Vol. 14(27), pp. 1154-1162, 4 July, 2019
DOI: 10.5897/AJAR2019.13945
Article Number: 7F98A6261322
ISSN: 1991-637X

http://www.academicjournals.org/AJAR

ACADEMIC JOURNALS
expand your knowledge

African Journal of Agricultural Research

Full Length Research Paper

Screening of rice germplasms under salt stress by phenotypic and molecular markers

Nguyen Thị Lang[1], Pham Thi Thu Ha[2]*, Nguyen Thu Tra[2] and Bui Chi Buu[3]

[1]High Agricultural Technology Research Institute (HATRI) for Mekong Delta, Vietnam.
[2]Faculty of Applied Sciences, Ton Duc Thang University, Ho Chi Minh City, Vietnam.
[3]Institute of Agricultural Science (IAS) for Southern, Vietnam.

Received 11 February, 2019; Accepted 17 May, 2019

The evaluation of salinity tolerance of rice germplasms based on phenotype and genotype of 100 rice germplasm was identified at the seedling stage at the High Agricultural Technology Research Institute for Mekong Delta, Vietnam. Screening of the phenotype was done with 30 day old seedlings using salinized concentrations (EC = 8 and 15 dS/m). Results show that the growth of the varieties, the higher the salt concentration, the lower the survival date, the lower the tree height, the higher the root length, and the weight of the stem and roots were all significant positive correlations in this study. These indicators are also closely correlated with each other. This shows that saline conditions greatly affect the survival, growth, and development of rice. Genotypic analysis on the RM223 and RM3252 molecular markers recorded polymorphism on both indicators. Among rice germplasms, the proposed varieties including Pokkali, OM4900, HATRI144, HATRI60, OM5704, HATR162, HATRI131 and HATRI132 were found to have salt tolerance for evaluation future of the breeding program. Thus, the RM233 and RM3252 used in this study are proved available to identify salt tolerance for rice breeding.

Key words: Phenotype, genotype, simple sequence repeat (SSR) markers, salt stress, seedling stage, rice.

INTRODUCTION

Rice is one of the most important grains grown and accounts for more than one-third of the world's food (Munns, 2002; Wassmann et al., 2009). However, climate change, particularly the greenhouse effect, the atmospheric temperature warms and melting ice at the two poles, will create flooding in the lowlands. Seawater intrusion into the mainland is increasing rapidly, causing serious consequences for rice production in Vietnam and other countries in the world, especially in the Mekong Delta. Farm land is affected by the saline intrusion of over 400 million hectares, accounting for 1/3 of the world's cultivated land. It is often accompanied by the phenomenon of alkaline and wetland soils (Gale, 2002). In Vietnam, in 2008 the area of rice cultivation reached 7.4 million hectares, of which the Mekong River Delta had 2.9 million hectares with 800,000 ha of saline soil. In recent years, global urbanization and climate change have affected agricultural land, especially drought and saline intrusion. Therefore, developing high-yielding rice varieties that are salt tolerant could be the best effort for

*Corresponding author. E-mail: phamthithuha@tdtu.edu.vn.

these areas.

Research on the development of saline tolerant in plants has been at the forefront of international projects in genetic technology. The breeding crop of salt-tolerant rice would likely be an economical improvement and overcome the problems of salty soil. With the advancement of biotechnology, molecular markers can be used in breeding. Based on molecular markers associated with salt-resistant genes, breeders can identify genotypes for resistance and infection from an early stage. Determining the main gene position by microsatellite marker analysis can be used to develop new varieties (Mondal and Ganie, 2014). And appropriate strategies for breeding salinity tolerance and crop cultivation are seen as the most effective and economical way to increase paddy production in saline areas (Buu and Lang, 1995). Significant differences in injury rates between cultivars when grown under salt stress were detected by Ali et al. (2014) and the results were used for screening purposes.

Screening is also being done on different parts of the discovery of diverse genetic potential to determine salinity tolerance of rice genotypes. Vegetative stage and reproductive stage tolerance of the rice was expressed in tolerance at the seedling stage by Hariadi et al. (2015). There were some germplasms with high salt tolerance offering. In spite of this, these common germplasms have many undesirable traits. The highly tolerant, and often used as donors in breeding for salt tolerance, was Pokkali which is tall, photosensitive, low yielding, and has red kernels (De Leon et al., 2016). Conventional breeding is time-consuming and depends on environmental conditions. Molecular markers are technologies that provide the possibility of applying the genetic map (Islam, 2004), and studying genetic diversity in germplasms. The most efficient descriptors to screen the salt tolerant genotypes were simple sequence repeat (SSR) markers RM8094, RM336 and RM8046, which have a higher polymorphic information content coupled with higher marker index value (Ali et al., 2014). Five SSR markers (RM1287, RM8094, RM3412, RM493 and RM140) and two EST markers (CP3970 and CP6224) were linked to Saltol QTL on chromosome 1 were detected by Niones (2004). The RM3412 marker has been shown to be useful for marker-assisted selection of *Saltol* QTL (Naresh et al., 2014). The present study determined the phenotype of rice germplasms used salinized conditions (EC= 8 and EC = 15 dS/m) at the seedling stage and identified salt tolerance rice germplasms from 100 varieties by SSR markers.

