Uptake of potassium in relation to sodium (K^+/Na^+) was also computed using data of Na^+ and K^+ ions. Concentration of chloride in the leaves was measured with an automated chloride meter Corning-EEL 920 (Corning Labware and Equipment, Corning, NY, USA) and directly calibrated in mg L^{-1}.

Statistical/Biometrical Analyses

Variances and orthogonal comparisons: Effects of generations in NaCl salinities were tested using analysis of variance technique (Steel *et al*., 1997) using GenStat v 10.2 (GenStat, 2008). Coefficients for the partitioning of the sum of squares among six generations were constructed according to the rules given elsewhere (Little and Hills, 1978). Sum of squares for these comparisons were calculated using following function (Little and Hills, 1978).

$SS = (\Sigma c_i Y_{i.})^2/r\Sigma c_i^2$

Where, SS, sum of square of comparisons; Σ, summation; c_i, comparison coefficients; $Y_{i.}$, generation totals; r, replicates.

Generation mean analysis: The data were further subjected to generation mean analysis (Mather and Jinks, 1982) using computer software computerized by Dr. H.S. Pooni, School of Biological Sciences, University of Birmingham. A weighted least square analysis (Mather and Jinks, 1982) was performed on the generation means commencing with the simplest model using parameter 'm' only. Further models of increasing complexity (md, mdh, etc.) were fitted if the chi square value was significant. The best fit model was the one which had significant estimates of all parameters along with non-significant chi square value. For each trait the higher parent was always taken as P_1 in the model fitting.

Heterosis, inbreeding depression, heritability in narrow sense, genetic advance and correlation: Magnitude of heterosis in F_1 (HF_1), inbreeding depression in F_2, narrow-sense heritability (h^2_N) estimates, expected genetic advance in the next generation and correlation was computed as explained elsewhere (Ali, 2010).

Results

Variability in Response to NaCl Stress

Mean squares and partitioned generation variances into various components of P_1 vs P_2, P's vs F_1, BC_1 vs BC_2, F_2 vs BC's, and P's, F_1 vs BC's, F_2 for various seedlings, ionic and yield related traits of six generations grown in saline and non-saline conditions is presented in Table 1. Six generations were significantly ($P \leq 0.01$) different for all traits measured in control, 10 and 15 dS m^{-1} of NaCl salinities. P_1 vs P_2 component was significant ($P \leq 0.05$-0.01) for all the traits measured in saline and non-saline conditions except fertile tillers, grains per spike, grain yield per plant in non-saline conditions while K^+/Na^+ ratio in saline and non-saline conditions. Significant P's vs F_1 component was found for root length, biomass, Na^+, and K^+ uptake in saline and non-saline conditions, for grains per spike in both salinities, for Cl^- in low salinity and for fertile tillers, grain yield and K^+/Na^+ ratio in high salinity. BC_1 vs BC_2 component was significant for grain yield in saline and non-saline conditions, for grain number, 100 grain weight and K^+ in non-saline conditions, for biomass and fertile tillers in non-saline and low salinity, and for Cl^- in high salinity only. F_2 vs BC's contrast was significant for grains per spike and K^+/Na^+ ratio in saline and non-saline conditions, for Na^+ in non-saline condition only, for grain yield, biomass, 100 grain weight and K^+ in non-saline and high saline conditions, and for root length in both the salinities. P's, F_1 vs BC's, F_2 was significant for grain yield in saline and non-saline conditions, for root length, biomass, grains per spike, Na^+, and Cl^- in non-saline and low salinity, for fertile tillers in non-saline and high salinity, for K^+/Na^+ ratio in low salinity, and for 100 grain weight in both the salinities. Mean values of six generations is given in Table 2.

Gene Effects of NaCl Tolerance

Estimates of different types of gene effect (Table 3) clearly illustrate the variation for various traits in saline and non-saline conditions. Only additive [d] and dominant [h] gene effects were statistically significant for Cl^- in 15 dS m^{-1} NaCl salinity and 100 grain weight in non-saline condition. Additive [d] and additive × dominant epistasis [j] gene effects were found for fertile tillers in 10 dS m^{-1} of NaCl salinity. Additive [d], dominant [h] and additive × additive epistasis [i] gene effects were pronounced for grains per spike in low salinity. Additive [d], additive × additive epistasis [i] and dominant × dominant epistasis [l] gene effects were significant for Cl^- in non-saline conditions. Dominant [h], additive × additive epistasis [i] and dominant × dominant epistasis [l] gene effects were found for grains per spike in non-saline condition. Root length in non-saline condition and biomass in low salinity were controlled by additive [d], dominant [h], additive × additive epistasis [i] and additive × dominant epistasis [j] gene effects. Additive [d], dominant [h], additive × additive epistasis [i] and dominant × dominant epistasis [l] gene effects controlled K^+ in non-saline and K^+/Na^+ ratio in low salinity. Additive [d], dominant [h], additive × dominant epistasis [j] and dominant × dominant epistasis [l] gene effects controlled 100 grain weight in 10 dS m^{-1} NaCl. Additive [d], additive × additive epistasis [i], additive × dominant epistasis [j] and dominant × dominant epistasis [l] gene effects controlled grain yield in 10 dS m^{-1} NaCl. All measurements of remaining traits in saline and non-saline conditions showed significant χ^2.

