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Research Article

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SCREENING RICE FOR SALINITY TOLERANCE BY PHENOTYPIC IN POPULATION OM1490/POKKALI//OM1490 CROSS AT SEEDLING STAGE

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Abstract

Breeding for salinity tolerance in rice requires dependable screening techniques. A population was developed from a cross between OM1490 (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). Introgression from Pokkali variety into the hybrid population was recorded with 12,90% recognition for salinity tolerant gene at the survival level of rice plant in salt concentration of EC=8DS/m and 4,30% of survival rice plant at the concentration of EC=15 dS/m. Analysis of 95 individuals from the BC3F3 population of OM1490/Pokkali//OM1490 cross, the 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 = 0,8 and 15 dS/m. This demonstrates the expression of traits affecting closely when analyzing the resistant varieties with various salt concentrations. Through BC3F3 generation, the salt tolerant lines will continue to grow to be evaluated at the vegetative and growth stages, and be taken into breeding programs.

Key Words: Rice, Salinity tolerance, screening, seedlings stage, correlation

INTRODUCTION

Salinity is one of the major abiotic stresses limit the crop production severely (Shannon, 1998). At present, salinity is the second type of stress and is the most predominant interference to rice production after drought (Gregorio, 1997). Nearly 95 million hectares of arable land in worldwide are facing salinity problem and estimated to damage crop up to 50% of fertile land by the 21st mid-century (Nazar *et al*., 2011; Huyen, *et al*., 2013; Ghassemis – Golezani *et al*., 2010). Salt tolerance is generally a sustained growth of the plant in the soil environment impregnated with NaCl or other salt combinations. The response of the rice plant to salinity was observed in all the various stages of its development, that is at early seedling, vegetative and reproductive stages (Mohammadi-Nejad *et al*, 2010; Aref and Rad, 2012). Several studies indicated that rice is tolerant during germination, and becomes very sensitive during early seedling stage (2-3 leaf stage) (Pearson *et al*., 1966; IRRI, 1967). A Recent study, indicated that salinity reduced tillering, spikelet filling, florets per panicle, 1,000 grain weight, grain yield, harvest index, shoot and root dry matter (De Leon *et al*., 2015).

The difference in salt tolerance between vegetative and reproductive stage is 19.4% at and 13% in the case of Doc Phung (Lang *et al*., 2001a). 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 varieties (Takehisa *et al*., 2004). The selective screening of some lines for tolerance at the seedling stage is still underway. Thus, it is very difficult to evaluate phenotype of salinity tolerance in rice under field condition.

To enhance salinity tolerance in rice, a screening method for salinity tolerance could be readily acceptable when based on a simple criterion for selection, it provides rapid screening of many materials and reproducible results. Screening is done under the controlled condition to minimize environmental effects. The objective of this was the evaluation of backcross population for salt tolerance in rice. This requires that the trait is generally an important objective in rice breeding programs.

MATERIALS AND METHODS

Plant materials

This experiment was carried out on the greenhouse at Department of Genetic and plant breeding at Cuu Long Delta Rice Research Institute Viet Nam.The population was developed by backcrossing between OM1490 (high yielding variety and short duration) as the female

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parent with Pokkali as male parent (a donor in many breeding programs and salt tolerance related studies). The ninety-three lines of BC3F2 OM1490/Pokkali //OM1490 of Lang *et al*, (2001b) were used for phenotypic at BC3F3 generation.

Screening salinity tolerance at the seedling stage

Salt treatment is applied at the 2–3 leaf stage, after 1–2 weeks of seedling establishment following the guidelines proposed by in nutrient solution Yoshida (1986). The experiment was in the form of a split-plot design with 3 replications. On each soft tray, we drilled 100 holes with diameter 1 cm, in a 10 x 10 matrix. The bottom was covered with nylon mesh to keep the grain. The spongy tray was placed in a 40 x 50 cm plastic tray with a capacity of about 3-5 liters of water.

Preparation of solution components.

Preparing correct solution components is extremely important to avoid lack of nutrients and formation of mineral toxins affected the salinity of salt. The solutions can be stored for 3 months at room temperature or longer-term storage at 40C. Saline solution was created by adding NaCl to the nutrient solution and adjusting desired electrical conductivity EC (0, 8 and 15 dS/m). Three to five liters of nutrient solution culture is needed for each tray following (Lang *et al*., 1999).

