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Assessment of Variation for NaCl Tolerance in Spring Wheat (*Triticum aestivum* L.)

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ABSTRACT

On the basis of root and shoot length the response of four-week-old wheat seedlings of 45 genotypes was assessed in three salinity levels i.e., control, 10 and 20 dS m^{-1}, developed in solution cultures. The 45 genotypes responded differently to increasing salinity levels in the growing medium. The genotypes 18194-II and DN-4, which produced longer roots than those of LU-26S, appeared as the most tolerant, whilst 18180-II, 18205-I and DN-18 as the most sensitive genotypes. The estimates of broad sense heritabilities for absolute root length were 0.52, 0.46 and 0.32 in control, 10 and 20 dS m^{-1} respectively, whilst those for relative root length were 0.23 and 0.28 in low and high salinities respectively.

Key Words: Salinity tolerance; genetic variability; wheat; heritability

INTRODUCTION

Wheat is grown both in arid and semi-arid regions of the world. Increasing wheat production under abiotic stress conditions has become important in recent years, since wheat production in areas with optimum growing conditions does not meet the needs of the increasing population. The problem of soil salinity is of frequent occurrence in irrigated areas of the world (Shannon, 1984). Pakistan lies within the subtropical region and has semi-arid to arid climate and about 6.3×10^6 hectares of irrigated land has become salt affected to varying degrees (Qureshi & Barrett-Lennard, 1998).

The biological/genetic engineering approaches have been advocated to deal with salinity problem. The later approach is too costly, due to escalating cost of labour and energy (Shannon, 1984), so it does not appear economically feasible to follow it for developing countries like Pakistan. Evolution/breeding of salt-tolerant and high yielding wheat varieties developed through genetic modifications to improve their adaptation to salt stressed conditions is a possible alternative to tackle this problem (Epstein *et al*., 1980; Qureshi *et al*., 1990). The presence of variability for salt tolerance in the species is a prerequisite to breed salt tolerant wheat varieties. This paper examines the genetic variability for salt tolerance in wheat.

MATERIALS AND METHODS

Plant material and screening protocol. Seeds of 45 genotypes differing for their genetic make up and origin were collected from different sources. Out of these, 28 lines/genotypes were obtained from PGRI (Plant Germplasm Resources Institute, Islamabad, Pakistan), 14 genotypes from CIMMYT, Mexico through PARC (Pakistan Agricultural Research Council, Islamabad, Pakistan), and 3 genotypes/varieties from the germplasm maintained in the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan.

The seeds of 45 genotypes were grown in iron trays filled with acid washed gravel. The young seedlings at the two-leaved stage were transferred to aerated half strength Hoagland solution (Hoagland and Arnon, 1950) in 3 large iron containers (118×88×30cm) internally lined with polythene sheet. Seedlings of each genotype were held in position through foam-plugged holes made in thermo pal sheets floating over 200 L culture solutions. Each of the 45 genotypes was planted in quadruplicate in the two NaCl treatments i.e. 10 dS m^{-1}, 20 dS m^{-1} and one without salt (control). The appropriate salinity level in the two containers were developed after two days of transplanting the seedlings and completed in four equal NaCl doses i.e., one dose/day. The pH of the solutions, ranging from 6.0 to 6.5, was maintained daily using 1N HCl and/or NaOH solutions. The NaCl solutions in the containers were changed after two weeks. After four weeks growth, root length (cm) and shoots length (cm) of 6 seedlings of each genotype in each replication were measured. Based upon the measurements of these characters, the responses of genotypes were compared using values of absolute salt tolerance (Dewey, 1962) and relative salt tolerance (Maas, 1986). Relative salt tolerance of 45 genotypes were computed according to the following formula:

$$\text{Relative salt tolerance} = \frac{\text{Value of a character in NaCl}}{\text{Value of a character in control}} \times 100$$

Statistical analysis of salt tolerance. The values of absolute salt tolerance and indices of salt tolerance of 45 genotypes were subjected to ordinary analysis of variance using general linear model of SPSS 8.0 for Windows: Advance Statistics 1994.