Estimates of heterosis, inbreeding depression, narrow sense heritabilities and expected genetic advance for various seedling, ionic and yield related traits of six wheat generations grown in saline and non-saline conditions are presented in Table 4. The magnitude of positive heterosis was significant for Cl^- in non-saline condition, for root length, biomass and K^+ in 10 dS m^{-1} and for K^+/Na^+ ratio and Cl^- in 15 dS m^{-1}. Significant negative heterosis was found for biomass in 15 dS m^{-1}. Negative inbreeding depression was significant for root length, Na^+ and Cl^- in control and both the salinities, for grains per spike in control and 10 dS m^{-1}, and for biomass in non-saline conditions. Positive significant inbreeding depression was observed for K^+ in control and both the salinities, for K^+/Na^+ ratio in control and 10 dS m^{-1}, for tillers per plant in control and 15 dS m^{-1}, and for grain yield in 10 dS m^{-1} only. 100-grain weight showed positive significant inbreeding depression in 10 dS m^{-1} while negative significant in 15 dS m^{-1}.

Narrow sense heritability was high (> 0.70) for

Table 1: Mean squares with partitioned generation variances for various seedling, ionic and yield related traits of six bread wheat generations grown in non-saline (control) and two NaCl salinities

		Root length (cm)			Biomass (g)			Fertile tillers per plant			Grains per spike			100 grain weight (g)		
Sources of variation	DF	Control	10 dS/m	15 dS/m	Control	10 dS/m	15 dS/m	Control	10 dS/m	15 dS/m	Control	10 dS/m	15 dS/m	Control	10 dS/m	15 dS/m
Generations	5	37.24^{**}	36.65^{**}	47.02^{**}	0.304^{**}	0.013^{**}	0.029^{**}	1.536^{**}	0.945^{**}	0.334^{**}	31.56^{**}	208.31^{**}	179.09^{**}	0.0838^{**}	0.3712^{**}	0.106^{**}
P_1 vs P_2	1	37.95^{**}	67.94^{**}	96.96^{**}	0.45^{**}	0.008^{*}	0.059^{**}	0.135^{NS}	1.949^{**}	0.437^{**}	0.28^{NS}	431.97^{**}	494.68^{**}	0.1536^{**}	0.2904^{*}	0.073^{*}
P's vs F_1	1	26.57^{*}	52.12^{**}	29.18^{*}	0.244^{*}	0.011^{**}	0.018^{*}	0.0018^{NS}	0.370^{NS}	0.898^{**}	0.94^{NS}	173.54^{**}	203.21^{**}	0.0072^{NS}	0.0578^{NS}	0.005^{NS}
BC_1 vs BC_2	1	1.59^{NS}	0.54^{NS}	4.70^{NS}	0.223^{*}	0.026^{**}	0.001^{NS}	1.215^{*}	2.160^{**}	0.025^{NS}	22.12^{*}	28.65^{NS}	36.02^{NS}	0.1262^{**}	0.0794^{NS}	0.010^{NS}
F_2 vs BC's	1	13.78^{NS}	37.32^{**}	96.74^{**}	0.223^{*}	0.003^{NS}	0.062^{**}	0.583^{NS}	0.192^{NS}	0.022^{NS}	101.96^{**}	108.49^{**}	85.28^{*}	0.1301^{**}	0.0004^{NS}	0.387^{**}
P's, F_1 vs BC's, F_2	1	106.29^{**}	25.35^{*}	7.49^{NS}	0.379^{**}	0.02^{**}	0.005^{NS}	5.746^{**}	0.054^{NS}	0.289^{*}	32.48^{**}	298.90^{**}	76.26^{NS}	0.0018^{NS}	1.4281^{**}	0.054^{*}
Error	12	3.62	3.77	4.8	0.030	0.001	0.003	0.144	0.075	0.023	3.12	17.83	15.91	0.0074	0.0331	0.011
		Grain yield per plant (g)			Na^+			K^+			K^+/Na^+ ratio			Cl^{-1}		
Sources of variation	DF	Control	10 dS/m	15 dS/m	Control	10 dS/m	15 dS/m	Control	10 dS/m	15 dS/m	Control	10 dS/m	15 dS/m	Control	10 dS/m	15 dS/m
Generations	5	1.18^{**}	1.31^{**}	1.53^{**}	11.30^{**}	16839^{**}	17899^{**}	102.90^{**}	807^{**}	224^{**}	62.40^{**}	0.688^{**}	0.0356^{**}	299.19^{**}	28089^{**}	37930^{**}
P_1 vs P_2	1	0.56^{NS}	2.27^{**}	1.19^{*}	21.89^{**}	43381^{**}	49550^{**}	146.42^{**}	2260^{**}	491^{**}	27.99^{NS}	0.240^{NS}	0.0074^{NS}	690.80^{**}	69338^{**}	150410^{**}
P's vs F_1	1	0.22^{NS}	0.27^{NS}	1.50^{**}	7.53^{*}	16359^{**}	24387^{**}	146.21^{**}	1247^{**}	219^{**}	10.40^{NS}	0.115^{NS}	0.0613^{**}	24.08^{NS}	31165^{**}	2006^{NS}
BC_1 vs BC_2	1	0.60^{*}	1.85^{**}	0.78^{*}	0.06^{NS}	23^{NS}	1398^{NS}	123.13^{**}	170^{NS}	6^{NS}	5.70^{NS}	0.054^{NS}	0.0006^{NS}	8.35^{NS}	8406^{NS}	30246^{*}
F_2 vs BC's	1	3.95^{**}	0.0061^{NS}	3.28^{**}	19.85^{**}	3346^{NS}	7831^{NS}	96.33^{**}	199^{NS}	303^{**}	256.51^{**}	1.980^{**}	0.1058^{**}	101.10^{NS}	2^{NS}	1755^{NS}
P's, F_1 vs BC's, F_2	1	0.59^{*}	2.14^{**}	0.90^{*}	7.18^{*}	21086^{**}	6328^{NS}	2.44^{NS}	157^{NS}	100^{NS}	11.38^{NS}	1.051^{**}	0.0032^{NS}	671.61^{**}	31536^{**}	5233^{NS}
Error	12	0.11	0.11	0.13	1.11	1384	1490	10.16	71	19	6.12	0.049	0.0032	24.92	2309	3493

