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Effect of Salinity on Morphological and Physiological Characteristics in Correlation to Selection of Salt Tolerance in Rice (*Oryza sativa* L.)

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ABSTRACT

In order to study salt tolerance in rice (*Oryza sativa* L.) seven genotypes consisting land races, pure and improvement lines were tested for their salt tolerance at two levels of salinity, 8 dS m^{-1} Electrical Conductivity (EC) and tap water as control. Completely randomized block design with three replications. Cultivars of rice were screened for salinity tolerance at seedling stage. Data taken 6 weeks after salt application were reported and traits including root and shoot length and dry weights, biomass, K^+ percentage, Na^+ percentage, Na^+/K^+ ratio, Chlorophyll Index (CI) and leaf area were measured. However, with time, salt injury symptoms were clearly visible in all plants growing in all levels of salt and showing different symptoms. The degree of injury was greater in the highest salt concentration (8 dS m^{-1}). The symptoms appeared mostly in older leaves and the upper portion of the leaves rolled and withered away. Salt injury symptoms varied with concentration of salt and between cultivars. The relative salt sensitivity of cultivars was not consistent across salt levels indicating genotypes differences in threshold levels of salt tolerance. All plant parameters decreased significantly in all genotypes with increasing salinity. Potassium/sodium ratios of the youngest three leaves (K/Na) were determined by flame photometry at the late vegetative stage. The chlorophyll index, leaf area, roots and shoot length, dry weights and biomass decreased significantly in all genotypes of rice.

Key words: Salt tolerance, rice genotypes, chlorophyll index, Na^+/K^+ ratio, biomass, leaf area

INTRODUCTION

Salinity is a worldwide problem of serious nature in arid and semi-arid regions where most of the developed countries happen to fall. It is one of the most important factors in reducing crop yields in most of the countries of the world (Khan *et al*., 1999).

Plant growth are adversely affected by salinity, a major environmental stress that limits agricultural production (Lauchli and Grattan, 2007). Rice (*Oryza sativa* L.) is one of the most staple foods in the world the second main food consumed in Iran. It is rated as an especially salt-sensitive crop. The response of rice to salinity varies with growth stages. In the most commonly cultivated rice cultivars, young seedlings were very sensitive to salinity whereas, rice was more salt tolerant at germination than other stages (Zeng, 2000).

In rice, Munns (2002) have shown that Na-K selectivity of plant roots function to minimize the entry of Na into plants and maintain effective K uptake together with the mechanism of low salt port to expanding leaves is very important mechanism directly correlated with salt tolerance.

The effects of NaCl on chlorophyll index very depending on the concentration of salt, duration of treatment and age of leaves. With the increased duration of treatment or concentration of salt, chlorophyll content decreases, with that of salt-resistant cultivars being less affected than salt-sensitive cultivars (Faustino *et al*., 1996). The response of rice to salinity varies with growth stage. In the most commonly cultivated rice cultivars, young seedlings were very sensitive to salinity (Lutts *et al*., 1995).

Munns (2002) reported that some crops are moderately tolerant of saline conditions; many crops are negatively affected by even low levels of salt. Salinity affects plants at all stages of development and for some crops, sensitivity varies from one growth stage to the next. Several studies have shown that rice is tolerant during germination, but becomes very sensitive during early seedling. It again becomes more tolerant during vegetative growth with the sensitivity returning again during pollination and fertilization and finally it again becomes more tolerant at maturity (Lauchli and Grattan, 2007).

For genetic adaptation of crop to salinity it is essential that sufficient hertable variability exists within the target crop. Various mechanisms of salinity tolerance in rice have been identified (Flowers and Yeo, 1986). The effects of NaCl on chlorophyll content vary depending on the concentration of salt, duration of treatment and age of leaves. With the increased duration of treatment or concentration of salts, chlorophyll content decreases, with that of salt-resistant cultivars being less affected than salt-sensitive cultivars (Misra *et al*., 1997; Faustino *et al*., 1996).

The main objective of the study was to evaluate the salt tolerance at vegetative growth stages of some proposed salt tolerant rice cultivars and identification of salt tolerant ones and evaluate rice genotypes at seedling stage.

