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SODIUM CHLORIDE TOLERANCE IN RICE (*ORYZA SATIVA* L.) AT EARLY SEEDLING GROWTH: GENOTYPIC VARIABILITY, IDENTIFICATION AND SELECTION

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Abstract

Plant growth response of four week old seedlings of 48 rice lines/cultivars to four NaCl levels (0, 10, 15 and 20 dS/m) was assessed in solution culture. Genotypic responses were compared from measures of absolute and relative salt tolerances. Increasing salinity levels in the growing medium differentially reduced fresh shoot weight, seedling fresh weight and specific shoot length. Shoot length, root length, seedling length, fresh root weight, specific root length, dry shoot weight, dry root weight and seedling dry weight were less affected differentially. The rice genotypes originating from Pindi Bhatian exhibited significantly greater salinity tolerances overall than those originating from Faisalabad, Kala Shah Kaku and IRRI. Estimated broad sense heritabilities indicated that phenotypic variance exceeded that of genotypic by nearly two orders of magnitude. These findings indicate that the genetic improvement of salt tolerance in rice through selection will be problematic due to masking effects of the environment, and imply rigorous and careful selection of salt tolerant genotypes.

Introduction

Rice is the second most important cereal crop next to wheat (*Triticum* spp.). Although it has moderate tolerance to soil salinity, it can tolerate exchangeable sodium in appreciable amounts. Rice grows, as hydrophytes do, in a standing layer of water thus diluting the solution in the top soil layers. Due to its shallow root system, roots grow only superficially in the top soil layers. Some desalinization usually occurs prior to transplantation as a result of puddling. In arid and semi arid areas rice is often planted as a reclamation crop (Anon., 1980).

It has been reported that salinity affects rice plant to varying degrees from germination to maturity, however the effect may vary depending on the stage of plant development (Castro & Sabado, 1977; Aslam *et al*., 1994). Rice is tolerant during germination, very sensitive during early seedling stage, tolerant during vegetative stage, again becomes sensitive during pollination and fertilization, and then becomes increasingly tolerant towards maturity (Anon., 1967). The effect of salinity was also related to the stage of plant development at which salinity was imposed, concentration and nature of the salt, and the duration of salinization (Pearson *et al*., 1966; Aslam *et al*., 1993; Zeng *et al*., 2001).

Reclamation, drainage and water control can minimize the extent and spread of saline soils, but engineering and management costs are high. Increasing costs for water and energy accentuate the need for cost effective strategies. Breeding crops for salt tolerance is one example of such a strategy. It is a promising, energy efficient approach that can be meshed with water and land management alternatives. The adaptation of crops to salinity is a formidable challenge for plant breeders and geneticists to meet the increasing needs of food in the future.

Flowers *et al*., (1997) discussed the role and scope of plant breeding in the development of salt tolerant crop plants. Availability of ample genetic variability in a crop is considered to be a pre-requisite to launch an effective breeding program. The previous findings suggest that considerable genetic variation exists among rice cultivars but this variation has not been exploited advantageously (Ikehashi, 1979). Other reports support the feasibility of breeding for salt tolerance because there is no antagonism between high yield and salt tolerance (Shannon & Akbar, 1978; Gonzales and Ramirez, 1998). Keeping in view the magnitude and spread of salt affected soils throughout the world, the development of salt tolerant crops was seen as an important area of research. The present research determines the genetic variability, its transmissibility, and identification and selection of salt tolerant rice lines/cultivars to be used in future regular breeding programs.

Materials and Methods

Plant material and screening protocols: Grains of 48 rice lines/cultivars were obtained from different Rice Research Institutes in Pakistan including the Salinity Research Institute (SRI), Pindi Bhatian; Rice Research Institute (RRI), Kala Shah Kaku and Nuclear Institute for Agricultural Biology (NIAB), Faisalabad, Pakistan. The grains of 48 lines/cultivars were grown in iron trays filled with acid washed gravel. The young seedlings at the two-leaf stage were transferred to aerated half strength Hoagland solution (Hoagland & Arnon, 1950) in four large iron containers (118 x 88 x 30 cm). Each of the 48 lines/cultivars was planted in triplicate in the three NaCl treatments i.e. 10, 15 and 20 dS/m, and in one without salt (control). The appropriate salinity levels in the three containers were raised in four equal steps across four days i.e. one step/day, starting on 3rd day of seedlings transplantation in the containers. The pH of the solutions ranged from 6.0 to 6.5 and was adjusted daily using 1N HCl and/or NaOH solutions. The NaCl solutions in the containers were changed after two weeks. After four weeks growth, shoot length (cm), root length (cm), fresh shoot weight (g), fresh root weight (g), dry shoot weight (g) and dry root weight (g) of six seedlings of each line in each replication were measured. Seedling length (cm), seedling fresh weight (g) and seedling dry weight (g) were estimated from the data on the component parts of the seedling i.e., shoot and root. Specific shoot length (g cm^{-1}) and specific root length (g/cm) were estimated from the data on fresh weights and lengths of the shoots and roots respectively. The percent changes, in the characters measured in salinity, relative to control (relative salt tolerance) of 48 lines were computed and compared following Ali *et al*., (2007).