Table 2: Mean performance of six generations for various seedling, ionic and yield related traits of six bread wheat generations grown in non-saline (control) and two NaCl salinities

Generations	Root length (cm)	Biomass (g)	Fertile tillers per plant	Grains per spike	100-grain weight (g)	Grain yield per plant (g)	Na^+ (mmol L^{-1})	K^+ (mmol L^{-1})	K^+/Na^+ ratio	Cl^- (mg L^{-1})
			Control							
P_1 (4WLRG/1-8)	19.40	3.90	5.6	40.13	2.34	3.59	6.18	114.45	18.72	74.67
P_2 (4WLRG/1-12)	14.37	2.25	5.3	39.7	2.02	2.98	10	104.57	14.4	96.13
F_1	10.53	1.15	5.48	40.6	2.24	3.62	6.15	118.06	18.84	81.93
F_2	12.60	1.25	4.69	47.59	2.35	3.97	8.28	108.47	11.36	101.2
BC_1	11.57	0.99	4.6	42.37	2.24	2.88	5.03	119.94	23.66	92.91
BC_2	14.71	1.75	3.7	38.53	1.95	2.25	5.23	110.88	21.71	95.27
			10 dS m^{-1}							
P_1 (4WLRG/1-8)	17.93	1.19	4.47	38.77	2.22	3.42	99.65	106.56	1.14	262
P_2 (4WLRG/1-12)	11.20	0.97	3.33	21.8	2.66	2.19	269.71	67.74	0.74	477
F_1	9.67	0.41	4.33	39.6	2.27	3.17	94.24	112.12	1.18	244.67
F_2	10.90	0.47	4.14	46.45	1.81	2.2	113.35	108.03	0.84	412.33
BC_1	9.50	0.51	4.43	41.27	1.94	2.81	70.47	103.37	1.93	373.81
BC_2	12.52	1.00	3.23	36.9	1.71	1.7	74.42	92.72	1.74	448.67
			15 dS m^{-1}							
P_1 (4WLRG/1-8)	16.87	1.33	3.17	36.33	1.98	2.56	181.89	30.78	0.19	447.67
P_2 (4WLRG/1-12)	10.83	0.74	2.63	18.17	2.2	1.67	363.64	12.69	0.12	764.33
F_1	8.67	0.24	3.57	37.33	2.14	2.98	162.34	32.19	0.33	574.33
F_2	11.40	0.52	2.94	39.08	2.51	2.81	231.74	38.14	0.34	649.29
BC_1	9.63	0.51	2.9	35	2.11	1.89	279.05	26.8	0.12	548.67
BC_2	11.47	0.97	2.77	30.1	2.03	1.17	309.58	24.85	0.1	690.67

biomass and Na^+ in non-saline condition, for all characters except grains per spike and K^+ in 10 dS m^{-1} of salinity, and for grain yield, fertile tillers, 100 grain weight, K^+ and K^+/Na^+ ratio in 15 dS m^{-1}. Variation in root length, fertile tillers, 100 grain weight, grain yield, K^+/Na^+ ratio and Cl^- in control, K^+ in 10 dS m^{-1}, and biomass, 100 grain weight and Cl^- in 15 dS m^{-1} was moderately heritable (> 0.50 but<0.70). Grains per spike have heritability < 0.50 in saline and non-saline conditions. K^+ in control and root length in 15 dS m^{-1} was also less heritable (0.36). Cl^- has maximum genetic advance of 61.06 and 449.71 in control and 10 dS m^{-1}, respectively, whilst Na^+ has maximum genetic advance of 822.97 in 15 dS m^{-1}.