MATERIALS AND METHODS

The experiment was carried out at Agricultural College, Golestan University, Gorgan, Iran, during 2008. 7 rice genotypes (*Oryza sativa* L.) were tested for their salt tolerance at two levels of salinity, 8 dS m^{-1} Electrical Conductivity (EC) and tap water as control. Completely randomized block design with three replications. Seeds were heat-treated for 5 days in a convection oven set at 50°C to break dormancy. Surface sterilized seeds with sodium hypochlorite were placed in petridishes with moistened filter papers. Three pre-germination seeds were sown per hole on the Styrofoam sheet having in holes with a nylon net bottom. The sheets were floated on distilled water. After 3 days, the fully germinated seeds were subjected to salinization solution with EC 8 dS m^{-1} for two weeks (Yoshida *et al*., 1976). The pH was adjusted every 3 days to 5.5 by adding 1N NaOH or HCl. Electrical Conductivity (EC) in nutrient solutions was measured every 3 days. The nutrient solution was changed after 8 days. Data taken 6 weeks after salt application were reported and traits including root and shoot length and dry weights, biomass, Na^+/K^+ ratio, Chlorophyll Index (CI) and leaf area were measured. Potassium/sodium ratios of the youngest three leaves (K/Na) were determined by flame photometry (model Jenway-PFP7) at the late vegetative stage.

The standard evaluating score in rating the visual symptoms of salt toxicity established at International Rice Research Institute (Gregorio *et al*., 1997) was used with modifications to discriminate the susceptible from the tolerant and moderately tolerant genotypes.

Table 1: Standard Evaluation Score (SES) of visual salt injury at seedling stage

Tolerance	Score	Observation	Cultivars
Tolerant	3	Nearly normal growth but leaf tips of few leaves whitish and rolled	Gharib
Tolerant	3	Nearly normal growth but leaf tips of few leaves whitish and rolled	Shahpasand
Susceptible	7	Growth severely retarded, most leaves rolled, only a few are elongating tolerant	Khazar
Susceptible	7	Complete cessation of growth, most leaves dry: some plants dying	Cpidrod
Susceptible	7	Almost all plants dead or dying	IR50
Highly susceptible	9	Almost all plants dead or dying	IR28
Highly susceptible	9	Almost all plants dead or dying	IR29

Measurement of different growth parameters: Shoot height was measured from the root base to the tip of the longest leaf, excluding any dead portion. Root lengths were measured from the root-shoot junction to the tip of the longest root. Leaf area (green portion only) was measured by a leaf area meter (model MK2, DELTA-T DEVICES, Burwell, England). Plant samples were dried at 70°C for 72 h for measurement of Na^+ and K^+ content, by flame photometry.

Measurement of the chlorophyll Index: The effects of NaCl on chlorophyll content vary depending on the concentration of salt, duration of treatment and age of leaves. Chlorophyll Content (CC) was measured by Chlorophyll meter (Minolta, SPAD-502, Japan). The 2nd leaf from the shoot apex was measured at positions for the CC at the after 14 days of NaCl treatment. The CC was calibrated with a standard curve of total chlorophyll concentration according to the method by Shabala *et al*. (1998). Visual scoring of plants (SES) 3-9 scale was done (Table 1).

Data analysis: Statistical analysis of physiological data was performed using one-way ANOVA and the difference between the mean values was compared at 5% significant level using Duncan's Multiple Range Test. Seven rice cultivars were tested for their salt tolerance at two levels of salinity 0 and 8 dS m^{-1} Electrical Conductivity (EC) and tap water as control. Genotypes of rice were screened for salinity tolerance at seedling stage. Data taken 6 weeks after salt application were reported. The degree of injury was greater in the highest salt concentration (8 dS m^{-1}). Salt injury symptoms varied with concentration of salt and between genotypes.

RESULTS AND DISCUSSION

Screening for salinity tolerance at seedling stage: The results indicated that traits including seedling dry weight, shoot length and root length were influenced ($p<0.01$) by genotypes, salinity levels and also the interaction between genotypes and salinity levels. There were also significant differences between genotypes at different salt levels (Table 2).

Increasing salinity level reduced traits mentioned above however, the alteration process over these cultivars is very important as the salinity stress increases from 0 to 8 dS m^{-1} NaCl. Salinization started when seedling were 14 days old and had 4-5 green healthy leaves. After 3 days in salinized solution at 8 dS m^{-1}, initial of salt stress were observed in the oldest leaves which started to desiccate and roll inward, especially in the highly sensitive genotypes IR29. The effect of NaCl on root growth was different from that of shoots, fresh weight of roots of NaCl-treated plants of all genotypes increased compared with the non-treated control plants.

Growth parameters

Root Dry Weight (RDW) and Shoot Dry Weight (SDW): The effect of NaCl on weight of roots of NaCl-treated plants of all genotypes increased compared with the non-treated control plants.