Evaluation of salt stress symptoms

The modified standard evaluation system (SES) was used in rating the visual symptom of salt toxicity (IRRI, 1996). The survived plants were selected randomly for each replication from each plate of both control (EC as 0 dS/m) and treatments (EC as 8 dS/m and 15 dS/m), to record rate of survival, salinity level, plant height, root length, dry weight of the stem and dry weight of root during 30 days treatment. Scoring is relative and carried out according to the standard evaluation system developed by IRRI with a score 1 for tolerant and 9 for sensitive.

Table 1 Assessment scores of seedlings with respect to relative salt tolerance

Levels	Visual observatio	Relative tolerance
1	Normal growth; no leaf symptom	Highly Tolerant
3	Nearly normal growth; but occasional white leaf tips and rolled leaves	Tolerance
5	Growth severely retarded; most leaves rolled, few leaves elongate	Moderately tolerant
7	Complete cessation of growth; most leaves dry and some seedling death	Susceptible
9	Most seedling dead or dyin	Highly susceptible

Statistical Analysis

All measurements were conducted in triplicates. An analysis of variance (ANOVA) for all data was performed using the SAS 9.1 software. Correlation coefficients of different traits at seedling stage under salinized condition were also calculated.

RESULTS

Salt tolerance is a multi-gene factor and is greatly influenced by environmental conditions, so the salt gene screening analysis must combine evaluation of phenotype and genotype. The phenotype is the result of influence between genotype and environment. Therefore, it is very important to accurately measure phenotypes. A total 93 lines of population OM1490/Pokkali//OM1490 were used to evaluate tolerance to disadvantageous conditions (salt). Plants were distributed in a random matrix with three replications (Fig. 1).

Fig 1 The rice experiment distribution after stress NaCl with 10 days(A) and 30 days (B) in BC3F3 of OM1490/Pokkali//OM1490 population

Number of survival lines

Among the tested lines showed that the survival period of different lines is statistically different at 99% (**). Fig 2 shows these comparisons, which suggests the conditions of higher salinity stress affects rice growth. Out of 93 lines, 37 lines observed the highest survival rate at 8 dS/m about 23 to 25 days, and 12 lines survived about 27-29 days under salinized culture solution. Stress level increased adjusting salinity level to EC 15 dS/m, among tested 21-23 days and about 23-25 days indicated increase number of survival lines (25, 49 lines respectively). But at this salinity stress EC, it scored only 4 lines survived about 27-29 days under salinized culture solution.

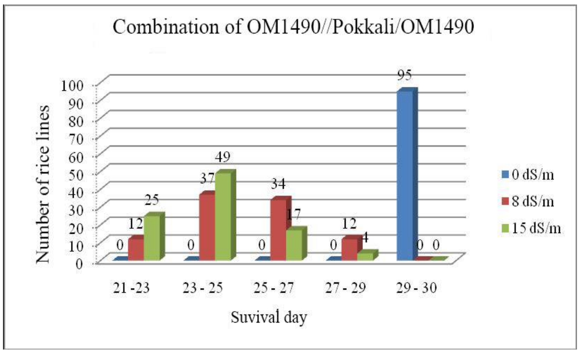

Fig 2 Survial rates of lines B_3F_3 OM1490/Pokkali//OM1490 after salinity stress.

Salinity Levels

A salty environment affects rice growth and development. Individual plants affected by salinity stress expressed yellow leaves, stem and roots were less developed than usual, and more severe infection could make the rice plant sick, even dry fire and died. The average mean score ranged from 1 (tolerant) to 9 highly susceptible (Fig. 3). Under EC conditions, the level showed a significant variation at 8 dS/ m of NaCl. Most of the lines through 30 days of screening in salt environment expressed dried leaf levels in a level of 7 to 9 (45 lines). There were 35 lines with a level of 5 to 7, 14 lines a level of 3 to 5 and one line a level of 1 to 3. While at 15dS/m, more lines were susceptible compared with the 8dS/m. Twenty-three lines had levels of 5 to 7 and 5 lines had a level of 3 to 5, no lines with expression of resistance (level 1-3).