Significant positive relationship was observed between 100 grain weight and grain yield in control, and tillers per plant and grain yield in 10 dS m^{-1} (Fig. 1). A strong negative association between Cl^- and grain yield in 10 dS m^{-1} was detected. In 15 dS m^{-1}, root length, biomass and K^+/Na^+ ratio were significantly and positively associated with grain yield whilst negative significant correlation was found between Na^+ and grain yield. Grain yield in control and in 10 dS m^{-1} showed medium positive association (r = 0.55), but significant ($P \leq 0.01$) correlation in 15 dS m^{-1} (r = 0.95). Grain yield in 10 and 15 dS m^{-1} salinity also appeared to show medium positive association (r = 0.67, data not presented). Root length in control had medium negative association with grain yield

Table 3: Components of generation means, m, [d], [h], [i], [j] and [l] for various seedling, ionic and yield related traits of six bread wheat generations grown in non-saline (control) and 2 NaCl salinities

m	[d]	[h]	[i]	[j]	[l]	χ^2	DF	Probability
Traits/Salinity levels								
Root length								
Control	28.81 ± 0.70	2.51 ± 0.43	-7.95 ± 1.11	-11.71 ± 0.79	-2.88 ± 1.16		1.77	1	0.25-0.1
10 dS/m	28.66 ± 1.21	1.21 ± 0.28	-19.56 ± 2.99	-15.35 ± 1.20		10.57 ± 2.07	49.11**	1	< 0.005
15 dS/m	17.96 ± 0.65	2.83 ± 0.36	-4.84 ± 1.00	-7.41 ± 0.74	-2.66 ± 1.11		392.01**	1	< 0.005
Biomass									
Control	7.3 ± 0.18	0.80 ± 0.052	-6.00 ± 0.44	-4.20 ± 0.17	0.60 ± 0.15	2.80 ± 0.30	0	0	
10 dS/m	1.5 ± 0.061	0.10 ± 0.031	-0.20 ± 0.087	-0.40 ± 0.07	-1.00 ± 0.10		0	1	
15 dS/m	1.1 ± 0.021	0.29 ± 0.024		-0.27 ± 0.034	-0.23 ± 0.079	0.15 ± 0.03	435.19	1	
Fertile tillers per plant									
Control	4.02 ± 0.16			0.92 ± 0.27	2.08 ± 0.36	1.19 ± 0.38	37.47**	2	< 0.005
10 dS/m	3.96 ± 0.07	0.58 ± 0.19			1.28 ± 0.53		5.92	3	0.25-0.1
15 dS/m	2.91 ± 0.06						9.19	5	0.25-0.1
Grains per spike									
Control	67.95 ± 6.03		-54.09 ± 15.53	-27.95 ± 5.83		26.74 ± 11.09	3.93	2	0.25-0.1
10 dS/m	54.34 ± 3.53	6.47 ± 1.28	-16.59 ± 5.84	-24.51 ± 3.78			3.68	2	0.25-0.1
15 dS/m	37.40 ± 0.83	6.79 ± 1.46		-12.35 ± 2.37			8.45*	3	0.05-0.025
100-grain weight									
Control	2.202 ± 0.032	0.186 ± 0.057					9.56*	4	0.05-0.025
10 dS/m	2.440 ±0.055	0.220 ± 0.055	-2.31 ± 0.24		-0.90 ± 0.22	2.14 ± 0.26	0.036	1	0.75-0.50
15 dS/m	2.450 ±0.089			-0.47 ± 0.14		-0.36 ± 0.18	15.89**	3	< 0.005
Grain yield per plant									
Control	3.33 ± 0.10			-0.62 ± 0.23			73.34**	4	< 0.005
10 dS/m	1.78 ± 0.15	0.62 ± 0.18		0.95 ± 0.24	1.05 ± 0.52	1.35 ± 0.32	1.81	1	0.25-0.1
15 dS/m	2.23 ± 0.06	0.64 ± 0.10		-0.95 ± 0.19			130.69**	3	< 0.005
Na^+									
Control	10.94 ± 0.57	1.68 ± 0.31	-7.26 ± 0.91	-4.10 ± 0.63	-2.78 ± 0.85		137.03**	1	< 0.005
10 dS/m	81.50 ± 3.88	78.74 ± 6.52		-87.48 ± 8.07	-127.27 ± 22.26		82.30**	2	< 0.005
15 dS/m	286.89 ± 15.80	86.61 ± 15.80				-120.36 ± 20.61	10.27*	3	0.025-0.01
K^+									
Control	81.85 ± 7.94	6.99 ± 1.78	70.26 ± 20.5	27.96 ± 7.52		-34.05 ± 13.94	1.33	1	0.25-0.1
10 dS/m	106.36 ± 0.98	19.65 ± 2.13		-21.77 ± 2.45	-18.54 ± 6.19		14.10**	2	< 0.005
15 dS/m	69.64 ± 5.11	4.83 ± 1.11	-88.55 ± 12.77	-48.20 ± 4.81		51.10 ± 8.42	9.84**	1	< 0.005
K^+/Na^+ ratio									
Control	13.91 ± 0.66			8.80 ± 1.18		7.57 ± 1.53	279.57**	3	< 0.005
10 dS/m	3.04 ± 0.27	0.20 ± 0.05	11.29 ± 0.67	3.98 ± 0.27		-7.08 ± 0.44	0.01	1	0.95-0.9
15 dS/m	0.24 ± 0.01	0.04 ± 0.01		-0.11 ± 0.02			69.27**	3	< 0.005
Cl^-									
Control	106.72 ± 2.90	8.98 ± 1.89		-21.93 ± 3.78		-24.9 ± 4.80	3.41	2	0.25-0.1
10 dS/m	428.36 ± 11.51	121.30 ± 14.52				-164.85 ± 22.80	22.01**	3	< 0.005
15 dS/m	625.73 ± 11.13	149.25 ± 23.60					3.45	4	0.50-0.10

