RESEARCH ARTICLE

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EVALUATION OF RICE BACKCROSS POPULATIONS FOR SALT TOLERANCE USING PHENOTYPIC ANALYSIS

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SUMMARY

Selection of elite rice cultivars through backcross as well as based on quantitative genetics, and interaction of rice cultivars were evaluated by genetic index at the High Agricultural Technology Research Institute (HATRI) for Mekong Delta, Vietnam. A population was developed from a cross between OM10252 (high yielding variety and short duration from Cuu Long Delta Rice Research Institute (CLRRI) as female parent and Pokkali as male parent (a donor in many breeding programs and salinity tolerance related studies from Indian). Analysis of 95 individuals from the BC_3F_3 population of OM10252 /Pokkali// OM10252, the plant height trait, root length, dry weight of stem, dried weight of roots had the positive and high correlation with the survival time of rice plants at EC = 0 dS/m, 8 dS/m and 15 dS/m. The line BC_3 F_4-17 gave good survival at 4‰ condition in different areas. A final comment is that both field management and improved cultural practices were extremely important for sanity in rice. For generations, salt-tolerant lines will continue to grow and will be evaluated in the vegetative and growth stages, and included in breeding programs.

Key words: Salt stress, rice lines, seedling and stages, breeding, *Oryza sativa* L.

Key findings: The special line (BC3F4-17) to salinity stress at 4% in the field condition. The finding, develop high yielding new line with significantly improved tolerance to salinity at the different region in Vietnam.

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INTRODUCTION

Rice (*Oryza sativa* L.) is sensitive to salinity, which affects one-fifth of irrigated land worldwide. Breeding for salt tolerance has been reviewed by several workers. Progress in salinity tolerance breeding is slow due to the following limited knowledge in the genetics of tolerance, complexity of the several tolerance mechanisms involved, inadequate screening techniques, low selection efficiency and poor understanding of salinity and environmental interactions. Through recent developments in molecular marker analysis, it is now feasible to analyze both the simply inherited as well as quantitative traits, and then identify individual genes controlling the trait of interest. Several studies indicated that rice is tolerant during germination, become very sensitive during early seedling stage and the difference score salt tolerance between vegetative and productivity stage in the case 19.4% and 13% at reproductive stage from Doc Phung (Lang *et al*., 2001).

Tolerance to salinity is desirable at both the vegetative and reproductive stage, and tolerance at the two stages does not seem to be correlated. At the vegetative stage, large differences have been observed in tolerance levels. A major QTL named *Saltol* controlling 64–80% of the phenotypic variability from the tolerant cultivar Pokkali was identified on chromosome 1 (Bonilla *et al*., 2002), and the QTL seems to be present in other cultivars (Takehisa *et al*., 2004). This selective and screening some lines from tolerance at the seedling stage are still underway. The current focus on screening selected salt tolerance should result in the identification of an increasing number of gene salt tolerances in rice. Takahashi (1974) reported that newly improved *indica* and *japonica* cultivars had similar germination trends under 1.5% NaCl but no germination at 2.5% NaCl. The traditional *indica* cultivars had higher germination percentage than improved cultivars.

Rice plants were more sensitive during the early seedling stages than at later stages (Akbar and Yabuno, 1974). The significant decrease in growth parameters such as dry matter, seedling height, root length and the emergence of new roots was observed at an electrical conductivity value of 5-6 dSm^{-1} (Heenan *et al*., 1988). Salt stress at early seedling stage shows a severe reduction in effective leaf areas as the first symptom. In low-stress condition, the dry weight of some cultivars often increases for some time and then decreases due to reduced leaf area. At higher stress, dry weight of shoot and root decrease almost proportionally. The objective of this study was to investigate the effect of salinity stress at seedling and reproductive stages in the development of salinity and tolerance.

MATERIALS AND METHODS

The populations of 95 plants of BC3F3 were developed from OM10252/Pokkali //OM10252 (Lang *et al*., 2017a).