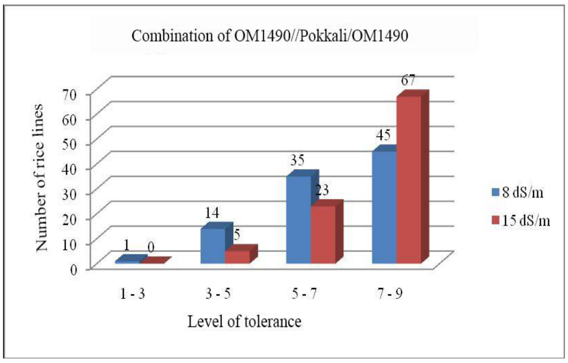

Fig 3 Salinity levels of lines B_3F_3 OM1490/Pokkali//OM1490 under salinized culture solution.

The salt injury level at 8 and 15 dS /m showed identical responses for the lines, indicating that 14 lines and 5 lines are moderate salt tolerance (level 3-5), while other varieties were classified as susceptible.

Plant Height

Plant heights significantly decreased (differences statistically significant at 99% (**) in salinized conditions compared with to plants grown in un-saline condition 0dS/m (Fig. 4). At the higher salt concentrations of EC 15 dS/m, most of the lines had plant height that decreased compared at 8 dS/m concentration, and at the concentration of 8dS/m, most of the lines had plant height that decreased compared with at concentrations 0dS/m. In population OM1490/Pokkali//OM1490, environment EC 0 dS/m, height majority concentrated in about 18-22 cm, whereas at EC 8 dS/m was in the range of 16-22 cm, at EC 15 dS/m was 16-20 cm. This suggests salt conditions will limit the growth and development of rice. To adapt to salt conditions, rice plants resistant to stress will grow rapidly to break out past the salt sensitive stage, gradually adapting to harsh environments. The salinity sensitive lines will grow slowly, gradually dry fire and died.

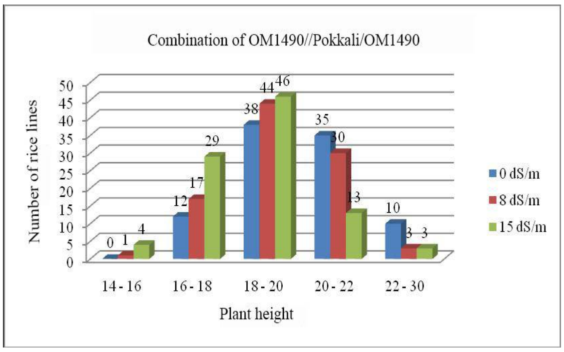

Fig 4 Plant height of lines B_3F_3 OM1490/Pokkali//OM1490 under salinized culture solution

Root Length (RL)

The root length was pronounced among lines (Fig. 5) with mean reductions of concentrations 0dS/m, 8dS/m and 15dS/m were statistically different at the 99% level (**). At the higher salt concentrations, root length was reduced more. The length of roots was very sensitive to salt, and the pattern of variation was complex when screening in a nutrient environment. In populations OM1490/Pokkali//OM1490, with concentration 0dS/m root length ranged from 6-9cm for 7 lines, 9-12cm for 53lines, 12 - 15 cm for 31 lines and 15-18cm for 4 lines. An average of root length from 9-12cm, about 50 % of lines had decreased number of lines in saline conditions. At EC 8dS/m, root lengths were observed for 18 lines (6-9 cm), 51 lines (9-12 cm), for 23 lines (12-15cm) and for 3 lines (15-18 cm). At EC 15dS /m, root lengths were observed for 30 lines (6-9cm), for 47 lines (9-12cm), for 16 lines (12-15 cm) and for 2 lines (15-18 cm).

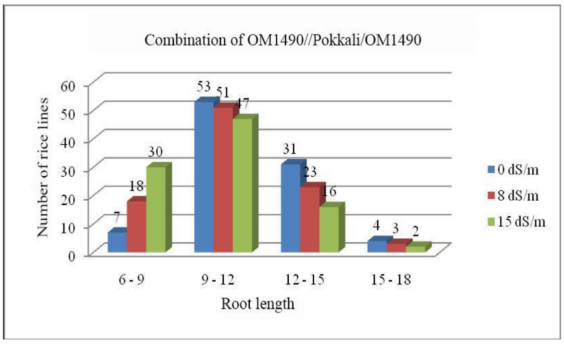

Fig 5 Plant height of lines B_3F_3 OM1490/Pokkali//OM1490 under salinized culture solution.