The models with significant (*) χ^2 were not adequate for the estimated parameters thus suggesting raising of succeeding generations in order to obtain the best fit model

in 10 dS m^{-1} (r = - 0.68), however, it had highly significant association with that in 15 dS m^{-1} (r = 0.94, data not presented).

Discussion

Involvement of additive and non-additive types of genes in the expression of salinity tolerance, in terms of biomass, fertile tillers, grains per spike, 100 grain weight, grain yield and K^+/Na^+ ratio in 10 dS m^{-1} of NaCl salinity, showed salt tolerance to be polygenic complex trait (Table 3). Only additive gene effects for Cl^- uptake were found in 15 dS m^{-1}. Significant genetic models for most of the traits in 15 dS m^{-1} elucidated the complexity of gene interactions and suggested further careful investigation via tri-genic or higher gene interaction models. Differences in quantity of NaCl in the nutrient solution or soil beds affected the expression of the genes (Table 3) and changed patterns of trait association (Fig. 1). The positive and negative sign of best fit genetic models for biomass, fertile tillers, grains per spike, 100 grain weight, grain yield and K^+/Na^+ ratio in 10 dS m^{-1} showed the contribution of increasing and decreasing alleles aggregated in the higher and lower parents, respectively. Positive [d] component for all these traits, positive [h] and [i] for K^+/Na^+ ratio and positive [i], [j] and [l] for grain yield indicated the major contribution of increasing additive and non-additive alleles from higher parent (4WLRG/1-8) in the character expression in 10 dS m^{-1}. Negative [h] and [j] for 100 grain weight, negative [h] and [i] for grains per spike, and negative [h], [i] and [j] for biomass showed involvement of decreasing alleles of dominant and additive × additive epistatic genes from lower parent (4WLRG/1-12) in the character expression in 10 dS m^{-1}. The high magnitude and negative direction of [l]

Table 4: Estimates of heterosis, inbreeding depression, heritability and genetic advance for various seedling, ionic and yield related traits of six bread wheat generations grown in non-saline (control) and two NaCl salinities

	Heterosis			Inbreeding depression		
Traits	Control	10 dS/m	15 dS/m	Control	10 dS/m	15 dS/m
Root length	1.13 ± 0.57	1.74 ± 0.65	-0.20 ± 0.55	-4.18 ± 0.27	-1.85 ± 0.28	-0.80 ± 0.26
Biomass	0.23□0.10	0.12□0.04	-0.016□0.004	-0.84□0.045	-0.12□0.05	-0.063□0.035
Fertile tillers per plant	-0.12 ± 0.29	-0.14 ± 0.31	0.40 ± 0.26	0.79 ± 0.13	0.19 ± 0.15	0.63 ± 0.14
Grains per spike	0.47 ± 2.46	0.83 ± 2.79	1.00 ± 2.71	-6.99 ± 1.36	-6.85 ± 1.33	-1.75 ± 1.25
100-grain weight	-0.10 ± 0.074	0.050 ± 0.082	0.16 ± 0.14	-0.11 ± 0.069	0.46 ± 0.065	-0.37 ± 0.076
Grain yield per plant	0.03 ± 0.35	-0.25 ± 0.23	0.42 ± 0.24	-0.35 ± 0.17	0.97 ± 0.13	0.17 ± 0.11
Na^+	-0.03 ± 0.41	-5.41 ± 7.80	-19.55 ± 15.83	-2.13 ± 0.22	-19.11 ± 6.17	-69.40 ± 22.93
K^+	3.61 ± 3.42	5.56 ± 2.77	1.41 ± 2.16	9.59 ± 1.61	4.09 ± 1.53	-5.95 ± 1.05
K^+/Na^+ ratio	0.12 ± 1.20	0.040 ± 0.096	0.14 ± 0.028	7.48 ± 0.56	0.34 ± 0.059	-0.010 ± 0.023
Cl^-	7.26 ± 2.74	-17.33 ± 17.44	126.66 ± 46.35	-19.27 ± 2.58	-167.66 ± 16.20	-74.96 ± 22.65
	Heritability			Genetic advance		
Traits	Control	10 dS/m	15 dS/m	Control	10 dS/m	15 dS/m
Root length	0.57	0.73	0.36	4.71	6.28	3.00
Biomass	0.81	0.79	0.61	0.95	0.74	0.42
Fertile tillers per plant	0.61	0.71	0.70	2.37	2.90	1.88
Grains per spike	0.41	0.47	0.43	18.38	16.84	17.14
100-grain weight	0.69	0.80	0.56	1.70	1.79	1.40
Grain yield per plant	0.65	0.77	0.94	3.07	3.06	2.92
Na^+	0.82	0.78	0.98	5.58	165.13	822.97
K^+	0.36	0.65	0.89	17.71	31.68	30.69
K^+/Na^+ ratio	0.61	0.95	0.86	10.37	1.84	0.67
Cl^-	0.67	0.78	0.61	61.06	449.71	248.08