Table 2: Mean squares of main effects and interactions for agronomic parameters of rice cultivars

Sources	d.f.	Root dry weight (g)	Shoot dry weight (g)	Chlorophyll content (Chlorophyll index)	Leaf area (cm^2)	Biomass (g)	Na^+ / K^+ ratio
Salt (S)	1	0.021**	1.0005**	46.49**	88.01**	1.0018**	15.63**
Cultivar (c)	6	0.001**	0.0010**	24.04**	08.79**	0.0013**	0.13**
C×S	6	0.0002**	0.0001**	16.22**	01.80**	0.0039**	0.101**
S(R)	4	0.00017^{ns}	0.00007^{ns}	0.015^{ns}	0.09^{ns}	0.00006^{ns}	0.0003**

** Significant at 0.01 significance level in F-tests

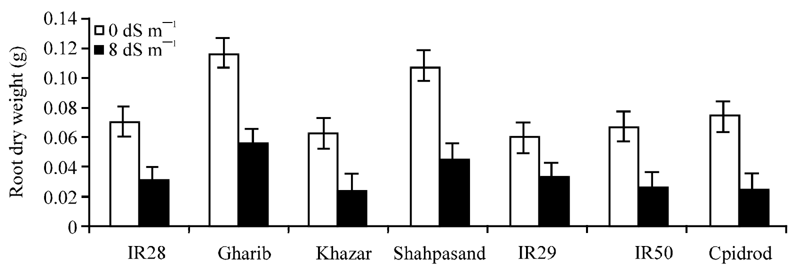

Fig. 1: Effects of root dry weight (g) in seedling stage in all genotypes of root dry weight (g) at 0 and 8 dS m^{-1}. Values are means of seven plants and bars indicate standard errors

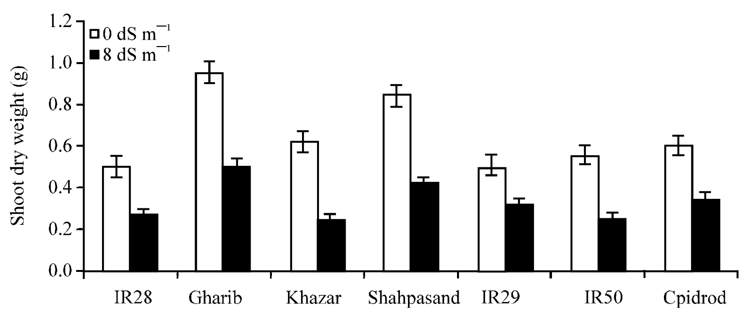

Fig. 2: Effects of shoot dry weight (g) in seedling stage in all genotypes of shoot dry weight (g) at 0 and 8 dS m^{-1}. Values are means of seven plants and bars indicate standard errors

The dry weight of root of all cultivars slightly decreased (Fig. 1). Shoot dry weight was relatively less affected than RDW. A deleterious effect of salt on SDW was observed at all salt concentrations on all the genotypes (Fig. 2). There was difference between the genotypes. At 8 dS m^{-1} smallest reductions were observed in gharib and shahpasand. Ashraf and Bhatti (2000) showed that salt tolerant varieties had more plant growth Water stress condition, which may be due to the adoptability of salt tolerant varieties to stress environments.

Shoot length: Salinity decreased shoot length of all the cultivars. Shoot length after 6 weeks of salinization were significantly reduced with respect to the controls. There were also significant differences between cultivars at different salt levels. The height reduction was observed in khazar at 8 dS m^{-1} and the lowest reduction was observed in gharib (Fig. 3).

Biomass production: There was a significant effect of salinity levels on the biomass production of rice (Fig. 4). The height reductions were observed in khazar and IR50 at 8 dS m^{-1} and the lowest

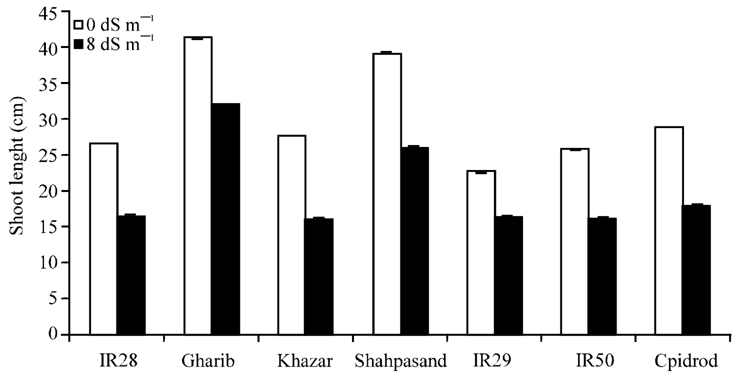

Fig. 3: Effects of Shoot length in seedling stage in all genotypes of plant height at 0 and 8 dS m^{-1}. Values are means of seven plants and bars indicate standard errors