Dry weight of the stem

In the same way as plant height and root length, dry weight of stem was greatly influenced by environmental salt. Dry matter accumulation in rice plants was reduced due to slow nutrient movement, and the ability to form and accumulate dry matter in the plant decreased leading to decrease to the dry weight of the plant. However, for resistant lines, the formation and accumulation of dry matter still occurred normally, so dry weight was not much diminished.The dry weight of the lines in different concentrations EC 0dS/m, 8dS/m and 15dS/m was significantly different, at the 99% level (**). Dry weight of shoot observed in the lines of population OM1490/Pokkali//OM1490 at concentrations EC=15dS/m was lower than shoots grown in 8dS/m, 0dS/m solutions, and dry weight of shoots in EC=8dS/m was lower than weight of shoots grown at a concentration 0dS/m, specifically as follows: at concentrations 0dS/m dry weight of stem was from 8-12mg in 3 lines, 12-16 mg in 63 lines, 16-20 mg in 26 lines, 20-24 mg in and 24 - 26 mg one line. At concentration EC 8dS /m dry weight of shoot was from 8-12mg in 6 lines, 12-16 mg in 60 lines, 16-20 mg in 28 lines, from 24-26 mg in 1 line. At concentration with EC=15dS/m dry weight of shoot was from 8-12 mg in 11 lines, 12-16 mg in 57 lines, 16-20 mg in 26 lines, and from 24-26 mg in one lines.

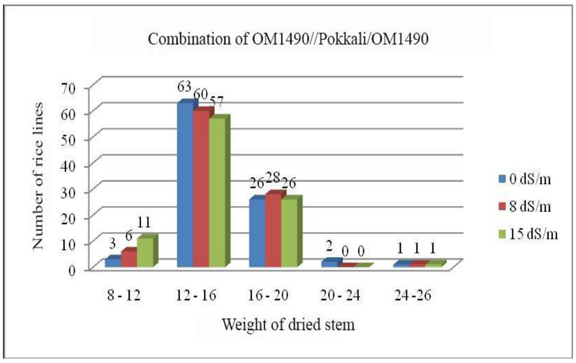

Fig 6 Dry weight of the stem of lines B_3F_3 OM1490/Pokkali//OM1490 under salinized culture solution.

Dry weight of root

Differences in the dry weight of roots at concentrations 0dS/m, 8dS/m and 15dS/m were also statistically significant at 99% (**), with differences within lines and between the concentrations of various salts. The dry weight of roots was also affected by salt tolerance. At concentration EC 0dS/m dry weight of roots was greater than the dry weight of roots at concentration EC 8dS/m and the dry weight of roots at concentration EC 8dS/m was greater than the dry weight of roots at concentration EC 15dS/m. By analyzing the dry weight of root of 93 rice lines of the population OM1490/Pokkali //OM1490 we found the difference between the lines and in different concentrations 0dS/m, 8dS/m and 15dS/m. At EC 0 dS/m, dry weight of root ranged from 3.0-6.0 mg/plant, while at EC 8 dS/m weights were at 2.0-5 mg.

Salt conditions affected the growth of rice plants. The higher the salt concentration, the greater decrease in growth of rice plants, meaning that plant height, root length, dry weight of the plant, and dry weight of root also decreased. Due to the genetic characteristics of each line, some lines had lower salinity tolerance, but plant height, root length, dry weight of stem, dry roots were equivalent, or slightly higher than stronger resistant lines. However, if judging salinity tolerance of the same line, then these traits at EC 0 dS/m was higher at EC 8 dS/m and EC 8 dS/m higher than EC 15 dS /m.