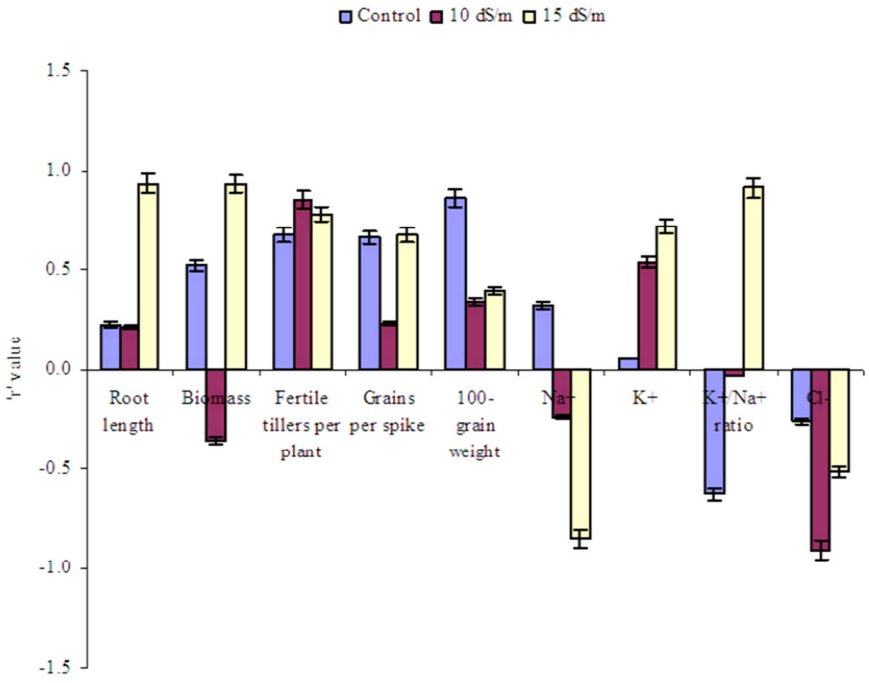

Fig. 1: Association of grain yield with various traits measured in non-saline (control) and two NaCl treatments

component for K^+/Na^+ ratio in 10 dS m^{-1} cautioned careful trait utility for improving salt tolerance.

Strong positive associations of root length, biomass and K^+/Na^+ ratio and strong negative association of Na^+ uptake with grain yield in 15 dS m^{-1} shows the simplicity of their use with potential improvement in salt tolerance through breeding but their respective non-significant correlation coefficient in 10 dS m^{-1} and significant genetic models in 15 dS m^{-1} indicated complexity in the inheritance pattern. Further, moderately high correlation (r = 0.67) between salt tolerance performance in terms of grain yield under 10 and 15 dS m^{-1} and high correlation (r = 0.95)

between control and 15 dS m^{-1} (data not presented) suggested involvement of common genes which would be expressed in saline and non-saline conditions, as well as existence of some additional genes, which would be expressed in salinity only. Earlier high correlation between performance under 10 and 20 dS m^{-1} treatment than those between each treatment and control were found and existence of additional genes was suggested, in tomato, that would be expressed only under saline conditions (Saranga *et al.*, 1992; Asins *et al.*, 1993). Increase in narrow sense heritability in salinities than control is due to increase in additive variance that might be resulted due to expression of additional or hidden genes in salinities that would not be expressed in non-saline conditions (Shannon, 1984). Positive heterosis and negative inbreeding depression in salinities for some traits is encouraging but negative heterosis and positive inbreeding depression for grain yield in 10 dS m^{-1} is not encouraging for simple selection breeding. Further, negative heterosis and negative inbreeding depression for Na^+ and Cl^-, undesirable traits, indicates complexity in breeding for salt tolerance.