Screening salt tolerance at the seedling stage

The screening at seedling stage was prepared nutrient solution following Yoshida (1981) while the hydroponic condition used followed Gregorio *et al*. (1997). According to the SES of IRRI (Gregorio *et al*., 1997) a score from 1-9 was used for classifying the genotypes based on visual salt damage where, 1 = highly tolerant and 9 = highly susceptible.

On each spongy tray we drill the holes with diameter 1 cm, length 10-hole and width 10-hole. Preparing plastic tray has a capacity of about 3-5 liters of water with a diameter of 40 x 50 x 4 cm to put into spongy tray. Preparing correctly solution components is extremely important to avoid a lack of nutrients and forming minerals toxins affected the salinity of salt. Experiment of screening salt in nutrient solution Yoshida (1981). The solutions can be stored for 3 months at room temperature and if we want to use long term, we storage at 4^0C. Saline solution was created by adding NaCl to the nutrient solution and adjusting desired electrical conductivity EC (0, 8 and 15 dS/m), nutrient solution culture needed for each tray is 3 - 5 liters following (Lang, 1999).

Fifteen cultivars were established to test salt tolerance within the cropping system of three crops per year. The seeds were planted using with 20 x 20 cm spacing. The fertilizers applied at the rate of 100:60:40 N:P:K kg ha^{-1}. Weed control was done by applying a post-emergence herbicide. Yield per plant was the average weight per plant, measured in grams of bulked harvested grain from the 5 m^2/plot.

Statistical analyses

Analysis of variance (ANOVA) was performed to determine by Excel. Significant $P < 0.05$ means were separated with DUNCAN's Multiple range Test using Crop Stat 7.2 software.

RESULTS AND DISCUSSION

Phenotypic screening for salinity tolerance at seedling stage

In the first experiment, the effect of salinity was significant on all measured traits (Figure 1) in EC 8 and 15 dS/m. Ninety-five lines representing a range of tolerance to salt response were selected for the study (Figures 2, 3 and 4).

The levels of salinity tolerance ranged from 1 (tolerant) to 9 highly susceptible (Figure 2). Under EC conditions, the level showed a significant variation at 8 dS/m of NaCl. The results observed wide variation in phenotypes from tolerant (levels 1-3) to susceptible (level 9) lines using modified SES of IRRI standard. Most of the lines through 30 days of screening in salt environment expressed dried leaf levels in a level of 7 to 9 (34 lines). There were 42 lines with a level of 5, 15 lines a level of 3 and four lines a level of 1. While at 15 dS/m, lines were more susceptible than compared with the 8dS/m. Seven lines had level of 5 and 1 line had a level of 3, no lines with expression of resistance (level 1).

As expected, tolerant lines were less affected by salt stress compared to susceptible lines for different agronomic characteristics such as plant height, root length, dry weight of term, and dry weight of root dry

(Figures 3 and 4). Five lines had showed greater plant height reduction under the salinity stress at 8 dS/m (Figure 3). Salinity also decreased root length of lines. At seedling stage, fifty lines showed higher root length reduction at 8 dS/m (Figure 3). As well as plant height and root length, dry weight of stem greatly influenced by environmental salt. Salt-making ability of dry matter accumulation in rice plants reduced due to nutrient movement slowed down, the ability to form and accumulate dry matter in plant decreased and leads to the dry weight of plant decreased. Dry weight

