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Salt Tolerance Evaluation of Rice (*Oryza sativa* L.) Genotypes Based on Physiological Characters Contributing to Salinity Resistance

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Abstract. Seven newly developed rice cultivars i.e., KS-133, DR-83, DR-64, BR-601, Gomal, JP-5 and Gomal-6, were evaluated for salinity tolerance in a glasshouse along with three varieties of known salinity tolerance i.e., KS-282 (tolerant), IR-6 (medium tolerant) and Basmati-385 (susceptible). Based on the survival percentage at 50 mol/m^3 sodium chloride salinity imposed at seedling stage, rice cultivars KS-133, Gomal, and DR-83 showed high survival comparable to that of salinity tolerant cultivars like KS-282, and were thus placed in tolerance range. Survival percentage of JP-5, Gomal-6 and DR-64 remained in medium tolerance range (35 to 38%) as that of IR-6. The rice cultivar BR-601 showed only 13% survival and was found to be as sensitive towards salinity as Basmati-385. The results of rice survival in saline medium showed good uniformity and the check varieties showed results corresponding to those found elsewhere. Sodium (Na^+) and potassium (K^+) concentrations in the third leaf showed variations among different rice cultivars under salinity. There was an inverse correlation between varietal leaf Na^+ *vs* survival percentage (r = -0.808) and Na^+ *vs* leaf chlorophyll (r = -0.857). The correlation between K^+ and final survival percentage was direct (r = 0.744) and also leaf chlorophyll *vs* survival (r = 0.952). The shoot fresh and dry weights were greater in the rice genotypes having higher final survival percentage under saline conditions. Therefore, in addition to final survival percentage, the higher shoot fresh and dry weight under salinity could be also used as criterion for evaluation of salinity tolerance of rice.

Keywords: salinity, rice, chlorophyll, salinity tolerance

Introduction

Salinization of agricultural soils is one of the major abiotic stresses reducing crop productivity worldwide. Over 6 % of the global land area and over 20% of the irrigated land are currently affected by salinity (Munns, 2005). As irrigated system supplies roughly one-third of the world food supply, therefore, addressing the problem of salinity is of great concern especially with an increasing global population. Rice is one of the most important food crops, but the yield of the grain is very susceptible to salinity (Akbar *et al.,* 1985). In Pakistan out of 6.8 million hectares of salt-affected land, over 1.5 million hectares are under rice cultivation (Khan, 1998). Thus, selection of rice cultivar having salt tolerant potential that would grow over a range of soil salinity is a prerequisite for generating income for rice farmers having sizable salt-affected lands.

Salinity resistance in rice is a complex character and many factors contribute to such resistance as occurs in species. Physiological studies suggest that in rice, restriction of sodium entry, higher potassium uptake, plant vigour, tissue tolerance to absorbed ions and water-use efficiency are the main factors contributing towards salinity resistance (Yeo *et al.,* 1990). Reduction in shoot growth under salinity limits the volume of tissue for the uptake of newly arriving salt and once it starts, the situation worsens. Accumulation of Na^+ and Cl^- in the leaves has been found to reduce photosynthetic activity, with ultra-structural and metabolic damage (Flowers *et al.,* 1985). Salinity tolerance in rice can be enhanced by reducing the influx of excessive amounts of sodium chloride in the transpiration stream. The salt concentration in the shoot can be reduced by lowering sodium transport to the shoot and/ or increasing plant vigour. Normally the more vigorous plants under non-saline conditions show greater resistance to salts (Yeo and Flowers, 1986). Some traditional cultivars and landraces are more tolerant to various abiotic stresses than elite cultivars. These cultivars are good source of tolerant traits; however, they generally have poor agronomic traits.

