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Screening of salinity tolerance of rice at early seedling stage

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ABSTRACT

The study was conducted at International Rice Research Institute (IRRI) to assess the response of dry season hybrid seeds including parental checks FL478 and NSIC Rc222 to salt tolerance (12 dS/m) at the seedling stage of rice using IRRI screening techniques. The total number of seeds were two hundred thirty-one and irrigated, flood prone, heat tolerance, salinity and problem soils, aerobic and anaerobic germination, rainfed lowland and South Asian samples were used for this experiment. Among them, only 1.73% populations (4 irrigated) were identified as tolerant, 18.18% moderately tolerant, 37.26% sensitive and 46.86% were highly sensitive. Moderately tolerant plants were found from irrigated, flood prone, salinity and problem soils, aerobic germination, anaerobic germination and rainfed lowland and South Asian genotypes. As there was Brown Plant Hopper (BPH) infestation in this experiment, the tolerant genotypes may also be tolerant to BPH and for that reason results were distorted for other genotypes. Therefore, study should be conducted under controlled environment to ascertain the level of salt tolerance of the moderately tolerant populations. Besides, the identified tolerant genotypes (4) should be further tested with 18 dS/m to determine their supremacy to salt tolerance at the seedling and reproductive stages and QTL analysis could be performed to determine the effects of each genomic region of the trait of interest.

Key Words: *Salinity tolerance, Seedling stage, IRRI, FL478 and NSIC Rc222*

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I. Introduction

Rice is a diploid (2n= 2x= 24) glycophyte, of tropical origin, and is currently the model crop cereals (Jenkins *et al.,* 2008). About 90% of the rice in the world is grown in Asia (nearly 640 million tons) and 85% is devoted for human consumption (IRRI, 1997). Various abiotic stresses greatly affect rice yield and among the abiotic stresses, salinity is the second most prevalent soil problem in rice growing countries of the world and is considered as a serious threat to increased rice production worldwide (Gregorio, 1997). For this reason, millions of hectares in the humid regions of South and Southeast

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Asia are technically suited for rice production but are left uncultivated or generally are grown crops with very low yields. (IRRI, 2006). Rice is tolerant during germination, becomes very sensitive during early seedling stage (2-3 leaf stage, when 2-3 leaves appeared), and it gains tolerance during vegetative growth stage, becomes sensitive during pollination and fertilization, and then becomes increasingly more tolerant at maturity (Pearson *et al.,* 1966; IRRI, 1967; Lutts *et al.,* 1995). Hence, for better understanding the response of the rice plant to salinity , it is imperative that the effects of salinity stress be observed in all the development stages, that is at early seedling, vegetative and reproductive stages of rice plant. (Gregorio *et al.,* 1997) The detection of salinity induced injuries, however, are very complex to identify even under controlled environments . The visual symptoms of salt stress may still be the most appropriate for mass screening (Gregorio *et al.,* 1997). As the salinity is one of the major threat to crop productivity, development of salt tolerant varieties has been considered as an important effort to feed the millions living in such adverse environments. Breeding for salinity tolerance in rice requires reliable screening techniques and IRRI has developed two screening techniques for using at seedling stage, and vegetative and reproductive stages of rice (Gregorio, 1997). The present study was undertaken to document salinity tolerant at the seedling stage of some dry season (DS) hybrid (HB) population of rice using IRRI screening techniques. The objective of this study was to identify the tolerant and sensitive genotypes from DS hybrid seed samples (231 seeds) which included irrigated, flood prone, anaerobic germination, salinity and problem soils, aerobic, rainfed lowland and South Asian and some other seed materials.

II. Materials and Methods

The experiment was conducted at the Phytotron (glass house) and Green House (NG 02) of the International Rice Research Institute (IRRI), Philippines during December 2013 to January 2014. Total two hundred thirty one (231) rice genotypes were screened for salt tolerance at seedling stage in hydroponics system using IRRI standard protocol (Gregorio, 1997). In this experiment, FL478 and NSIC Rc222 were used as salt tolerant and sensitive checks, respectively. The screening was done under controlled environmental conditions having 29^0C/21^0C day/night temperature, 70% relative humidity and natural daylight inside the Phytotron. But later on, the whole population was transferred to the green house (NG 02) due to shut down of Phytotron for annual management.

