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Salinity Tolerance of Progenies between Korean Cultivars and IRRI's New Plant Type Lines in Rice

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ABSTRACT

To select new germplasm for salinity tolerance from new plant type (NPT) breeding lines, the sixty F_4 lines selected from the crosses between Korean cultivars and IRRI's NPT lines were evaluated for salinity tolerance at the seedling stage with salinized culture solution (EC=12 dS/m) in the controlled conditions. Two NTP lines derived from a cross between 'Ilmibyeo' and 'IR66152-AC5-1', 'HR15258-7-1' and 'HR15258-27-1', were found to have good tolerance. The salinity tolerance of the lines was compared to their parents and the sensitive ('IR29') and tolerant ('Pokkali') checks in three salinity levels, no salinity (control) and an EC of 12 and 16 dS/m. Visual salinity score, shoot Na^+ and Na-K ratio in two NPT lines was significantly low compared with the parents and IR29. Indicating that salinity tolerance of the lines might be derived from a transgressive segregation. The relative water content of the lines was higher than Pokkali, and the dry weight of shoot and root was proportionally decreased to salinity score and salinizing concentration. The visual salinity scores were significantly correlated with shoot Na concentration, Na-K ratio, relative water content, and reduction of dry weight ($P<0.01$). Their tolerance was attributed to root and shoot characteristics that led to high shoot water content, thus diluting the toxic effect of salts.

Key words : japonica rice, new plant type, salinity tolerance, seedling test.

Soil salinity is one of the important stresses to limit rice growth and productivity in many areas of the world. Salt tolerance is economically an important breeding goal for many breeders. In the reclaimed ricelands of the western coast of Korea, rice yields have reduced due to the soil salinity. The salinity damage could be reduced by the transplanting because the transplanting rice seedlings are old enough, grown for 30~45 days after seeding on the normal soil, to tolerate the level of salinity in these fields. It has known that rice is tolerant for salt stress during germination but becomes very sensitive during the early seedling stage (Akbar & Yabuno, 1974). Therefore, rice cultivars with salinity tolerance at the seedling stage should be improved. Most of rice varieties are moderately sensitive to salt with the exception of some wild and traditional varieties (Yeo et al., 1990). The mechanism of salinity tolerance is caused by both osmotic imbalance and Cl^- accumulation (Akbar, 1975). Recently, the cause of salt injury is due to the excessive sodium uptake (Clarkson & Hanson, 1980; Munns, 1993). The typical characteristics of salinity tolerance in rice were found that Na exclusion and an increased absorption of K would maintain a good Na-K balance in the shoot (Won et al., 1992; Gregorio & Senadhira, 1993). It has reported salinity tolerance would control by polygenes (Akbar & Yabuno, 1975; Skriver & Mundy, 1990). Since a high degree of salinity tolerance is found only in indica cultivars, such as Pokkali, Nona Bokra and Kararata, its transfer from indica to japonica is extremely difficult. The salinity tolerance is coinherited agronomically with non-desirable characters in their progenies, or the lack of inherited tolerance is happened in the selection processes of lines with agronomically useful characteristics. It is, therefore, necessary to look for new source of genes for salinity tolerance from the diverse genotypes. Recently, the incorporation of the new plant type (NPT) traits into the improved Korean cultivars is one of the main objectives of rice breeding program in Korea to increase the yield potential. The NPT breeding program of Korea have conducted in collaboration with the International Rice Research Institute (IRRI). The NPT traits of IRRI lines have been deriven from the tropical japonicas, commonly called bulus. It has been reported that some bulus possess tolerance to multiple soil stresses including coastal salinity (IRRI 1988). Additionally, the advanced NPT lines of IRRI could be used as the parents for hybridization with Korean cultivars. We have tested that there are salinity tolerant lines in the materials derived from three crosses. The selected NPT lines were conducted to compare with those parents under different saline condition through the seedling growth.

MATERIALS AND METHODS

Plants were selected from the F_3 pedigree nursery of crosses between Korean cultivars (temperate japonicas) and advanced NPT lines of IRRI. The nursery was planted in Iksan, Korea, in 1996. During maturity, they were evaluated for other agronomic characteristics and 60 lines were selected. 15 lines are from the cross HR15277 (IR66805-114/IR66806-11), 30 lines from HR15258 (Ilmibyeo/IR66152-AC5-1), and 15 lines from HR15260 (Dongjinbyeo/ IR66152-AC5-1). The salinity tolerance of

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those entries has been evaluated during the seedling stage for 15 days on January, 1997, in the IRRI phytotron glasshouse by the salinized culture solution method (Lee & Senadhira, 1996). The conditions of phytotron glasshouse have maintained with 29/21℃ day/night temperatures, 70% relative humidity, and natural daylight. IR29 (indica, sensitive), Daegudo (japonica, sensitive) and, Pokkali (indica, tolerant) were used as the checks.