Maximizing the salt tolerance of crop species mainly depends on two factors: availability of genetic variation to tolerance and exploitation of the genetic variation by screening and selection of plants with superior performance under the applied stress (Yamaguchi and Blumwald, 2005; Shannon *et al.,* 1994). Sensitivity of rice to salinity varies with the stage of growth. Generally, it is very sensitive to salinity stress at

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young seedling stage and less at reproductive stage (Lutts *et al.*, 1995). Hence, for selection a specific growth stage that is more sensitive to salinity stress should be targeted. Rice is considered to be generally salt sensitive; there is genetic variation for salt tolerance at critical stages in the cultivated gene pool (Moradi *et al.*, 2003; Yeo and Flowers, 1983). Therefore, selection of highly salt tolerant rice cultivars could be expected to provide useful material for breeding and for experimental comparison with unselected lines, in order to examine possible mechanism of salt tolerance. In the present study, we have reported the screening of some newly developed rice cultivars for overall performance (survival) and other physiological characters, such as leaf chlorophyll, Na^+ and K^+ concentrations and biomass accumulation under salt stress.

Materials and Methods

Plant material and growth conditions. The experiment was conducted in a glasshouse with temperature controlled between 25 ºC to 35±3 ºC. Seven newly developed rice cultivars, along with three varieties of known salinity tolerance were used in the study. The three rice varieties of known salinity tolerance used as check were; KS-282 (salt resistant), IR-6 (moderate resistant) and Basmati-385 (salinity sensitive). Sterilized rice seeds were germinated and seedlings were grown in a sand culture. Seven days old seedlings were transplanted into black boxes filled with 5 L rice culture solution (Yoshida *et al.*, 1976). The solution was renewed once a week. Each cultivar was replicated thrice in separate boxes having 30 plants per replicate. At day 11, the solution was salinized with NaCl at a concentration of 50 mol/m^3 (total electrolyte concentration resulting in electrical conductivity of 6 ds/m). The concentration of 50 mol/m^3 NaCl is an established and useful working level for eliciting a wide range of varietal response (Yeo *et al.*, 1990).

Leaf sodium, potassium and chlorophyll concentrations. Six days after salinization, third leaves of 10 plants of each cultivar was analyzed for sodium (Na^+), potassium (K^+) and chlorophyll concentration as described by Din (1997). Chlorophyll contents were extracted in 80% ethanol and calculated according to Arnon (1949).

Plant growth and survival tests. After salinization for 24 days, 30 plants (10 from each replicate) of each cultivar were harvested and the shoot fresh and dry weights were recorded. The number of dead plants was recorded every day after the first plant had died and continued till more than 50% plants of the sensitive check variety, Basmati-385, were dead; a plant was considered dead when it was totally bleached. The final survival was calculated by subtracting the number of dead plants from the total number of plants and expressed as percentage.

Results and Discussion

Survival rate was used as an indicator of genotypic performance to salinity. Based on the survival in saline medium, the cultivars were divided into three tolerance ranks. Survival of KS-133, Gomal and DR-83 was as high as that of KS-282 and therefore, these three varieties were ranked as tolerant. Survival percentage of JP-5, Gomal-6 and DR-64 was 35-36% as that of IR-6, and so these were ranked as medium tolerant, while, BR-601 showed only 13% survival as that of the sensitive, Basmati-385 (Table 1).

The concentrations of Na^+, measured 5 days after salinization, showed differences in various rice cultivars (Fig. 1). There was an inverse correlation between leaf Na^+ and survival

Table 1. Ranking of salinity tolerance of rice cultivars based on the survival under NaCl salinity (50 mM)

Salinity rank	Rice genotypes	Survival (%)
Resistant	KS-133	66
Resistant	Gomal	72
Resistant	DR-83	56
Resistant	KS-282	63
Medium	JP-5	36
Medium	Gomal-6	36
Medium	IR-6	35
Medium	DR-64	29
Sensitive	Bas-385	13
Sensitive	BR-601	13