Firstly, the seeds were disinfected with NaOCl solution (200 ml NaCl in 1 liter water) and incubated for 48 hours to enhance germination. Eleven trays were filled up with tap water and styrofoam seedling floats were placed on the tray. Two pre-germinated seeds were sown in each hole of Styrofoam seedling float and the seedling floats were then covered with a lid for 3 to 4 days to promote germination in the dark. The tap water was replaced with Peter nutrient solution after six days of seeding and was salinized by adding crude salt to obtain EC of 12 dS/m. The volume of the Peter solution was adjusted to the level of touching the seedling float at two days interval. The pH was adjusted to 5 as well as EC was also adjusted with 12 dS/m synchronizing with the Peter solution. Tap water was added to the solution when EC was higher than 12 dS/m and NaCl was added when EC was lower than 12 dS/m. In the similar way, HNO_3 was added to the solution when its pH was more than 5.0 and NaOH was added into the solution when pH was less than 5.0. Standard Evaluation System (SES) for salinity tolerance scoring was done when salt sensitive check was almost going to die or already dead. In this experiment, salt sensitive check, NSIC Rc222 was dead after two weeks of salinization. Scoring was done according to the modified standard evaluation system used in rating of the visual symptoms of salt toxicity injury (IRRI, 1997) (Table 01).

Table 01. Standard evaluation system (SES) for scoring of visual salt injury at seedling and reproductive stages in rice.

Score	Observation	Tolerance
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish & rolled	Tolerant
5	Growth severely retarded, most leaves are rolled; few elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dry; some plants dying	Sensitive
9	Almost all plants dead or dying	Highly sensitive

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III. Results and Discussion

The basic principle in screening of salinity tolerance in rice at the seedling stage was the ability of the seedlings to grow in salinized culture solution (Gregorio, 1997). In this experiment, all genotypes including checks were grown in normal condition and exhibited 100% survival in absence of salt stress (Figure 01). Different degrees of salt injuries (scores 3 to 9) and growth retardation was observed in all lines under salt stressed condition. The genotypes showed wide variation in comparison with FL478 having score 3 and NSIC Rc222 having score 9 (Figure 02 and 03). Gregorio et al. (2002) and Jubay (2012) also observed wide variations in Pokalli (tolerant) and IR29 (sensitive).

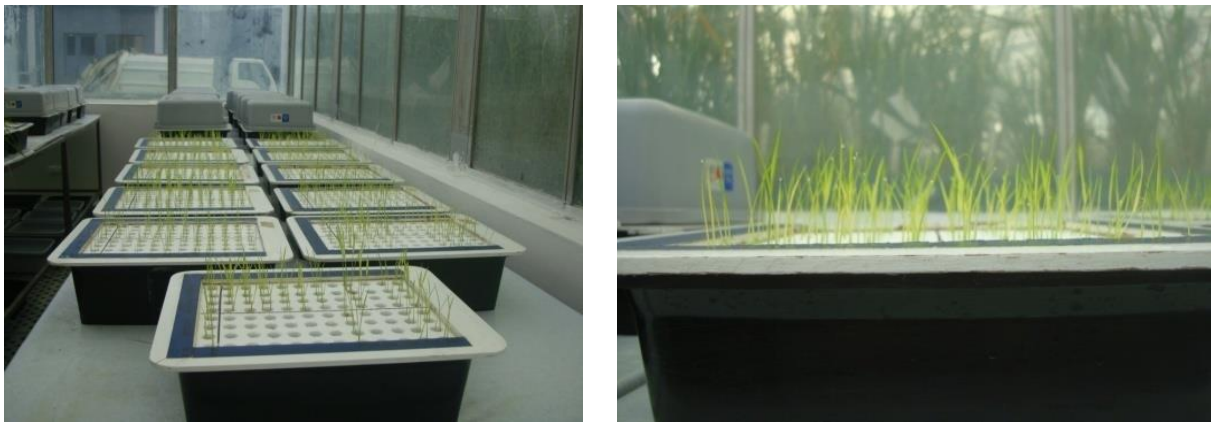

Figure 01. Experimental set up for screening of 231 dry season populations along with checks FL478 and NSIC Rc222 in the Phytotron of IRRI showing normal growth before salinization.

Figure 02. FL478 exhibits tolerant to salinity at 12 dS/m.

Figure 03. NSIC Rc222 exhibits highly susceptible to salinity at 12 dS/m.

Salt injury symptoms were shown after 7 to 8 days from the first day of salinization. The main symptoms were leaf rolling and formation of the new leaves, leaf rolling and whitening of the tips which lead to complete cessation of the growth and finally drying the leaves. Jubay (2012) and Islam (2004) observed similar salt injury at the seedling stage of rice plant in Pokalli and IR29. Among all the genotypes, the number of most salinity tolerant were four (4), moderately tolerant were forty-two (42), eighty-six (86) and ninety-nine (99) were sensitive and highly sensitive respectively (Table 02).

The most salinity tolerant genotypes were from irrigated lines; HB9303, HB9305, HB9330, HB9335 with scoring 3. These genotypes were 1.73% of the total genotypes (Table 02) and average percentages of plant damaged under this category was 17.5% (Figure 04). The genotypes which were moderately tolerant, sensitive and highly sensitive were 18.18, 37.23 and 46.86 percent of the total population (Table 02), and the average percentages of plant damage observed under those categories were 46.07, 76.16 and 97.98 percent, respectively (Figure 04).