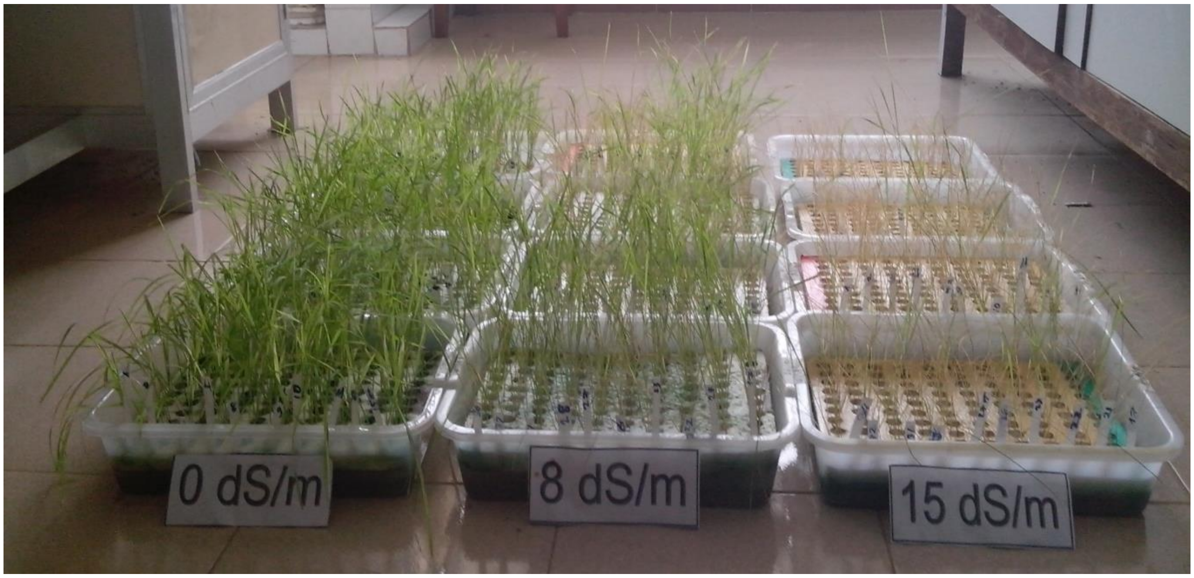

Figure 1. Screening result at the stage 30 days in environment 8 dS/m và 15 dS/m.

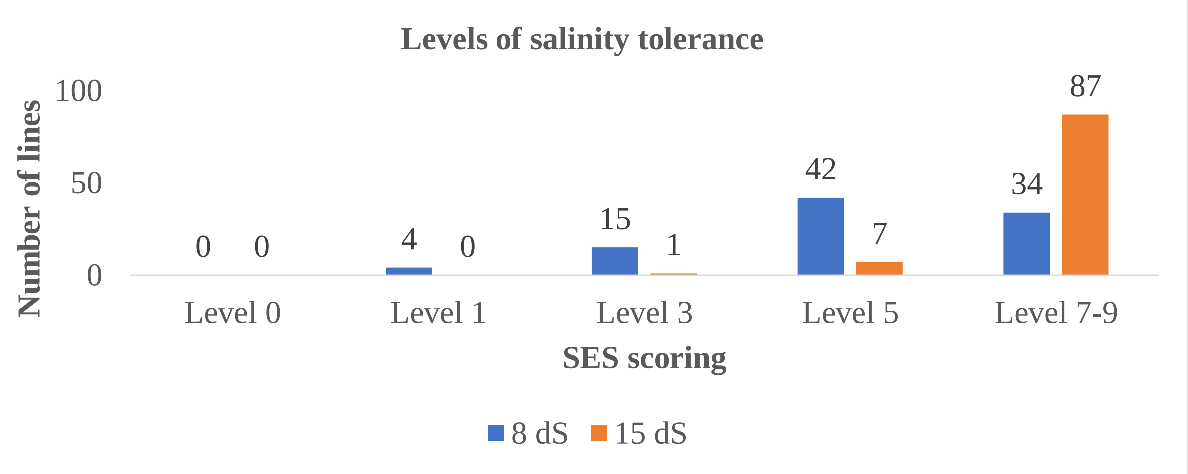

Figure 2. Salinity levels of lines BC_3 F_4 OM10252 /Pokkali//OM10252 under salt stress.

of stem the lines recorded at concentrations 15dS/m lower than 8dS/m, 0dS/m. The fifty lines showed higher dry weight of stem ranged from 13-16 mg at 8 dS/m (Figure 4). Among these lines only one line was measured dry weight of root (4 mg) (Figure 4).