Fig. 1. Absolute root lengths (cm) of 45 wheat genotypes grown in control and 2 salinity levels

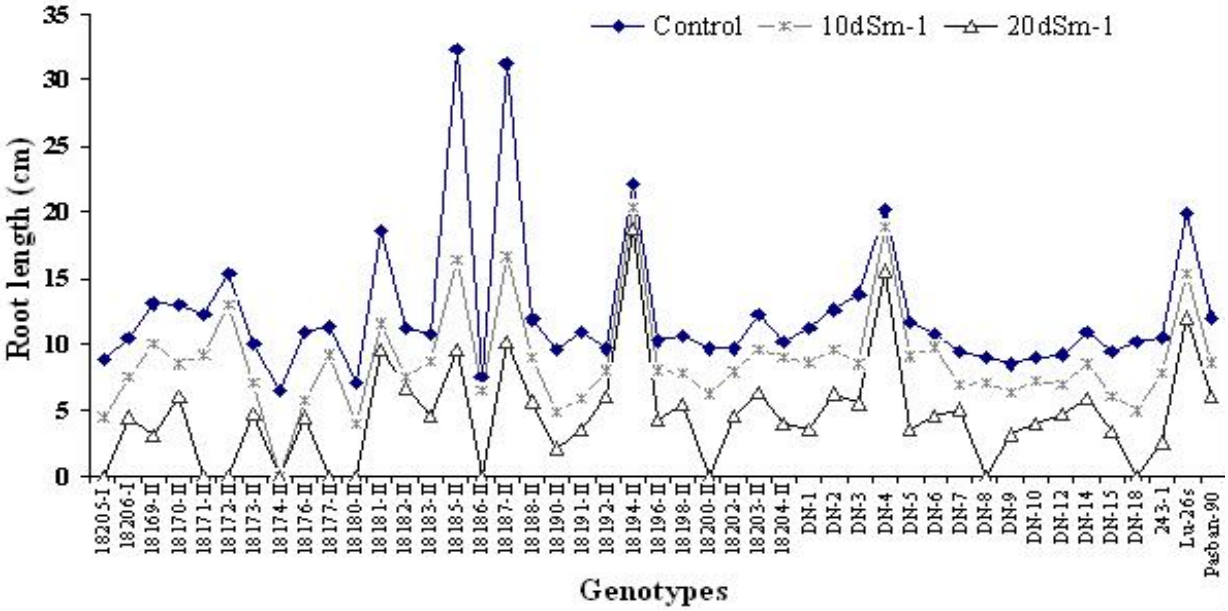
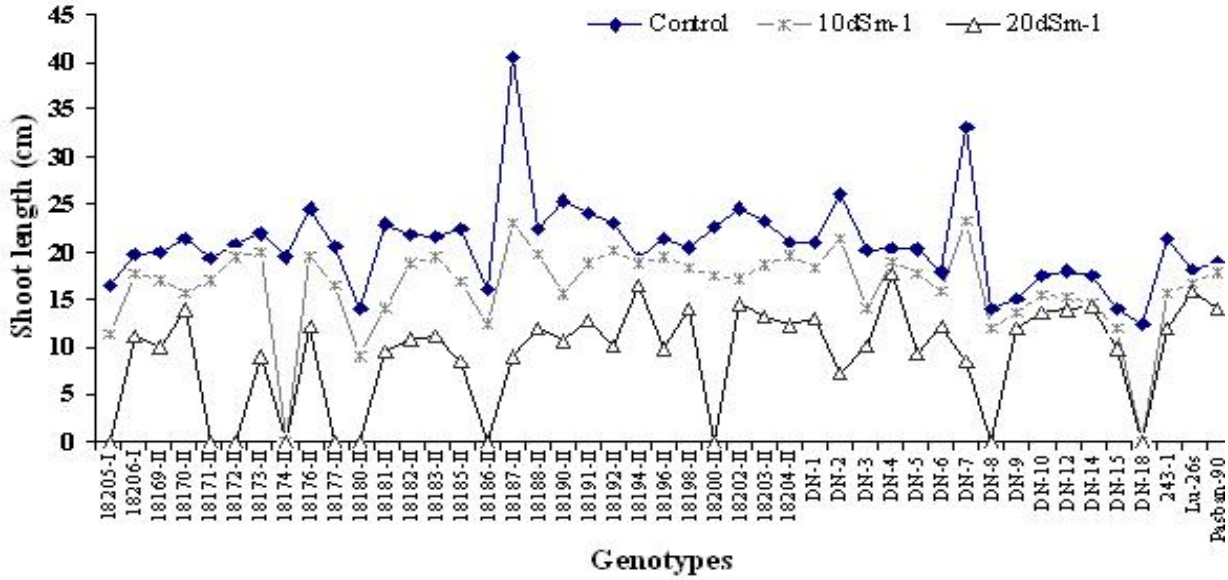