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Table 02. Salinity tolerance level of the 231 dry season populations

Population	Tolerant	Moderately Tolerant	Sensitive	Highly Sensitive	Total
Irrigated	4	18	29	15	66
Flood Prone	0	3	6	9	18
Anaerobic germination	0	4	0	1	5
Salinity and problem soil	0	10	20	15	45
Aerobic	0	6	25	32	63
Rainfed lowland & South Asia	0	1	1	14	16
Heat tolerant	0	0	5	10	15
Others	0	0	0	3	3
Total population	4	42	86	99	231
Population (%)	*1.73*	*18.18*	*37.23*	*46.86*	*100*

Among the 42 moderately tolerant populations, 18 genotypes were found from irrigated, 3 genotypes from flood prone, 4 genotypes from anaerobic, 10 genotypes from salinity and problem soil, 6 genotypes from aerobic and only 1 from rainfed lowland and South Asian materials (Table 02). There were no promising genotypes in moderately salinity tolerance group from the heat tolerance populations (15 genotypes) indicated that heat tolerant populations used in this study were not tolerant to salinity. About 67% heat tolerant populations were highly sensitive (Table 02) and exhibited 100% damage due to salinity. Total number of sensitive and highly sensitive genotypes were identified in this study were eighty-six (86) and ninety-nine (99), respectively and those were mostly from the aerobic, irrigated and salinity and problem soil populations used in this study (Table 02). The probable reason of absence of tolerant lines in salinity and problem soils populations may be due to Brown Plant Hopper (BPH) attack in the green house.

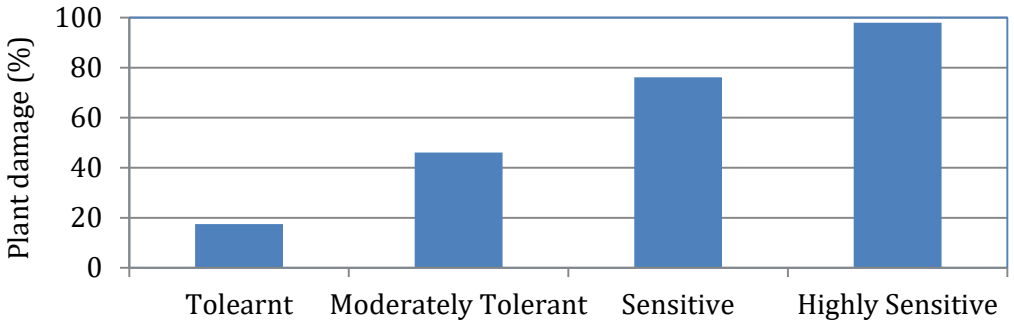

Figure 04. Percentages of total population showed different level of salinity tolerance.

More than 80% of the population falls under the category of sensitive to highly sensitive level of salinity tolerance (Table 02). Four (4) samples or genotypes from anaerobic germination expressed moderately tolerant to salinity at 12 dS/m. Among the irrigated population, about 33% (22 populations) showed tolerant to moderately tolerant and about 67% (44 genotypes) was found sensitive to highly sensitive at 12 dS/m.

Salinity score of all DS populations were evaluated by using Standard Evaluation System (SES) scoring (IRRI, 2013) through visual observation (Gregorio *et al.,* 2002). A regression model was developed for salinity score and percent plant damage of the tested populations. The equation for salinity score derived from this study is:

$$Y = 1.6419 + 0.0733\ X \qquad (R^2 = 0.9956)$$

Where, Y = salinity score and X = percentages of plant damaged due to 12 dS/m salinity.

For further study, salinity score of these populations can be determined knowing the percentages of plant damage due to salinity stress of 12 dS/m using this equation.

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IV. Conclusion

Salinity tolerance seedling stage showed wide variation in phenotypes with salinity scores ranging from 3 to 9. Among 231 DS genotypes, 1.73% populations were found tolerant, 18.18% were moderately tolerant, 37.26% populations were sensitive and 46.86% were highly sensitive to salt stress. Among the genotypes, only four genotypes from irrigated population showed tolerant to salinity at 12 dS/m. As this experiment was affected by BPH , these four genotypes may also be tolerant to both salinity and BPH. Moderately tolerant populations were found from irrigated, flood prone, aerobic germination, salinity and problem soils, anaerobic germination, rainfed low land and South Asian samples. All other populations including heat tolerant samples were sensitive and highly sensitive at 12 dS/m salinity. Therefore, four tolerant genotypes found in this experiment could be further tested with 18 dS/m to determine their supremacy to salt tolerance at the seedling and reproductive stages of rice. In addition, QTL analysis should be performed to determine the effects salinity of each genomic region of the traits.

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