$$\text{Relative salt tolerance} = \frac{\text{Performance in control - Performance in salinity}}{\text{Performance in control}} \times 100$$

Statistical analysis: The values of absolute salt tolerance and indices of salt tolerance of 48 lines were subjected to ordinary analysis of variance in order to see whether the genotypic differences were significant. For this analysis general linear model of SPSS 8.0 for Windows: Advance Statistics, (Anon., 1994) was used. The genotypic and phenotypic components of variance were estimated and broad-sense heritability (h^2_{bs}) was determined following Falconer & Mackay (1996).

$$h^2_{bs} = Vg/Vp$$

Where, Vg and Vp are estimates of genotypic and phenotypic variances, respectively.

The correlation coefficients among selected pairs of variates studied under normal and salt stress conditions were computed using following function of Pearson (1896).

$$r_{ab} = cov._{ab} / \sqrt{(var._a \times var._b)}$$

Where,
r_{ab} = correlation coefficient between a and b variables;
$cov._{ab}$ = covariance between a and b variables;
$var._a$ and $var._b$= variances of a and b variables, respectively.

Results

Significant differences ($p \leq 0.01$) were observed among rice lines for all the traits and reduction in these traits due to increasing salinity levels (Table 1). The interaction terms (gen. × sal.) were significant ($p \leq 0.01$) for three of 11 characters only i.e., fresh shoot weight, seedling fresh weight and specific shoot length revealing that genotypes responded differently for these traits to increasing salinity levels in the growth medium. The non-significant interaction terms for the remaining traits indicated the similar response (reduction) of the lines to increasing salinity levels.

Heritability (broad sense) together with genotypic and phenotypic variances for significantly differential traits i.e., fresh shoot weight, seedling fresh weight and specific shoot length were estimated under 0, 10, 15 and 20 dS/m of NaCl stress (Table 2). Genotypic and phenotypic variances decreased as the salinity level in the solution increased except at 20 dS/m where these were greater than those in 15 dS/m but less than 10 dS/m and control. Estimates of heritabilities (bs) were almost equal for the three traits in saline and non-saline solutions. These estimates were moderate to high in control and salinity levels varying from 0.41 to 0.63. Phenotypic variance exceeded that of genotypic by nearly two orders of magnitude in all the cases.

Table 1. Mean squares of absolute values for various seedling traits of 48 rice genotypes grown in control and three salinity levels.

Source of variation	df	Shoot length	Root length	Seedling length	Fresh shoot weight	Fresh root weight	Seedling fresh weight	Specific shoot length	Specific root length	Dry shoot weight	Dry root weight	Seedling dry weight
Genotypes (Gen.)	47	320.70**	24.57**	324.31**	423.39**	82.33**	787.71**	0.0878**	0.1450**	21.05**	1.34**	30.34**
Salinity levels (Sal.)	3	14827.44**	385.88**	18014.73**	11521.85**	355.86**	15894.78**	1.0870**	0.3210**	119.13**	13.08**	208.86**
Gen. × Sal.	141	30.72NS	2.79 NS	33.06 NS	96.12**	5.96 NS	139.30**	0.0134**	0.0089 NS	1.81 NS	0.19 NS	2.56 NS
Within + Residual	384	28.83	4.17	29.47	50.73	7.62	86.00	0.0087	0.0118	2.58	0.27	3.57

*, ** and NS indicates significant differences at $p \leq 0.05$, $p \leq 0.01$ and non-significant ($p > 0.05$) respectively

Table 2. Estimates of phenotypic (Vp) and genotypic variances (Vg), and broad sense heritability (h^2_{bs}) of fresh shoot weight, seedlings fresh weight and specific shoot length of 48 rice genotypes grown in control and three salinity levels.