Fig. 2. Absolute shoot lengths (cm) of 45 wheat genotypes grown in control and 2 salinity levels



The root length data of 24 seedlings (6 from each of four replicates) of the 45 genotypes assessed under each NaCl concentration and control were analysed using an analysis of variance which partitioned total variances into its components. The variance due to between-genotypes and within-genotypes were used to calculate broad-sense heritability using the formula given by Falconer and Mackay (1996).

$$h^2_B = Vg/Vp$$

Vg and Vp are genotypic and phenotypic variances respectively.

RESULTS

The results of analysis of variance for absolute and relative salt tolerance showed significant genotypic differences ($p \leq 0.01$) in root and shoot lengths (Table I). Differences between the three NaCl concentrations were also significant ($p \leq 0.01$). The interaction terms (Gen. × Sal.) were significant ($p \leq 0.01$) for absolute root and shoot lengths and relative root length revealing that genotypes responded differently to increasing salinity levels in the growing medium. However, the mean squares resulted from

Table I. Mean squares for absolute and relative root and shoot lengths of 45 wheat genotypes grown in control and two NaCl concentrations

Source of variation	DF	Absolute values		DF	Relative values	
		Root length	Shoot length		Root length	Shoot length
Replications	3	0.722 ^{NS}	6.075^{NS}	3	850.598 ^{NS}	173.961^{NS}
Genotypes (Gen.)	44	54.700**	76.230**	44	8633.617**	782.425**
Salinity levels (Sal.)	2	69.722**	31.283**	1	8418.156**	1782.425**
Gen. x Sal.	88	19.548**	16.822**	44	2488.947**	220.925 ^{NS}
Within + Residual	403	4.827	6.788	267	394.301	325.846

*, ** and NS indicates differences significant at p≤0.01, p≤0.05 and non-significant respectively.

interactions (Gen. × Sal.) were non-significant (p ≥ 0.05) for relative shoot length suggesting that genotypes were affected similarly by increasing salinity for this trait.

The data on absolute values of salt tolerance showed that root length of 45 genotypes measured in control differed from each other, it was longest in 18185-II (32 cm) followed by 18187-II (31 cm) (Fig. 1). By contrast, root lengths of 18174-II, 18180-II, DN-9, 18205-I and DN-10 in control were shorter, measuring 7, 7, 9, 9, and 9 cm, respectively. When means of root length measured in low salinity were compared, different responses of the genotypes were revealed. Root lengths of 18194-II and DN-4 were 20 and 19 cm, respectively, whilst 18174-II did not survived, and 18180-II and 18205-I produced 4 and 5 cm roots respectively. In high salinity, root lengths of DN-4 and 18194-II were measured as 19 and 16 cm respectively, which were higher than that of LU-26S which produced 12 cm. Based upon mean root length in two salinities, genotypes 18194-II and DN-4 which produced longer roots than those of LU-26S, may be regarded as the most tolerant, whilst 18180-II, 18205-I and DN-18 were revealed as the most sensitive genotypes. Relative salt tolerance based upon root length data provides further estimates of the salinity tolerance of genotypes (Table II). The comparison of genotypes based upon relative root lengths showed that some of the genotypes were more tolerant than others even at 10 dS m^{-1} NaCl, The genotypes DN-4 gave 93%, and 18194-II gave 92% root length of the control solution whereas DN-18 was measured as only 49%. With increased salinity level in the growing medium (20 dS m^{-1}), the root length of all the genotypes were affected but to varying degrees and the differences between genotypes were again striking. Under 20 dS m^{-1} genotype 18194-II with 85% index of salt tolerance appeared to be affected lesser than 18190-II. Root length of Pasban-90 was 50% of the control under 20 dS m^{-1}.