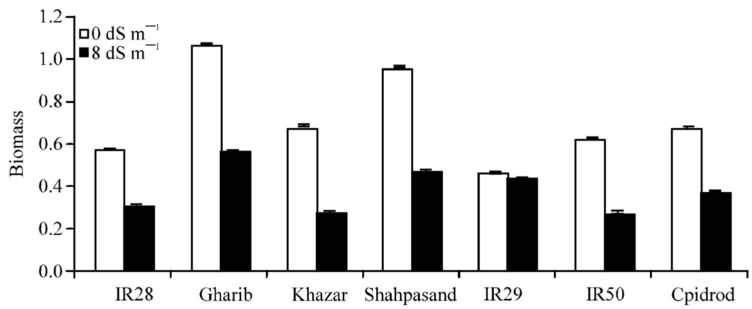

Fig. 4: Effects of biomass in seedling stage in all genotypes of biomass at 0 and 8 dS m^{-1}. Values are means of seven plants and bars indicate standard errors

reduction was observed in gharib and shahpasand. Therefore, the variety gharib and shahpasand are suitable for the cultivation in the saline soils in the coastal regions of Iran.

Leaf Area (LA): LA was highly sensitive to salt with about 50% reduction even at the lowest concentration. With increasing salinity, LA decreased progressively and there was a negative correlation between salt concentration and LA (Fig. 5). LAs were about 50.10 and 10% of the control. However, there were differences between cultivars.

Na^+/K^+ ratio: With salinity treatment the concentration of Na^+ significantly increased in all genotypes. The most sensitive IR50 accumulated the greatest, whereas the most tolerant gharib the lowest amount of Na^+. Concentration of K^+ on the other hand, decreases in response to NaCl except in gharib which showed a small insignificant increase. It appeared that concentration of Na^+ in shoots negatively, while K^+ positively correlated with the level of salt tolerance. The increase in Na^+ and decrease in K^+ resulted in the increase in Na^+/K^+ ratio in response to NaCl and the ratio was negatively related to the level of salt tolerance (Fig. 6). Similar observations were reported by Grieve and Fujiyama (1987) and Shannon and Grieve (1998). In rice, several authors have shown that Na-K selectivity of plant roots functions to minimize the entry of Na^+ into plants and maintain effective K^+ uptake together with the mechanism of low salt transport to expanding leaves is very important mechanism directly correlated with salt tolerance (Munns, 2002).

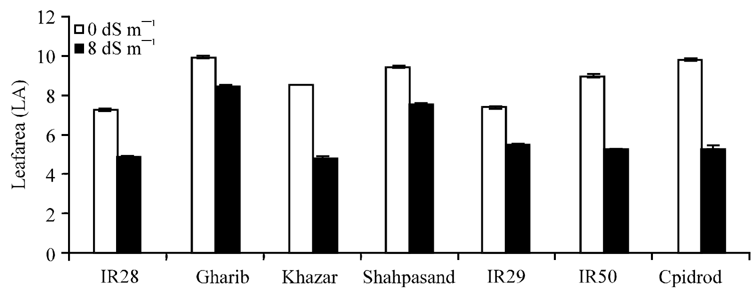

Fig. 5: Effects of leaf area in seedling stage in all genotypes of leaf area at 0 and 8 dS m^{-1}. Values are means of seven plants and bars indicate standard errors

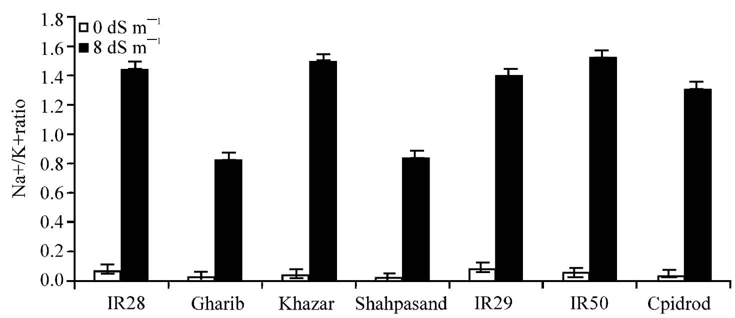

Fig. 6: Effects of NaCl on Na^+/K^+ ratio in seedling stage in all genotypes of NaCl at 0 and 8 dS m^{-1}. Values are means of seven plants and bars indicate standard errors

After 7 days of NaCl treatment, lowest leaves at the lower part of the NaCl-treated plants of most cultivars, except gharib and shahpasand, have rolled and dried. There are 3-5 green leaves remaining in some cultivars, except IR29, IR28, khazar and IR50 in which only 2-3 green leaves remained.