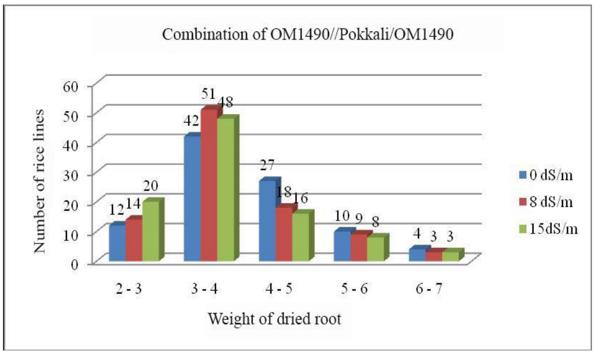

Fig 7. Dry weight of the stem of lines B_3F_3 OM1490/Pokkali//OM1490 under salinized culture solution (mg)

Correlation analysis

The coefficient of correlation among different phenotypic characters from the experiment is presented in Table 2. An evaluation of the correlations between the characteristics of the population OM1490/Pokkali//OM1490, results showed the environmental 0 dS/m, the plant height was closely correlated with the dry weight of stem while correlations between other characteristics were less strong. At EC 8 dS/m, plant height (r = 0.792**) and dry weight of stem (r = 0.757**) were positively but salinity tolerance level (r = -0.970**) was showed significant negative correlation with survival time. The similar finding also at EC 15 dS/m, positive and high correlation of dry weight of stem with survival time was obtained in this study. The survival time had significant negative correlation with levels of salt tolerance (r = -0.973**) but positive relation with root length (r = 0.642**), and plant height (0.794**). The dry weight of root showed a low correlation with survival time. Plant height recorded positive significant association with the dry weight of stem both of EC 8 dS/m and 15 ds/m (0.792** and 0.760**, respectively). Character root length observed the negative significant association with levels of salt tolerance (-0.644**) at EC 15 dS/m. The dry weight of stem had significant negative correlation with Levels of salt tolerance both of EC 8 dS/m and 15 ds/m (-0.769**and -0.820**, respectively). From the regression studies, it was clear that the higher the salt concentration is, the shorter the survival days, stem length and root length become, and stem and root dry weights become lighter. However, the criteria of root length only showed low correlations, which may have been due to an imperfect collection of the roots. The correlations between criteria in an environment of EC 8 dS/m were higher than at the EC 15 dS/m environment, which suggests that salt stress in higher concentrations may cause greater variation.

Table 2 The correlation among the assessment criteria characters of the salinity tolerance lines at the seedling stage.

Population OM1490/Pokkali//OM1490	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) EC=0 ds/m	-	1				
EC=8 DS/m	0.792**					
EC=15 DS/m	0.794**					
Root length (cm) EC=0 ds/m	-	0.580*	1			
EC=8DS/m	0.478*	0.551*				
EC=15 DS/m	0.642**	0.511*				
Dry weight of stem (mg) EC=0 ds/m	-	0.819**	0.562*	1		
EC=8DS/m	0.757**	0.792**	0.552*			
EC=15 DS/m	0.812**	0.760**	0.566*			
Dry weight of root (mg)EC=0 ds/m	-	0.594*	0.461*	0.575*	1	
EC=8DS/m	0.591*	0.530*	0.410*	0.533*		
EC=15 DS/m	0.496*	0.388ns	0.384ns	0.500*		
Levels of salt tolerance EC=0 ds/m	-	-	-	-	-	1
EC=8DS/m	-0.970**	-0.780**	-0.464*	-0.769**	-0.576*	
EC=15 DS/m	-0.973**	-0.773**	-0.644**	-0.820**	-0.477*	

Note:

Correlation coefficient from: ± 0.01 to ±0. 4: Low correlation (ns)
Correlation coefficient from: ± 0.4 to ±0.6: Average correlation (*)
Correlation coefficient from: ± 0.6 upwards: high correlation (**)
Correlation coefficient is positive number: Correlation is a positive correlation.
Correlation coefficient is negative number: Correlations is inversely correlation.

DISCUSSION

Salt tolerance is a multi-gene factor and is greatly influenced by environmental conditions, so the salt gene screening, mono-gene traits are easy to measure and observe, but not in all cases. The phenotype is the result of influence between genotype and environment. Therefore, it is very important to accurately measure phenotypes. Using a population of plants allows repeated phenotypes, which will increase the accuracy of measurement, particularly for traits susceptibility to environmental change. The analysis of the phenotype is the largest investment (Lang *et al*., 2001b).