Similar to root lengths, shoot lengths of 45 genotypes also differed in control solution. Shoot length of 18187-II was longest (40 cm) followed by18190-II (25 cm) in control, and reduced significantly under NaCl stress, having mean values only 16 and 13 cm, respectively (Fig. 2). In comparison, shoot length of genotype 18194-II (19 cm) in control, was measured 18 cm in salinities and thus affected less due to salinities, similar contrast in responses of other genotypes namely LU-26S, DN-4 and DN-9 was evident, all of them produced mean shoot lengths ranging 12 to 18 cm. In 10 dS m^{-1}, 18180-II was affected the most with a tolerance index of 65%, and by contrast, 18194-II and DN-4 had the greatest index of tolerance i.e. 98 and 93%, respectively (Table II). The data also showed that at higher salinity levels, the relative behaviour of the genotypes also changed. Although shoot length decreased markedly in 20 dS m^{-1}, pronounced differences between genotypes were still evident. Genotypes 18192-II and DN-4 having an index of tolerance of 87%, showed almost same tolerance in 20 dS m^{-1} as shown by LU-26S (87%). However, from overall assessment of the genotypes, 18194-II with a mean value of 92% and DN-4 with 90% appeared to be the most tolerant.

In 10 and 20 dS m^{-1} salinity levels, estimated genetic variances for the absolute root length were 2.28, 0.67 and estimates of h^2_B were 0.46, and 0.32, respectively. Genetic variances for relative root length were 137.31, 136.15, and estimates of h^2_B were 0.23, and 0.28, respectively. Variation in root length in control solution also had a significant genetic basis with an estimated h^2_B value of 0.52.

DISCUSSION

Obviously salinity tolerance is necessary at the whole plant level through the whole life cycle to seed production in grain producing species and for herbage production in forage species. It has been shown in several crops that tolerance at the seedling stage also reflects enhanced salinity tolerance at the adult plant level. This has been used as a mean for selecting for enhanced salinity tolerance in maize (Ashraf & McNeilly, 1990), rice (Shannon *et al*., 1998), wheat (Salam *et al*., 1999) and in cotton (Azhar & Ahmad, 2000). Maiti *et al*. (1996) have reported that variation in seedling response to salinity (and to drought) reflects potential grain yield at maturity. This implies that at least as a mean for preliminary selection for salinity tolerance in these species, screening of seedlings is a productive method, assuming that variability at the seedling stage is genetically based. Once genotypes are identified, the breeder or geneticist can use the advanced screening procedures either to improve salt tolerance or to study its heritability. For the development of the most promising plant material of any crop, research workers are obliged to collect this information before selecting desirable plants for breeding material.

Dewey (1962) used absolute values to make comparisons of genotypic responses to salinity; While Maas (1986) used relative values for this purpose. The analyses of variance of the data showed different behaviour of the genotypes to salinity. This indicated that different genes are responsible for salt tolerance (Salam, 1993).

In previous work LU-26S had been described as salt

Table II. Relative root and shoot lengths (%) of 45 wheat genotypes grown in control and two salinity levels