Through phenotypic evaluation of backcross hybrids OM10252/ Pokkali/OM10252 about the level of response tolerant to salinity with three different salt concentrations EC = 0 dS/m, 8 dS/m, and 15 dS/m can be divided into three different groups: group of salt tolerant lines, slight susceptible lines and susceptible lines. Lines of good salinity tolerance included BC$_3$F$_4$-17, BC$_3$ F$_4$-18, BC$_3$ F$_4$-23, BC$_3$ F$_4$-28, BC$_3$F$_4$-40, BC$_3$F$_4$-46, BC$_3$F$_4$-53, BC$_3$F$_4$-54, BC$_3$F$_4$-56, BC$_3$F$_4$-60, BC$_3$F$_4$-

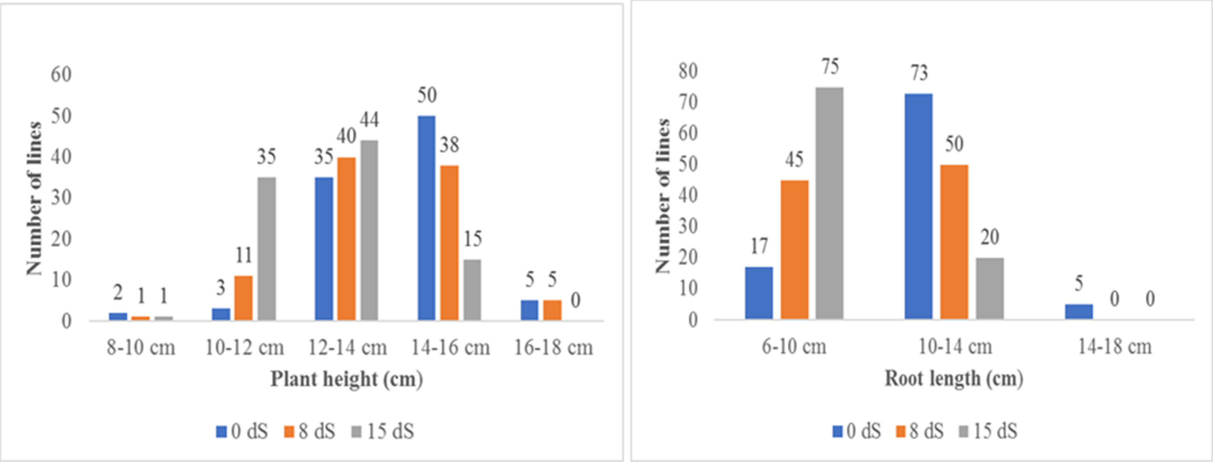

Figure 3. The plant height and root length of rice lines under different concentrations 0dS/m, 8dS/m and 15dS/m.

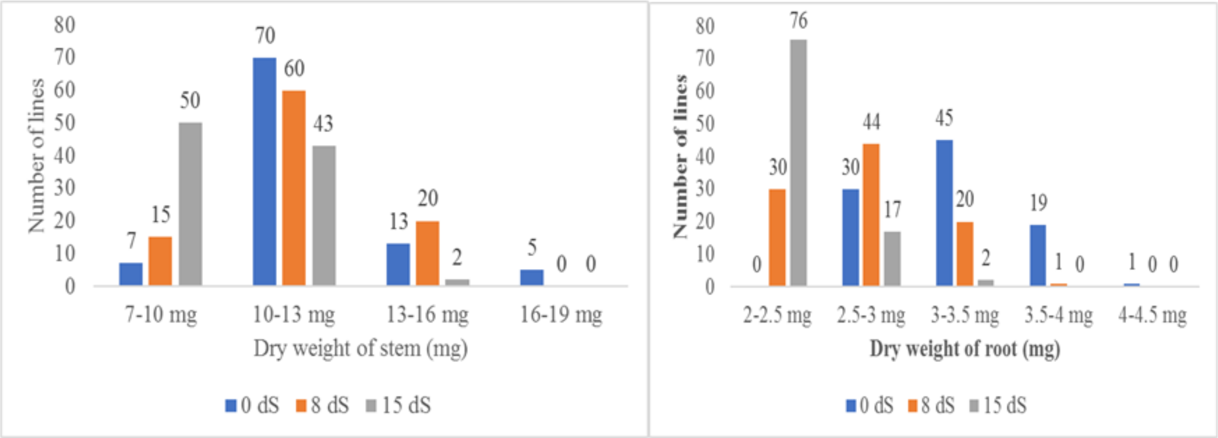

Figure 4. The dry weight of stem and dry weight of root of rice lines under different concentrations 0dS/m, 8dS/m and 15dS/m.