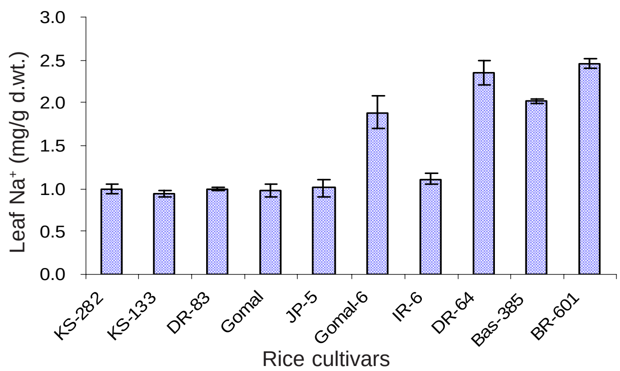

Fig. 1. Mean Na^+ concentration (mg/g dry weight) of third leaf of rice cultivars under NaCl (50 mol/m^3) salinity. Leaves were sampled 5 days after salinization. Each bar represents standard error of the mean.

(r = -0.808). Leaf K^+ showed good correlation with the salinity tolerance of rice cultivars (Fig. 2) where correlation with survival under salinity was (r=0.744, Table 2). Total chlorophyll contents measured in the third leaf after salinization was greater in the tolerant as compared to the sensitive (Fig. 3). The shoot fresh and dry weight showed good correlation with the final survival under NaCl salinity (Table 2). KS-133, Gomal and DR-83 had greater shoot fresh and dry weights and DR-64 had the least (Fig. 4).

In this study survival was used as a quantification of genotypic performance to salinity. This assessment criterion is used in the field for evaluation of salinity damage during mass screening of rice cultivars (IRRI, 1996). Based on the survival in saline medium, the cultivars were divided into three tolerance classes (Table 1). Generally, survival or visual assessment of salt damage is the criterion for overall measurement of plant performance. The characteristics would normally be chosen on their better correlation with overall performance. Yeo *et al.* (1990) concluded that survival under salinity strongly correlates with the salinity resistance of rice. In the present research study, the survival experiment was repeated three times and the results showed good uniformity; the check

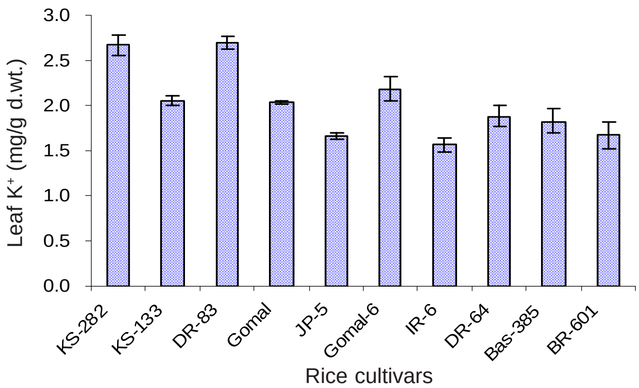

Fig. 2. Mean K^+ concentration (mg/g dry weight) of third leaf of rice cultivars under NaCl (50 mol/m^3) salinity. Leaves were sampled 5 days after salinization. Each bar represents standard error of the mean.

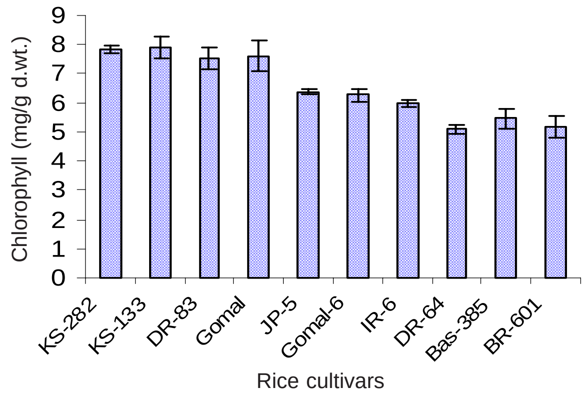

Fig. 3. Chlorophyll concentration (mg/g dry weight) of third leaf of rice cultivars under NaCl salinity (50 mol/m^3). Leaves were sampled 5 days after salinization. Each bar represents standard error of the mean.