Sr. No.	Genotypes	Relative root length (%)				Relative shoot length (%)			
		Control	10 dS m^{-1}	20 dS m^{-1}	Mean of 2 salinities	Control	10 dS m^{-1}	20 dS m^{-1}	Mean of 2 salinities
1	18205-I	8.83	51.18	-	25.59	16.50	68.18	-	34.06
2	18206-I	10.50	71.42	40.47	55.94	19.80	89.65	56.46	73.05
3	18169-II	13.12	76.22	23.70	49.96	20.00	85.00	50.00	67.50
4	18170-II	13.00	65.23	46.30	55.76	21.50	73.26	64.77	69.01
5	18171-II	12.25	75.51	-	45.92	19.37	87.76	-	43.88
6	18172-II	15.33	84.80	-	42.40	20.83	93.61	-	46.81
7	18173-II	10.00	70.40	46.60	58.50	22.00	90.91	40.90	65.90
8	18174-II	6.50	-	-	-	19.50	-	-	-
9	18176-II	10.98	53.09	40.98	47.03	24.50	79.59	77.14	78.36
10	18177-II	11.33	80.85	-	40.42	20.66	79.86	-	39.93
11	18180-II	7.12	67.44	-	33.72	14.00	64.64	-	32.32
12	18181-II	18.66	61.63	50.91	56.27	22.83	91.98	77.75	84.86
13	18182-II	11.25	67.37	60.00	63.68	21.75	86.76	72.78	79.77
14	18183-II	10.75	81.40	42.32	61.86	21.62	90.19	51.75	70.97
15	18185-II	32.25	50.64	38.76	44.70	22.50	75.15	38.08	56.61
16	18186-II	7.50	86.67	-	43.33	16.00	78.13	-	39.06
17	18187-II	31.25	56.38	38.40	47.39	40.37	64.40	49.54	56.97
18	18188-II	11.88	75.75	48.01	61.88	22.50	92.22	82.22	87.22
19	18190-II	9.50	51.26	22.94	37.10	25.41	60.99	41.79	51.39
20	18191-II	11.00	51.51	32.63	42.07	24.00	78.46	53.12	65.79
21	18192-II	9.66	82.81	62.11	72.46	23.00	91.83	86.96	89.39
22	18194-II	22.10	92.17	84.84	88.50	19.25	98.03	85.71	91.87
23	18196-II	10.25	78.04	41.46	59.75	21.50	90.70	84.27	87.48
24	18198-II	10.62	73.44	51.22	62.33	20.50	82.93	72.24	77.58
25	18200-II	9.62	64.96	-	32.48	22.52	77.70	-	38.85
26	18202-II	9.72	81.48	46.81	64.14	24.50	83.67	63.34	73.50
27	18203-II	12.25	77.55	51.67	64.61	23.25	84.51	65.33	74.92
28	18204-II	10.12	88.93	39.52	64.22	21.00	93.38	67.38	80.38
29	DN-1	11.25	76.62	31.20	53.91	21.00	87.47	78.57	83.02
30	DN-2	12.62	75.27	49.20	62.23	26.00	85.58	82.69	84.13
31	DN-3	13.75	81.82	40.36	61.09	20.12	70.12	50.29	60.20
32	DN-4	20.21	93.46	76.94	85.20	14.37	93.37	86.83	90.10
33	DN-5	11.62	78.49	30.89	54.69	20.25	87.65	76.04	81.84
34	DN-6	10.75	90.70	42.97	66.83	17.87	88.81	68.21	78.51
35	DN-7	9.37	76.41	50.46	63.43	33.00	79.27	69.17	74.22
36	DN-8	9.00	79.11	-	39.56	14.00	85.71	-	42.86
37	DN-9	8.50	74.94	37.70	56.32	15.00	91.07	79.13	85.10
38	DN-10	8.97	79.82	45.27	62.54	17.50	87.83	78.05	82.94
39	DN-12	9.16	76.41	51.85	64.13	18.00	84.72	76.83	80.78
40	DN-14	11.00	77.27	53.54	65.40	17.50	82.86	81.43	82.14
41	DN-15	9.37	64.03	36.60	50.31	14.00	85.71	70.07	77.89
42	DN-18	10.13	49.25	-	24.58	17.21	72.10	-	36.02
43	243-1	10.50	73.81	24.95	49.38	21.50	91.86	79.07	85.46
44	Lu-26s	19.87	77.15	60.39	68.77	18.12	92.43	87.47	89.95
45	Pasban-90	12.00	71.83	50.00	60.91	19.00	93.94	74.47	84.20