Table 1. The correlation among traits of the salinity tolerance at the seedling stage.

Traits	Survival days	Plant height (cm)	Root length (cm)	Dry weight of stem (mg)	Dry weight of root (mg)	Levels of salt tolerance
Survival days	1					
Plant height (cm)	-	1				
EC=0 ds/m						
EC=8 DS/m	0.845**					
EC=15 DS/m	0.701**					
Root length (cm)	-	0.542*	1			
EC=0 ds/m						
EC=8DS/m	0.536*	0.419*				
EC=15 DS/m	0.725**	0.542*				
Dry weight of stem (mg)	-	0.712**	0.632*	1		
EC=0 ds/m						
EC=8DS/m	0.741**	0.562**	0.489*			
EC=15 DS/m	0.845**	0.741**	0.541*			
Dry weight of root (mg)	-	0.742*	0.541*	0.523*	1	
EC=0 ds/m						
EC=8DS/m	0.516*	0.650*	0.452*	0.642*		
EC=15 DS/m	0.501*	0.355^{NS}	0.214^{NS}	0.632*		
Levels of salt tolerance	-	-	-	-	-	1
EC=0 ds/m						
EC=8DS/m	-0.845**	-0.623**	-0.547*	-0.766**	-0.478*	
EC=15 DS/m	-0.845**	-0.712**	-0.547**	-0.865**	-0.541*	

*, ** Correlation is significant at the 0.05, 0.01 level, respectively, Correlation coefficient is negative number: Correlations is inversely correlation, Correlation coefficient is positive number: Correlation is a positive correlation, Correlation coefficient from: ± 0.01 to ±0. 4: Low correlation (NS)

68, BC$_3$F$_4$-80, and BC$_3$F$_4$-81. These lines need to confirm with genotype with the ability to good tolerance to saline conditions.

For salt stress, rice plant has different reactions depending on the characteristics of each line. However, when considering the correlations of the agronomic-biological characteristics of the lines showed they also have close correlation with each other the same with population OM1490/Pokkali (Lang *et al*., 2017b). Correlation analysis showed that survival day had positive significant correlation with plant height, root length, dry weight of stem, dry weight of root in 6 and 12 ds/m and negative and significant correlation with levels of salinity tolerance in 8 dS/m and 15 dS/m (Table 1).

This suggests that salinity conditions greatly affect the survival, growth, and development of rice. Also, plant height had positive and negative significant correlation with dry weight of stem and levels of salt tolerance, respectively. Correlation between dry weights of stem with levels of salt tolerance was negative significant in 8 and 5 ds/m. The phenotype is the result of influence between genotype and environment. Therefore, it is very important how to accurately measure phenotypes. Our study for two stages of rice was done at the seedling and reproductive stages**.** Firstly, the results indicated that the phenotype traits should be selected first in the different concentration of salty. In this study, salt tolerant seedlings were well-known from the complex

Table 2. Evaluation of salinity tolerant lines in farmer's fields with level of salty at 4%.