Component	Fresh shoot weight (g)	Seedling fresh weight (g)	Specific shoot length (g/cm)
Control			
Vg	108.82	186.84	0.015
Vp	256.51	434.72	0.036
h^2_{bs}	0.42	0.43	0.417
10dS/m			
Vg	30.00	46.84	0.008
Vp	49.55	89.27	0.013
h^2_{bs}	0.61	0.52	0.625
15dS/m			
Vg	12.35	23.41	0.004
Vp	30.25	47.55	0.009
h^2_{bs}	0.41	0.49	0.423
20dS/m			
Vg	18.44	30.11	0.004
Vp	36.24	59.66	0.008
h^2_{bs}	0.51	0.50	0.500

Of the 48 rice genotypes tested for salinity tolerance, 28 displayed non-significant changes in fresh shoot weight at the low salinity concentration of 10 dS/m NaCl (Table 3). At 15 dS/m NaCl, 11 of the 48 genotypes exhibited non-significant differences in shoot growth. These genotypes included SRS-61, SRS-62, SRS-64, SRS-504, SRS-505, SRS506, DM-1-30-34-2002, NIAB-IRRI-9, SRI-8, SRI-52, and SRI-54. However, only 4 genotypes i.e., SRS-62, SRS-504, SRI-8, and SRI-52 displayed significantly unaltered shoot growth at highest salinity concentration of 20 dS/m (Table 3). The highest proportion of genotypes tolerant of the low salinity level (10 dS/m NaCl) originated from Pindi Bhatian. These genotypes had significantly greater salinity tolerances overall than those originating from Faisalabad, IRRI, and Kala Shah Kaku (Table 4).

Table 3. Percent change in fresh shoot weight from control of 48 rice genotypes grown in control and three salinity levels. Values are scaled from highest to lowest change in 10 dS/m with those significant at * p≤ 0.05 indicated. NS = not significant.

S. No.	Genotypes	Control	10 dS/m	15 dS/m	20 dS/m
1.	EF-1-2-54-2002	57.90	72*	69*	76*
2.	EF-1-25-30-2002	54.20	70*	75*	77*
3.	SRI-56	32.97	66*	73*	75*
4.	Shaheen	52.57	60*	70*	72*
5.	DM-1-25-4-2002	54.80	60*	69*	70*
6.	EF-1-30-54-2002	27.90	59*	65*	73*
7.	EF-2-20-3-2002	33.07	57*	59*	68*
8.	SRI-8	47.00	51*	67*	73*
9.	Super Basmati	25.13	43*	51*	57*
10.	SRS-501	48.77	50*	57*	67*
11.	DM-1-30-9-2002	46.33	50*	65*	71*
12.	EF-2-30-1-2002	23.10	48 NS	60*	63*
13.	SRI-51	22.77	48 NS	48*	53*
14.	Bas. 2000	27.43	48*	63*	66*
15.	IRRI-26	36.27	47*	51*	56*
16.	Bas. 385	38.70	46*	63*	84*
17.	DM-24	36.07	46*	65*	68*
18.	SRS-502	59.67	45*	49*	64*
19.	SRI-55	23.03	45 NS	51*	62*
20.	SRS-66	27.53	45 NS	55*	60*
21.	DM-1-30-53-2002	47.57	44*	55*	64*
22.	EF-1-20-51-2002	29.37	43 NS	50*	62*
23.	KS-282	52.17	42*	54*	57*
24.	IRRI-6	46.53	41*	51*	69*
25.	SRI-13	29.33	40 NS	39*	63*
26.	Bas. 370	26.57	40 NS	48*	51*
27.	Kashmir Basmati	48.07	37*	71*	72*
28.	SRI-57	36.17	35 NS	41*	62*
29.	SRI-16	35.13	35 NS	56*	60*
30.	SRS-63	21.50	35 NS	49*	54*
31.	SRI-53	19.13	34 NS	44*	48*
32.	SRS-65	30.77	33 NS	44*	52*
33.	SRS-506	33.10	32 NS	37 NS	49*
34.	SRS- 505	26.97	31 NS	37 NS	40*
35.	PB-95	39.07	28 NS	60*	64*
36.	DM-1-30-34-2002	23.60	26 NS	31 NS	44*
37.	Bas. Pak	29.33	25 NS	60*	61*
38.	Bas. 198	25.57	20 NS	43*	53*
39.	NIAB-IRRI-9	30.67	20 NS	34 NS	61*
40.	SRS-504	26.57	19 NS	32 NS	33 NS
41.	EF-1-30-17-2002	20.63	17 NS	57*	61*
42.	SRS-61	20.47	17 NS	35 NS	38*
43.	SRI-8	12.50	13 NS	26 NS	30 NS
44.	SRS-503	33.07	12 NS	42*	44*
45.	SRI-52	13.27	12 NS	24 NS	27 NS
46.	SRI-54	14.33	9 NS	35 NS	46*
47.	SRS-64	29.50	8 NS	27 NS	67*
48.	SRS-62	10.53	3 NS	7 NS	11 NS

Table 4. Statistical comparisons of relative salt tolerances of genotypes from different geographic locations.