tolerant (Ashraf, 1994; Farooq *et al*., 1995).Therefore, genotype LU-26S was used as check to compare salinity tolerance of wheat germplasm. The absolute data presented in Figures 1 and 2 for root length and shoot length respectively, showed that root lengths of the 45 genotypes were affected seriously as compared to their shoot lengths, showing that root is the most sensitive organ of plant. Similar suggestions have been given by Levitt (1980), Noor *et al*. (2001) and Okusanya and Ungar (1984). It had been observed that growth and production of cytokinins in roots were immediately stopped and under severe stresses root growth was ceased altogether (Bottger, 1978), thus root measurement is a reliable indicator of measuring salt tolerance of a species. The data exhibited marked differences in genotypic responses to salinity and some of the genotypes were shown to be higher salt tolerant than others. Based upon root and shoot length data genotype 18194-II and DN-4 (Fig. 1) seems to be more tolerant than LU26S and in contrast genotypes 18180-II, 18205-I and DN-18 were identified as salt sensitive. This data also suggest that there is a considerable phenotypic variation in salinity tolerance in wheat germplasm. These intervarietal variations suggested that improvement might be achieved through selection or by crossing tolerant and sensitive genotypes. The different behaviour of genotypes could be attributed to the differences in gene frequencies and their interaction with the environment (Maas, 1986; Cheeseman 1988).

Table III. Components of variance and broad sense heritability (h^2_B) of NaCl tolerance at seedling stage

Component	Absolute root length			Relative root length	
	Control	10 dS m^{-1}	20 dS m^{-1}	10 dS m^{-1}	20 dS m^{-1}
$\delta^2_b = Vg$	5.26	2.28	0.67	137.31	136.15
$\delta^2_b + \delta^2_w = Vp$	10.06	4.95	2.11	585.89	485.52
$h^2_B = Vg/Vp$	0.52	0.46	0.32	0.23	0.28

Although salt tolerance indices of root and shoot length reveals different rates of decrease in genotypic responses to low and high salinity levels, the interaction Gen. x Sal. being non-significant exhibited the decrease to be similar in all the genotypes. Azhar and McNeilly (1989) observed non-significant effects of salinity on shoot weight. The indices of salt tolerance of genotypes measured under 10 and 15 dS m^{-1} reveal considerable decrease in the characters, genotype 18194-II and DN-4 exhibit little decrease and thus are more salt tolerant than 18180-II, 18205-I and DN-18 (Table II). Shannon (1984) has suggested that variation in plant vigor in non-saline condition may account to some degree for variation in salinity tolerance, high vigor and high tolerance being correlated. This suggestion may be substantiated by the response of the genotypes to salinity (Table II). Genotypes 18194-II and DN-4 being vigorously growing genotypes in nonsaline condition (root length), expressed high mean tolerance index of 89 and 85%, respectively and thus agree with that of Shannon (1984). In contrast genotype 18185-II and 18187-II measuring longer root lengths in control, exhibited decrease in tolerance index, 45 and 47%, respectively. Genotype LU-26S which is slow growing genotype in control, yet it is observed as moderately tolerant, suggesting that high tolerance to environmental stress and high yield in non stalinized conditions appeared to be mutually exclusive (Rosielle & Hamblin, 1981). The genotype DN-18 that is also a slow growing genotype in control retained 49% of the root length under low salinity and did not survive at high salinity level. This behavior of the genotype suggest that there is no clear relationship between plant vigor in control and growth in salinized solution and did not appear to agree to the suggestion of Shannon (1984) and agreed with the suggestion of Rosielle and Hamblin (1981). Thus based upon root length data reported here three genotypes namely 18194-II, DN-4 and LU-26S appeared to be more salt tolerant than the others while three genotypes namely 18180-II, 18205-I and DN-18 appeared to be salt sensitive ones.

The estimates of broad sense heritability calculated using root length measurement data were high (Table III). Similar studies had been reported in wheat (Ali *et al*., 2002) and in cotton, (Azhar & Ahmad, 2000; Noor *et al*., 2001). The trend of reduction in extent of genetic variation with the increase in salinity level may be due to the low intrinsic capability of the genotypes to tolerate salts at higher concentrations. Root length being more heritable was used as selection criteria to identify salt tolerant and sensitive genotypes.

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