The tolerant lines identified in the first experiment were evaluated through compairing them with their parents and the checks of sensitive (IR29) and tolerant (Pokkali). Three salinity levels, no salinity (control), 12 and 16 of EC dS/m, were applied with RCB design in the IRRI phytotron glasshouse with three replications in March 1997. Each entry in a replication had 10 seedlings. After 15 days of salinization, tolerance was examined using the scale proposed by Lee & Senadhira (1996) and the fresh weights of seedlings were measured.

Roots and shoots were separated and dried at 70℃ for 72h. The dried shoot samples were analyzed for Na and K concentration by the atomic absorption spectrophotometer. The relationships between visual score and Na and K concentrations and Na-K ratio were examined by the regression analysis. Water content of stressed seedlings compared to non-stressed seedlings was calculated for each salinity level as follows:

$$\text{Relative water content (\%)} = \frac{\text{Fresh weight of shoot} - \text{Dry weight of shoot at salinity level}}{\text{Fresh weight of shoot} - \text{Dry weight of shoot at control}} \times 100$$

Similarly, percent reduction of shoot/root dry weight at each salinity level was calculated as follows:

$$\text{Reduction \%} = \frac{\text{Shoot/root dry weight at control} - \text{Shoot/root dry weight at salinity level}}{\text{Shoot/root dry weight at control}} \times 100$$

Relative water content, percent reduction in root/shoot dry weight, and Shoot Na and K concentrations and Na-K ratio were subjected to ANOVA and the differences were compared by Duncan's Multiple Range Test (DMRT).

RESULTS

The 60 lines of three crosses were evaluated for salinity tolerance at the seedling stage. There were considerable variations according to crosses, and it was the widest in HR15258 (japonica/NPT lines; Ilmibyeo/IR66152-AC5-1) among three crosses (Table 1). IR29, sensitive check, displayed browning or chlorosis of the old leaf tips in third day after the seedlings put to 12 dS/m of EC. About two weeks later, most of plants were died and displayed the dying status. However, Pokkali, tolerant check, wasn't visible any symptoms even after fifth day in the same condition. About ten days later, most of leaf tips displayed wilting, browning or chlorosis. Some susceptible lines were more sensitive than IR 29. The NPT lines of HR15258 cross had more tolerance than that of other two crosses. Out of the 30 lines of HR15258 cross tested, two were highly tolerant (comparable to Pokkali), one was tolerant, twenty-one were moderately tolerant, and six were sensitive. In the other two crosses, HR15277 and HR15260, there were no tolerant lines. Three lines of HR15277 and four lines of HR15260 were expressed moderate tolerance and all others were sensitive. The visual symptoms of two NPT lines of HR15258 cross were 3.2 and 3.4 (Table 2). Their salinity tolerance was similar to that of Pokkali.

The visual symptoms of injury were neater in the second experiment than in the first experiment (Table 3). Pokkali was reclassified as a highly tolerant, while the NPT lines fell into the moderately tolerant category. However, they were significantly more tolerant than that of their parents, showing the moderately sensitive. Even at higher salinity (EC=16 dS/m), the two NPT lines retained their tolerance level, while their parents became sensitive. The water content of seedlings at 12 dS/m of EC compared to the unsalinized plants was highest in the NPT lines, and significantly higher than Pokkali (Table

Table 1. Variation of salt tolerance in F_4 generation derived from the three crosses at 12 EC dS /m of saline condition for 15 days at the seedling stage.

Tolerance rating (visual score)†	No. of line (%)‡		
	HR15277	HR15258	HR15260
Highly tolerant (2.8~3.5)	0	2(6.7)	0
Tolerant (3.6~5.0)	0	1(3.3)	0
Moderately tolerant (5.1~7.0)	3(20.0)	21(70.0)	4(27.0)
Susceptible (7.1~9.0)	12(80.0)	6(20.0)	11(73.0)
Total	15	30	15

† Visual score was divided to 1~9 scales with score 1 as most tolerant (Lee & Senadhira, 1996).
‡ HR 15277 (IR66805-114 /IR66806-11, Tropical japonica /Tropical japonica), HR 15258 (Milyang 122 /IR66152-AC5-1, Japonica /Tropical japonica), HR 15260 (Dongjinbyeo /IR66152-AC 5-1, Japonica /Tropical japonica)

Table 2. Visual rating of salinity tolerance of the advanced NPT lines selected and check cultivars under 12 EC dS /m of saline condition for 15 days at the seedling stage.

Cultivar /line	Visual score† (mean±SD)	Tolerance rating
HR15258-7-1	3.2 ± 0.6	Highly tolerant
HR15258-27-4	3.4 ± 0.8	Highly tolerant
Daegudo (japonica, sensitive check)	8.0 ± 0.6	Sensitive
IR29 (indica, sensitive check)	7.0 ± 1.2	Sensitive
Pokkali (indica, tolerant check)	2.8 ± 0.4	Highly tolerant

† Visual score was divided to 1~9 scales with score 1 as most tolerant (Lee & Senadhira, 1996).