In rice, the concentration of salt in leaves is found to cause different degrees of toxicity in different varieties, which is termed tissue tolerance (Ismail *et al*., 2007). Mondal and Borromeo, (2016) observed at the seedling stage of four genotypes from irrigated population tolerant to salinity at 12 dS/m. A rice plant that was dry over 70% of its surface was considered dead. The survival period of a variety was calculated at the average number of survival days of the rice individual in three replications. Survival was screened in three levels of salinity (0, 8 and 15 dS/m). The variance between replications of the environment 15 dS/m was higher than the environment EC 8 dS/m, which suggests that conditions of higher salinity stress affect rice growth. This suggests salt conditions will limit the growth and development of rice. To adapt to salt conditions, rice plants resistant to stress will grow rapidly to break out past the salt sensitive stage, gradually adapting to harsh environments. The salinity sensitive lines will grow slowly, gradually dry fire and die. The results indicated that the injury scores were significantly influenced by salt conditions. The high concentration of salt was selected better tolerance lines. The lines had different root lengths and length was also different between concentrations. At the higher salt concentrations, root length was reduced more. The length of roots was very sensitive to salt, and the pattern of variation was complex when screening in a nutrient environment. It had been reported that salinity caused some morphological changes like reduction of shoot (Bhowimk *et al*., 2007), root length (Mishra *et al*., 1995), and restriction of rooting (Evers *et al*., 1997).

Jamil *et al*, (2007) reported that decreased plant growth at high salinity concentration. In the same way in this study as plant height and root length, dry weight of stem was greatly influenced by environmental salt. Dry matter accumulation in rice plants was reduced due to slowed nutrient movement, and the ability to form and accumulate dry matter in the plant decreased leading to decreased to the dry weight of the plant. However, for resistant lines, the formation and accumulation of dry matter still occurred normally so dry weight was not much diminished Salt conditions affected the growth of rice plants. The higher the salt concentration, the greater to decrease in growth of rice plants, meaning that plant height, root length, dry weight of the plant, and dry weight of root also decreased. Due to the genetic characteristics of each line, some lines had lower salinity tolerance, but plant height, root length, dry weight of stem, dry roots were equivalent, or slightly higher than stronger resistant lines. However, if judging salinity tolerance of the same line, then these traits at EC 0 dS/m was higher at EC 8 dS/m and EC 8 dS/m higher than EC 15 dS/m.

When subjected to salt stress, rice plants had very 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 had the correlation with each other closely.

The correlations between criteria in an environment of EC 8 dS/m were higher than at the EC 15 dS/m environment, which suggests that salt stress in higher concentrations may cause greater variation. Survival time was highly correlated with criteria such as plant height, dry weight of stem, dry weight of root and salinity tolerance level. Plant height was highly correlated with the dry weight of stem and level of salinity tolerance. The remaining characteristics were weaklier correlated. Lines of good salinity tolerance at EC 15dS/m include: lines 53, 54, 18, 81, 60, 80, and 68. These lines need to confirm with genotype with an ability to tolerate saline conditions.

The ability of rice lines to respond to salinity differed greatly. However, in general, the higher the salt concentrations were, the lesser time the plant survived, the shorter the plant height, the longer the root length and the lesser the dry weight of stems and roots became. These criteria were also closely correlated with each other. This suggests that salinity conditions greatly affect the survival, growth, and development of rice.

An appropriate screening method that is effective in early stages of growth although various screening method under greenhouse condition, has been proposed, this method is very complex and even under controlled conditions, are expensive and time-consuming when screening large quantities of a population.

CONCLUSION

Through phenotypic evaluation of two populations of backcross hybrids, OM1490/Pokkali//OM1490 for tolerance to salinity at three different salt concentrations EC 0 dS/m, 8 dS/m, 15 dS/m the lines were divided into three different groups including a group of salt-tolerant lines, slightly susceptible lines, and susceptible lines. Lines of good salinity tolerance in line environment 15dS/m include: lines 53, 54, 18, 81, 60, 80, and 68. These lines need to confirm with genotype with an ability to tolerate saline conditions.These results, the strong correlation between the height trait, root length, dry weight of stem, dried weight of roots, and the survival time of rice plants at all three levels of salt concentration EC 0,8 and 15 dS/m emphasized features in a systematic way for tolerance to find new salinity tolerance lines.

Recommendations

Continue to evaluate varieties for good tolerance results in the seedling stage, flowering stage, and maturity. Evaluate whether some ions such as Na, K, Cl affect the population.

Evaluate and test with different primers, to more accurately determine salinity tolerance in lines. Screen salt tolerance in each variety in different concentrations to assess comprehensively.

Acknowledgements

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