Our results suggest that selection or screenings for salt tolerance breeding programs should be performed under saline conditions. These results agree with earlier report (Saranga *et al*., 1992) but in contrast with another report (Kelman and Qualset, 1991). Additive and non-additive gene effects for grain yield appeared to permit the development of recombinant inbred lines from the F_2 and backcrosses, containing the salt tolerant quantitative trait loci (QTL) of 4WLRG/1-4. This QTL couple with recurrent selection for specific combining ability of the 4WLRG/1-4 with a good wheat cultivar seems to be a good salt tolerant breeding strategy. Recent progress in molecular genetics and plant electrophysiology suggested the ability of a plant to maintain a high cytosolic K^+/Na^+ ratio to be critical to plant salt tolerance (Shabala and Cuin, 2008). So far, the major efforts of plant breeders have been aimed at improving this ratio by minimizing Na^+ uptake and transport to shoot. However, here significant differences between two parents (P_1 vs P_2) for phenotypic/physiological data and non-significant differences for K^+/Na^+ ratio did not allow to use this ratio as salt tolerant trait at least in the present instance and suggested further investigation for the inheritance of relationship between grain yield, salt tolerance and K^+/Na^+ ratio.

In conclusion, salt tolerance in spring wheat under 10 dS m^{-1} NaCl stress is a complex polygenic trait where both the fixable and non-fixable component of genetic variation is important. In wheat, designing the breeding programme for incorporating the salt tolerance and the statistical model used to locate the QTLs for salt tolerance, both additive and non-additive gene effects are focused (Carbonell *et al*., 1992). Concentrations of NaCl applied in this experiment have affected all types of gene actions of most of traits and thus suggested differences in gene regulation or interaction and/or involvement of additional or hidden genes. Further research in terms of precision phenotyping and genotyping for salt tolerance in terms of grain yield and K^+/Na^+ ratio is needed to be utilized as salt tolerant marker.

Acknowledgement

The authors gratefully acknowledge Higher Education Commission, Pakistan for providing funds through promotion of research grant to the University of Agriculture, Faisalabad, Pakistan. The authors have no conflict of interest to declare.

References

Ali, Z., 2010. *Analyzing and Understanding Genetic Problems: Classical and Conventional Approach.* VDM Publishing House Ltd, Mauritius, Germany

Ali, Z., A.S. Khan and M.A. Asad, 2002. Salt tolerance in bread wheat: Genetic variation and heritability for growth and ion relation. *Asian J. Plant Sci.,* 1: 420–422

Ali, Z., A.S. Khan, I.A. Khan and F.M. Azhar, 2005. Effect of NaCl on root growth and ionic relations of wheat. *J. Agric. Soc. Sci.,* 1: 123–125

Ali, Z., A. Salam, F.M. Azhar and I.A. Khan, 2007. Genotypic variation in salinity tolerance among spring and winter wheat (*Triticum aestivum* L.) accessions. *S. Afric. J. Bot*., 73: 70–75

Ali, Z., A. Salam, F.M. Azhar, I.A. Khan, A.A. Khan, S. Bahadur, T. Mahmood, A. Ahmad and R. Trethowan, 2012. The response of genetically distinct bread wheat genotypes to salinity stress. *Plant Breed*., 131: 707–715

Asins, M.J., M.P. Bretó, M. Cambra and E.A. Carbonell, 1993. Salt tolerance in Lycopersicon species. I. Character definition and changes in gene expression. *Theor. Appl. Gene*., 86: 737–743

Carbonell, E.A., T.M. Gerig, E. Balansard and M.J. Asins, 1992. Interval mapping in the analysis of non-additive quantitative trait loci. *Biometrics*, 48: 305–315

Flowers, T.J., 2004. Improving crop salt tolerance. *J. Exp. Bot*., 55: 307–319

GenStat, 2008. *GenStat Procedure Library Release PL18.1.* GenStat Release 10.2 (PC/Windows)

Gorham, J., E. McDonnell and R.G. Wyn Jones, 1984. Pinitol and other solutes in salt-stressed *Sesbania aculeata*. *Z. Pflanzenphys*, 114: 173–178

Gregorio, G.B. and D. Senadhira, 1993. Genetic analysis of salinity tolerance in rice (*Oryza sativa* L.). *TAG Theor. Appl. Gene*., 86: 333–338

Hoagland, D.R. and D.I. Arnon, 1950. The water culture method for growing plants without soil. Circular. *California Agric. Exp. Station*, 347: 1-32

Hollington, P.A., 2000. Technological breakthroughs in screening/breeding wheat varieties for salt tolerance. *In*: *Towards the: Salinity Management in Agriculture. Proceedings of the National Conference,* pp: 273–289. Gupta, S.K., S.K. Sharma and N.K. Tyagi (eds.). Central Soil Salinity Research Institute, Karnal, India

Huang, S., W. Spielmeyer, E.S. Lagudah, R.A. James, J.D. Platten, E.S. Dennis and R. Munns, 2006. A Sodium Transporter (HKT7) Is a Candidate for Nax1, a Gene for Salt Tolerance in Durum Wheat. *Plant Physiol*., 142: 1718–1727