Geographic location	10 dS/m	15 dS/m	20 dS/m
Faisalabad	46a	58a	66a
IRRI	44ab	51b	62a
Kala Shah Kaku	38bc	57ab	63a
Pindi Bhatian	33bc	44c	52b
F-ratio	$F_{3,44}$ = 39.7**	$F_{3,44}$ = 48.1**	$F_{3,44}$ = 37.3*

Values followed by dissimilar letters significantly different at * p≤0.05, ** p≤0.01.

Discussion

The solution culture technique followed in the present research work simply used four-week old seedlings to evaluate pattern of variability for salt tolerance in 48 diverse rice lines/cultivars, and provided useful information at the early stage of plant development. This method has been extensively used to study salt tolerance in rice (Aslam *et al*., 1993), and wheat (Qureshi *et al*., 1990; Khan *et al*., 2003b; Ali *et al*., 2007). A review of the literature shows that seedling stage is the most sensitive phase of plant development and most of the work on different crop species has been done at this stage, for example, in wheat (Kingsbury & Epstein, 1984; Qureshi *et al*., 1990; Salam *et al*., 1999; Ali *et al*., 2002, 2007; Khan *et al*., 2003b), forages (Ashraf *et al*., 1987), sorghum (*Sorgum bicolor*) (Azhar & McNeilly, 1987, 1989; Azhar *et al*., 1998), lucerne (*Medicago sativa*) (Al-Khatib *et al*., 1993), rice (Shannon *et al*., 1998), maize (*Zea mays*) (Rao & McNeilly 1999; Khan & McNeilly, 2000), and cotton (Akhtar & Azhar, 2001).

The expression of salt tolerance in rice was studied measuring 11 characters. Only three characters displayed differential genotypic behaviour for salt tolerance (Table 1). Therefore, broad-sense heritability was estimated for only these three characters because remaining characters did not exhibit genotypic differences in their responses with increasing salinity levels (Table 2). Although heritability estimates suggested much greater phenotypic than genetic variation in salinity tolerance among genotypes, there was a significantly greater preponderance of salt tolerant rice genotypes originating from Pindi Bhatian than from other geographic locations (Tables 3 and 4). Pindi Bhatian is highly salt affected area, most of the genotypes originating from there may carry salt tolerant genes due to natural selection and thus rice genotypes obtained from Soil Salinity Research Institute (SSRI) at Pindi Bhatian, Pakistan were more tolerant. The natural selection for salt tolerance in other crops like in wheat also operated. For example, Kharchia 65 a universally regarded as highly salt tolerant wheat landrace (Ali *et al*., 2007; Ashraf, 2002) from salt affected area, and extensively used at Central Soil Salinity Research Institute (CSSRI), Karnal, India. At CSSRI, most of the salt tolerant wheat genotypes were Kharchia 65 derivatives (Hollington, 2000). It is quite possible that rice genotypes from SSRI in the present study may carry some common salt tolerant progenitor. The frequency of salt tolerant genes from that progenitor increased in the succeeding derivatives due to either natural selection or human assisted selection.

The heritability values in a broad sense are useful as first approximations but not as definitive values for the improvement of salinity tolerance in rice. Heritability estimates were larger at 20 dS/m NaCl stress than non-stress condition. Increased heritability estimates under salt stress were also reported in tomato (Saranga *et al*., 1992; Foolad, 1996) and maize (Khan *et al*., 2003a). Saranga *et al*., (1992) speculated that increased heritability under increasing salinity levels might be a result of greater genetic variation due to the expression of genes associated with salinity tolerance and/or a smaller environmental variation. Bradshaw & Hardwick (1989) argued that hidden variation, previously unselected, could be uncovered when stress is applied, thus possibly increasing heritability. The substantially greater phenotypic variance indicated a strong masking effect of the environment which may make genetic improvement through selection of salt tolerant genotypes problematic (Singh & Narayanan, 2000; Ali *et al*., 2007). Reduction in genetic variability under stress, which has been reported previously in wheat (Singh & Chatrath, 1992; Ashraf, 1994; Ali *et al*., 2007), rice (Shereen *et al*., 2005) and sorghum (Azhar & McNeilly, 1989), suggests rigorous and careful selection of salt tolerant genotypes. Several genotypes which exhibited non-significant intra-genotypic variation in a pooled response at high salinity and control also exhibited non-significant intra-genotypic variation in a pooled response at low salinity and control, and thus appeared to be homogenous across the salinities (Table 3). These genotypes may contain more salt tolerant genes required to confer salt tolerance over a range of increasing salinities. These genotypes are precious resource to be used in further rice breeding programmes aimed at increasing salt tolerance.

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