Lines	Provinces						Means (ton ha^{-1})
	Bac Lieu	Soc Trang	Long An	Kien Giang	Ben Tre	Hau Giang	
BC3F4-17	4.45 a	4.36 e	5.48 a	4.47 b	5.78 b	5.90 e	5.07
BC3F4-18	3.41 g	4.72 b	4.57 h	4.53 a	5.22 f	6.40 c	4.81
BC3F4-23	3.14 j	3.11 n	4.42 i	3.73 g	4.97 k	4.70 j	4.01
BC3F4-28	3.81 cd	4.42 d	4.75 f	4.07 e	5.08 i	6.40 c	4.75
BC3F4-40	3.96 b	4.93 a	4.85 e	3.13 j	5.69 c	5.80 f	4.73
BC3F4-46	3.76 de	4.59 c	4.90 e	4.13 d	5.18 g	5.60 g	4.69
BC3F4-53	3.20 ij	4.00 g	5.29 b	3.93 f	5.83 a	5.80 f	4.68
BC3F4-54	3.67 f	3.44 k	4.96 d	4.27 c	5.62 d	5.20 i	4.53
BC3F4-56	3.71 ef	3.84 h	4.85 e	2.93 m	5.13 h	6.50 b	4.49
BC3F4-60	3.26 hi	3.96 g	4.63 g	3.67 h	5.03 j	6.30 d	4.47
BC3F4-68	3.41 g	3.38 m	4.24 j	3.40 i	5.71 c	6.70 a	4.47
BC3F4-80	3.64 f	3.79 i	5.18 c	3.00 l	5.70 c	5.50 h	4.47
BC3F4-81	3.39 g	4.07 f	4.47 i	2.80 n	5.42 e	6.50 b	4.44
OM10252	3.84 c	3.84 h	4.25 j	4.07 e	4.98 k	5.60 g	4.43
Pokkali	3.29 h	3.72 j	3.47 k	3.07 k	2.99 l	3.00 k	3.25
CV%	8.6	7.2	12.5	16.7	8.3	6.3	-
LSD$_{0.05}$	0.47	0.44	0.90	0.92	0.71	0.59	-

Means within columns followed the same letter are not significant different at 0.05 probability level using LSD test.

seedlings when grown in salt condition. As well as plant height and root length, dry weight of stem greatly influenced by environmental salt. In this study, the plant height showed significant positive association with plant biomass. This result was presented; the increasing plant height would greater biomass production (Asch *et al*., 2000). Such types of results were also found by several researchers who showed that the increase in salinity level reduced the seedling height (Javed *et al*., 2006; Maiti *et al*., 2006).

Salt-making ability of dry matter accumulation in rice plants reduced due to nutrient movement slowed down, the ability to form and accumulate dry matter in plant decreased and leads to the dry weight of plant decreased. However, for resistant lines, the formation and accumulation of dry matter still occur normally so dry weight was not much diminished. Bhowmik *et al*. (2007) found that salt stress changed phenotype traits of rice such as reduction of the root, shoot length and tiller number that leads to the reduction in biomass.

In the reproductive stage, a few lines could produce grain yield under salt stress. High or low grain yield of the lines was related with yield components to salt stress. Thus, evaluation for salinity tolerance does not affect the grain yield only; it is required that yield. Mean performance of thirteen lines for yield and yield-related traits is presented in Table 2. Performance stability is one of the most important properties of a genotype to be released as a variety to ensure wide adoption. To ensure this, we tested 13 lines at 6 different locations during the dry seasons of 2017-2018, using a randomized block design with three replications in each case. With salty control 4‰ duration, grain yield (t ha^{-1}) were presented in Table 2. The experiment was conducted in 6 provinces, Long An, Bac Lieu, Soc Trang, Ben Tre, Kien Giang, Hau Giang in Mekong Delta, Vietnam. The line BC3F4-17 was the best and was selected for two locations survival during 4‰ condition. A final comment is that both field management and improved cultural practices are also extremely important for salty in rice.

In the areas where rice lines were cultivated in direct at the reproductive stage. In the reproductive stage, a few genotypes could produce grain yield in salinity condition. High or low grain yield of the studied genotypes was related to yield components and different behaviors of rice genotypes to salt stress. To achieve high yield (HY) and yield stability through breeding, breeders have to develop high yielding rice cultivars with significantly improved tolerances to salinity.

CONCLUSION

Rice is more sensitive during the early seedling and later reproductive stages. There are several traditional cultivars which are salt tolerant but they have not good agronomic traits. So far, the importance of salt tolerance in backcross population used Pokkali, a salinity tolerant traditional cultivar with respectable results. Among the salt tolerance traits, the results were reported that under salt stress increased plant height was responsible for increasing the biomass production. These criteria were also closely correlated with each other. Conclusively, the few lines showed varied responsive to salt stress at the different region in Vietnam. This response was some lines and special one line (BC_3 F_4-17) to salinity stress at 4% in the field condition.

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