Table 3. Measurements of salinity tolerance level of NPT lines, their parents (Ilmibyeo and IR66152-AC5-1) and check cultivars grown under three levels of salinity at the seedling stage.

Salinity (EC in dS /m)	Cultivar /line	Visual score	Relative water content	Percent reduction		Shoot Na (%)	Shoot K (%)	Shoot Na-K ratio
				Shoot dry wt.	Root dry wt.			
1.3 (non-saline nutrient solution)	HR15258-7-1	–	100.0	–	–	0.07d	3.37a	0.02c
	HR15258-27-4	–	100.0	–	–	0.06d	3.27a	0.02c
	Ilmibyeo	–	100.0	–	–	0.07cd	3.37a	0.02c
	IR66152-AC5-1	–	100.0	–	–	0.13a	3.39a	0.04a
	IR29 (sensitive check)	–	100.0	–	–	0.09bc	3.06b	0.03b
	Pokkali (tolerant check)	–	100.0	–	–	0.09bc	3.05b	0.03b
12	HR15258-7-1	4.4c	53.5a	29.6c	49.5cd	1.77c	1.91b	0.93c
	HR15258-27-4	4.5c	57.4a	30.2c	46.2d	1.97bc	1.93b	1.02bc
	Ilmibyeo	6.7b	29.9c	51.5ab	68.3ab	2.23b	1.83b	1.23b
	IR66152-AC5-1	6.5b	33.3bc	52.5ab	71.7a	2.29b	1.83b	1.25b
	IR29 (sensitive check)	8.0a	26.0c	60.5a	76.7a	2.89a	1.77b	1.63a
	Pokkali (tolerant check)	3.2d	41.4b	44.8b	58.3bc	1.07d	2.61a	0.41d
16	HR15258-7-1	4.8c	27.1a	59.4e	67.8c	2.84b	1.51bc	1.88b
	HR15258-27-4	5.6c	26.0a	63.7de	67.5c	2.76b	1.45c	1.90b
	Ilmibyeo	8.8ab	7.8c	80.2b	85.0ab	5.99a	1.65b	3.64a
	IR66152-AC5-1	8.0b	13.8b	74.4c	82.0b	5.58a	1.53bc	3.64a
	IR29 (sensitive check)	9.0a	4.8c	86.9a	89.6a	5.92a	1.63b	3.64a
	Pokkali (tolerant check)	3.6d	26.7a	65.9d	70.5c	1.62c	2.46a	0.65c

• In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

3). At 16 dS/m of EC, the NPT lines and Pokkali were comparable. Shoot dry weight was reduced less in the NPT lines at 12 dS/m EC of salinity than that in Pokkali, but at increased salinity their reduction was similar to that in Pokkali. The NPT lines and Pokkali were similar with respect to root dry weight reduction at both levels of salinity. In general, all plant growth characteristics showed that, at an EC of 12 dS/m, the NPT lines were superior compared to the tolerant check, Pokkali.

IR29 (sensitive) had the highest Na concentration at 12 dS/m of EC, while Pokkali (tolerant) the lowest. The two NPT lines showed significantly lower Na concentrations than that of IR29, even if their Na concentrations were significantly higher than that of Pokkali. The two parents had slightly more Na than the do NPT lines but less than IR29. At the higher salt concentration, IR29 and the two parents showed the sensitive to absorb Na. The NPT lines had significantly lower concentrations of Na than do the parents, but significantly higher than Pokkali. K absorption from non-saline condition was significantly higher in the japonica cultivars/lines (NPT lines and their parents) compared to two indica cultivars (IR29 and Pokkali). K absorption of all lines was decreased with increasing the EC levels to 12 or 16 dS/m, except for Pokkali, showing slightly decrement of K absorption. NPT lines and their parents had K concentrations similar to sensitive check, IR29, at both 12 and 16 EC dS/m of salinity levels, except for the HR 15258-27-4 containing significantly less K concentration compared to the sensitive check, IR29. Na-K ratios of shoot under the non-saline condition were low although their ratios differed significantly among the entries (Table 3). At 12 EC dS/m of salinity, IR29 had the highest Na-K ratio, while Pokkali the lowest. Na-K ratio of two NPT lines were

Table 4. Correlation coefficient between salinity score and investigated characters grown under two levels of saline condition for 15 days at the seedling stage.