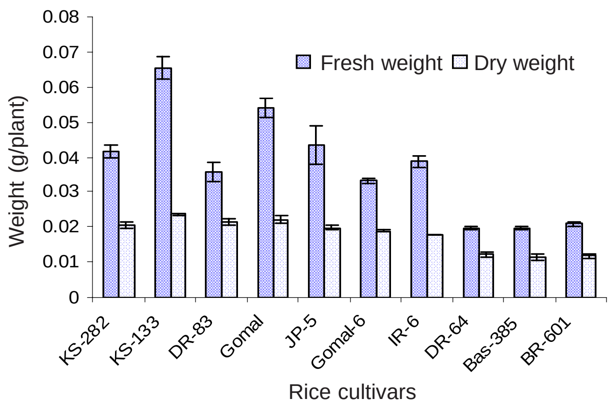

Fig. 4. Mean fresh and dry weights (g/plant) of shoots of rice cultivars, when grown for 24 days in NaCl (50 mol/m^3) salinity. Each bar represents standard error of the mean.

varieties, KS-282 and Basmati-385, showed corresponding salinity tolerance as found earlier (Khan and Abdullah, 2003) under field conditions.

In monocots, generally salinity tolerance is associated with the ability of plant to exclude Na^+ from shoot tissues (Tester

Table 2. Correlation coefficient (r) for relationship between overall performance (survival) and individual traits of the available data

	Shoot Na^+	Chlorophyll	SFW	SDW	K^+
Survival	0.808 (-)* (n = 30)	0.952 (+)* (n = 30)	0.762 (+) (n = 30)	0.886 (+) (n = 30)	0.744 (+) (n = 30)
Chlorophyll	0.857 (-) (n = 30)				

* = signs + and – represent the positive and negative correlation, respectively.

and Davenpor, 2003). In the present study, there were differences in the leaf Na^+ concentration among the rice cultivars; those having higher leaf sodium had poor survival rate (Table 1). Potassium accumulation also showed good correlation with the salinity tolerance of rice cultivars (r = 0.744). In rice, genotypic variations in Na^+ and K^+ uptake have already been reported; low concentration of Na^+ and higher K^+ were correlated with the salinity tolerance under salinity stress (Babu *et al.*, 2007; Kader *et al.*, 2006; Walia *et al.*, 2005). The chlorophyll contents measured in the third leaf after salinization was greater in the tolerant as compared to the sensitive varieties (Fig. 3). There was inverse correlation between leaf chlorophyll and Na^+ concentration (r = -0.857). Yeo and Flowers (1983) established inverse relationship between leaf chlorophyll and Na^+ concentration. The shoot fresh and dry weights were the greatest in the tolerant genotypes followed by the medium tolerant varieties and the least in sensitive rice cultivars (Fig. 4) and showed high correlation with the final survival under saline conditions (Table 2). Sankar *et al*. (2006) recorded the highest total biomass and vigour index in salt tolerant cultivars. The greater plant vigour (shoot fresh and dry weights) may provide dilution of salt concentrations with growth and tolerance within the tissues; these are the traits that may be expected to be helpful in achieving greater salinity tolerance (Yeo and Flowers, 1986). Richards (1983) concluded that vigour is essential for plant survival and productivity under saline environment. Akbar *et al.* (1985) identified more vigorous accessions, which were non-dwarf land races, as the most salt resistant in mass screening trials.

Conclusion

The data demonstrate that rice cultivars with low sodium and high potassium uptake could lead to higher survival of rice under saline conditions. The additional aspects of less chlorophyll damage under such conditions are the potential of resistant cultivars. Chlorophyll content could be used as an index of salt tolerance for selection of rice tolerance against salinity stress. It also demonstrates that amount of biomass of rice seedlings under NaCl salinity could be used as a criterion in ranking for salinity tolerance.

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