Jafari-Shabestari, J., H. Corke and C.O. Qualset, 1995. Field evaluation of tolerance to salinity stress in Iranian hexaploid wheat landrace accessions. *Gene. Res. Crop Evol*., 42: 147–156

James, R.A., C. Blake, C.S. Byrt and R. Munns, 2011. Major genes for Na+ exclusion, Nax1 and Nax2 (wheat HKT1;4 and HKT1;5), decrease Na+ accumulation in bread wheat leaves under saline and waterlogged conditions. *J. Exp. Bot*., 62: 2939–2947

Kelman, W.M. and C.O. Qualset, 1991. Breeding for salinity-stressed environments: recombinant inbred wheat lines under saline irrigation. *Crop Sci*., 31: 1436–1442

Khan, A.S., M.A. Asad and Z. Ali, 2003. Assessment of genetic variability for NaCl tolerance in wheat. *Pak. J. Agric. Sci*., 40: 33–36

Landry, B.S. and R.W. Michelmore (eds.), 1987. *Methods and Applications of Restriction Fragment Length Polymorphism Analysis to Plants*. Plenum press, New York

Little, T.M. and F.J. Hills, 1978. *Agricultural Experimentation: Design and Analysis*. John Wiley and Sons, Inc, New York, USA

Ma, Y.N., R.G. Wang, C. Wu, Y.W. Shi, Z.H. Wang, S.D. Xie, S.P. Zhou, H.M. Xu and X.L. Ren, 2012. Developmental analysis on genetic behavior of quality traits of flue-cured tobacco (*Nicotiana tabacum*) in multiple environments. *Int. J. Agric. Biol.*, 14: 345–352

Mather, K. and J.L. Jinks, 1982. *Biometrical Genetics*, 3rd edition. Chapman and Hall, London

Munns, R., 2005. Genes and salt tolerance: bringing them together. *New Phytol.*, 167: 645–663

Munns, R., 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot*., 57: 1025–1043

Munns, R., R.A. Hare, R.A. James and G.J. Rebetzke, 2000. Genetic variation for improving the salt tolerance of durum wheat. *Aust. J. Agric. Res*., 51: 69–74

Munns, R. and R.A. James, 2003. Screening methods for salinity tolerance: a case study with tetraploid wheat. *Plant Soil*, 253: 201–218

Ortiz, R., W.W. Wagoire, O. Stølen, G. Alvarado and J. Crossa, 2008. Combining ability and heterosis under pest epidemics in a broad-based global wheat-breeding population. *Plant Breed*., 127: 222–227

Qureshi, R.H., A. Rashid and N. Ahmed, 1990. A procedure for quick screening of wheat cultivars for salt tolerance. *In*: *Towards the: Genetic Aspects of Plant Mineral Nutrition.* pp: 315–324. Loughman, B.C. (ed.). Kluwer Academic Publishers, Amsterdam, The Netherlands

Rawson, H.M., R.A. Richards and R. Munns, 1988. An examination of selection criteria for salt tolerance in wheat, barley and triticale genotypes. *Aust. J. Agric. Res.*, 39: 759–772

Rayment, G.E. and F.R. Higginson (eds.), 1992. *Australian Laboratory Handbook of Soil and Water Chemical Methods*. Inkata Press, Melbourne, Australia

Rengasamy, P., 2006. World salinization with emphasis on *Aust. J. Exp. Bot*., 57: 1017–1023

Richards, R.A., 1983. Should selection for yield in saline regions be made on saline or non-saline soils? *Euphytica*, 32: 431–438

Royo, A. and R. Aragüés, 1999. Salinity-yield response functions of barley genotypes assessed with a triple line source sprinkler system. *Plant Soil*, 209: 9–20

Akhtar, S., M. Niaz, S.U. Rahman, M. Younas and M.Z. Iqbal, 2012. Somaclonal variation for development of salt tolerance in selected wheat (*Triticum aestivum*) cultivars. *Int. J. Agric. Biol.*, 14: 600–604

Saranga, Y., A. Cahaner, D. Zamir, A. Marani and J. Rudich, 1992. Breeding tomatoes for salt tolerance: inheritance of salt tolerance and related traits in interspecific populations. *Theor. Appl. Gene*., 84: 390–396

Shabala, S. and T.A. Cuin, 2008. Potassium transport and plant salt tolerance. *Physiol. Plant.,* 133: 651–669

Shannon, M.C., 1984. Breeding, selection and genetics of salt tolerance. *In*: *Towards the: Salinity Tolerance in Plants: Strategies for Crop Improvement*, pp: 231–254. Staples, R.C. and G.A. Toenniessen (eds.). John Wiley and Sons, New York, USA

Steel, R.G.D., J.H. Torrie and D.A. Dickey, 1997. *Principles and Procedures of Statistics: A Biometrical Analysis.* McGraw Hill Book Co, New York, USA

(Received 31 August 2013; Accepted 08 April 2014)