Characters†	Correlation coefficient	
	EC=12	EC=16 dS /m
RWC	−0.719**	−0.921**
RSDW	0.702**	0.865**
RRDW	0.781**	0.839**
Shoot Na^+	0.927**	0.931**
Shoot K^+	−0.730**	−0.506 *
Na-K ratio	0.924**	0.930**

† RWC : Relative water content, RSDW:Relative reduction of shoot dry weight, RRDW:Relative reduction of root dry weight, see Table 3.

*,** : Significant at 5 and 1% probability levels, respectively.

significantly higher than that of their parents. At 16 dS/m of EC, there were three groups showing significantly different Na-K ratios; the first was Pokkali having the lowest, the second was two NPT lines having medium, and the third was parents and IR29 having the highest value. The visual scores were significantly correlated ($P<0.01$) with shoot water content ($r=-0.72$), reduction of shoot/root dry weight ($r=0.70/0.78$), shoot Na concentration ($r=0.93$), shoot K concentration ($r=-0.73$), and Na-K ratio ($r=0.92$), except for the shoot K concentration ($P<0.05$).

DISCUSSION

We selected two lines for the salinity tolerance in F_4 generation between Korean cultivars and the advanced NPT lines from IRRI (Milyang 122/IR66152- AC5-1). The salinity tolerance of two selected lines had compared with their parents, tolerant check (Pokkali), and sensitive one(IR29). The two NPT lines showed highly tolerance compared to their parents, showing moderately sensitive. Salinity tolerance of the lines might be derived from a transgressive segregation. The tolerant category of the NPT lines was lower in second experiment than in the first experiment on the basis of Pokkali. This would possibly be due to the increased solar radiation on March. On the average, the solar radiation on March in Los Banos, IRRI, is about 21 $MJ/m^2/d$ compared to that about 13 $MJ/m^2/d$ on January. At 12 dS/m EC of salinity level, score for Pokkali increased from 2.8 in the first experiment to 3.2 in the second experiment, but remained within highly tolerant category.

In the unsalinized nutrient solution, the electrical conductivity was about 1.3 dS/m. This is due mainly to the P2O5 source (sodium phosphate) and NaOH and HCl used for pH adjustment. All entries absorbed small and non-toxic amounts of Na under this condition, but differences among test entries for Na concentration were significantly differed. The parents of the two salinity tolerant NPT lines identified were different from sensitive check, IR29, in shoot Na concentration and Na-K ratio at an 12 dS/m EC indicating that they possess some level of tolerance. Between the two parents, significant differences were observed only in shoot Na under non-saline condition that water content and shoot dry weight at high salinity (EC=16 dS/m). However, it appeared that these differences when appropriately combined could produce better genotypes. It has been reported that the salinity tolerant cultivars absorb more K when exposed to the stress (Gregorio 1991; Won et al., 1992; Gregorio & Senadhira, 1993). In this study, K absorption was reduced under saline conditions with the greatest reduction in sensitive cultivars and the least in tolerant cultivars. Under saline conditions, both types seem to attempt to absorb the needed amount of K but constrained by the entry of toxic Na. In tolerant cultivars, entry of Na to the shoot was reduced but at the cost of K absorption. Even highly tolerant cultivars like Pokkali cannot provide optimal K to the shoot under saline conditions. The visual scores were significantly correlated with shoot Na and K concentrations and Na-K ratio. Additionally, shoot Na and K concentrations and Na-K ratio can be used for the selection of salt tolerant lines in breeding program. Won et al. (1992) and Gregorio & Senadhira (1993) found that salt tolerant cultivars showed lower Na and higher K content and lower Na/K ratio than that of the salt sensitive ones.

The two NPT lines were significantly superior to their parents with respect to shoot water content, dry weight and Na-K ratio and root dry weight. The NPT lines were significantly better than Pokkali for shoot-and root-related traits. These traits appeared to have contributed to the tolerance measured in the NPT lines. The mechanism responsible for salinity tolerance in the two NPT lines is possibly the high shoot water content that diluted excess salts. Yeo & Flowers (1984) concluded that six different physiological mechanisms contribute to salinity tolerance in rice. They also found that the known tolerant cultivars such as Pokkali do not possess all the mechanisms. These findings suggest that the contribution of each mechanism to overall tolerance may be different and the known cultivars probably possess the major contributors. If so, there could be cultivars with one or few minor mechanisms but expressing sensitivity.

Some of the NPT traits such as vigorous growth, large and sturdy culm, and thick broad leaves could contribute to salinity tolerance (IRRI 1988). Therefore, screening of advanced lines of the NPT breeding program is worthwhile although the probability of isolating tolerant genotypes according to this study is only about 3 percent. Garcia et al. (1995) found that conventional selection for agronomic traits in the early generations reduced the frequency of low Na-transporting genotypes. Since such selection is unavoidable in the present NPT breeding program, we suggest that a program specific to coastal rice lands be initiated with crosses between the advanced NPT lines and salt tolerant japonicas, including the two

NPT lines identified here as the tolerant.

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