Growth and physiological responses: After 7 days in salinized solutions at 0 and 8 dS m^{-1}, growth of plants of all genotypes, except gharib and shahpasand, were retarded leading to the reduction in both fresh and dry weight of shoots. The distinction between the tolerant PK and other cultivars is clearly evident in changes in shoot dry weight.

In the two extreme cases, the most tolerant gharib and shahpasand which scored tolerant (score = 3) showed increase and the most sensitive IR29 and IR28 (score = 9) showed highest reduction. The result of the present study demonstrate that rice, in common with certain other cereals (e.g., wheat, com), is highly sensitive to salt with severe effects even at 4.0 dS m^{-1}.

Similar observations were reported by Grieve and Fujiyama (1987) and Shannon and Grieve (1998) reported that biomass production and plant density of rice decreased with increasing salinity over a range of even 0.5 to 4 dS m^{-1}. At 2.9 dS m^{-1} significant reductions in biomass production of rice occurred after only 14 days of salinization.

Although, plant height, leaf area, root and shoot fresh and dry weight all decreased significantly in all genotypes with increasing salinity, the magnitude of reduction varied between cultivars.

Roots are in direct contact with the surrounding solution. As such they are first to encounter the saline medium and are potentially the first site of damage or of defense under salt stress. Roots growth of rice in this study was severely inhibited by high concentrations of NaCl. This has been found by other workers in rice as well as in other crop (Dadkhah *et al.*, 2001; Cramer *et al.*, 1987). It was probably because salinity affected final cell size and as well as rate of cell production Azaizeh *et al.* (1992) thereby producing shorter roots. However, these results do not agree with those of Cramer and Nowak (1992), who reported that roots were less sensitive to salt than shoots. Leaf area, shoot and root fresh weight were relatively more affected and cultivar differences were more pronounced. These parameters could form the basis of a screening system. When plants are grown under saline conditions, as soon as the new cell starts its elongation process, the excess of salts modifies the metabolic activities of the cell wall causing the deposition of various materials which limit the cell wall elasticity. Secondary cell wall sooner, cell walls become rigid and consequently the turgor pressure efficiency in cell enlargement is decreased. Usually in the case of rice, after transplanting the previous roots die and new roots grow to support the plant. Inhibition of root growth by salinity reduces the volume of the growth media which can be explored by the roots and hence the availability and uptake of water and essential minerals (Kafkafi and Bernstein, 1996).

The diminished supply of nutritional elements to the shoot may also contribute to growth reduction of both root and shoot. The dry weight of roots IR29 slightly decreased, whereas those of the other cultivars were either unaffected or slightly increased. It is possible that the decrease in the observed shoot and root growth in salinized plants were due to several reasons.

CONCLUSION

Soil salinity, one of the most serious problems on planting areas, has the mast obstructive impact on crop production in the world. This crisis problem attracts many scientists to overcome this obstruction by improving salt-tolerant lines. These results indicated that almost all cultivars exhibited a reduction in traits evaluated in greenhouse stage as a result of an increase in salinity levels. The reduction in leaf area, chlorophyll index, biomass under saline conditions were also due to reduced growth as a result toxicity of sodium and chloride in the shoot cell as well as reduced photosynthesis. Reduction in chlorophyll concentrations is probably due to the inhibitory effect of the accumulated ions of various salts on the biosynthesis of the different chlorophyll fractions. Salinity affects the strength of the forces bringing the complex pigment protein liquid, in the chloroplast structure.

As the chloroplast in membrane bound its stability is dependent on the membrane stability which under high salinity condition seldom remains intact due to which reduction in chlorophyll was recorded Salt tolerance is not a function of single organ or plant attribute, but it is the product of all the plant attributes. Therefore, a genotype exhibiting relative salt tolerance for all the plant attributes may be ideal one. In this study, some genotypes showed tolerance to salinity for example gharib and shahpasand. Therefore, the variety gharib and shahpasand are suitable for the cultivation in the saline soils in the coastal regions of Iran.

Further study would be initiated with this basic information. By using these lines in breeding Programmed an improved ideotype of rice having higher chlorophyll concentration, more leaf area, early and better yield potential will be selected. This genotype possessing salt tolerance character will help in boosting up rice production in salt-affected soils. Therefore, a genotype exhibiting relative salt tolerance for all the plant attributes may be ideal one.

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