MATERIALS AND METHODS

Plant

Seeds of 100 rice germplasms (*Oryza sativa* L.) were collected in this study (Table 1) including one salt tolerant Indian variety "Pokkali" and one salt susceptible variety "IR28" as controls. The rice germplasms were received from Intentional Rice Research Institute (IRRI), Cuu Long Delta Rice Research Institute (CLRRI) and High Agricultural Technology Research Institute for Mekong Delta, Vietnam (HATRI).

Screening of phenotype

Rice germplasms were screened for salt tolerance at the seedling stage using IRRI standard modified by Lang et al. (2001). The evaluation was done using Yoshida et al. (1976) nutrition solution to obtain EC (8 and 15 dS/m). The experiment was conducted at the HARTI laboratory. The final scoring was observed after 30 days of salinization followed by a protocol of Gregorio et al. (1997). The characteristics as SES coring, survival day, shoot height, root length, plant dry weight, and root dry weight were observed at the seedling stage.

Genotyping using SSR markers

Modified CTAB was used to extract DNA from leaf samples of genotypes. Two primers as RM233 and RM3252 were chosen for the study (Lang et al., 2017) (Table 2). 30 µl polymerase chain reactions (PCR) reaction contained 15 ng DNA samples, 1.25 units Taq DNA polymerase, 0.2 µM primer, 1.7 mM $MgCl_2$ 0.17 mM dNTPs and PCR buffer. PCR profile was maintained as the initial denaturation at 94°C for 4 min, and then the reaction was subjected to 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 2 min with a final elongation step of 4 min at 75°C. Amplification products were resolved by electrophoresis on a 3% polyacrylamide gel within TBE 1X.

Data analysis

The data was analyzed using Excel and the software by CropStat 7.2. The correlation coefficient of among traits at the seedling stage also was calculated under salinized conditions.

RESULTS AND DISCUSSION

Screening rice germplasms by phenotype

In the first experiment, the salt tolerance of the rice germplasms in the seedling stage was assessed in two different salt concentrations (8 and 15 dS/m) (Figure 1). 100 varieties were purified in the saline phase. There were 8 varieties that died 100% before 30 days. According to SES scoring of IRRI standard protocol was modified by Gregoe et al. (1997). The scoring was used for the evaluation of the tolerance, moderate tolerance, and susceptible rice varieties. The levels surveyed after 30 days of completing the saline test were as follows: Among 92 remaining varieties, there were 10 varieties found with level 3 at 8 dS/m compared to Pokkali control variety, two varieties with level 5, 79 varieties with levels 7 and 9 (susceptible). In case of 15 dS/m, the varieties were burnt and died a lot compared to the 8 dS/m medium, only a few survived, but they also burned heavily. Most of the varieties in this environment exhibited very high levels of dry leaves, level 9 with 78 varieties, accounting for 84.78%. There are 13

Table 1. List of varieties used for the study.

No.	Name of varieties	Origin	No.	Name of varieties	Origin
1	OM4900	CLRRI	51	HATRI60	HATRI
2	OM1490	CLRRI	52	HATRI90	HATRI
3	AS 996	CLRRI	53	OM10413	CLRRI
4	M362	IRRI	54	OM10414	CLRRI
5	BASMATI	IRRI	55	OM10415	CLRRI
6	Basmati ĐB	IRRI	56	OM6614	CLRRI
7	OM6162	CLRRI	57	OM6843	CLRRI
8	Swarna Sub1	IRRI	58	OMCS2009	CLRRI
9	IR64Sub1	IRRI	59	OM5703	CLRRI
10	IRGA318-11-6-9-2B	IRRI	60	OM5704	CLRRI
11	IR78966-B-10-B-B-B-2	IRRI	61	HATRI62	HATRI
12	IR78913-B-10-B-B-B	IRRI	62	OMCS2008	CLRRI
13	IR75499-73-1-B	IRRI	63	HATRI31	HATRI
14	IR78913-B-19-B-B-B	IRRI	64	HATRI32	HATRI
15	AZUCENA	IRRI	65	OM63L	CLRRI
16	IR78933-B-24-B-B-2	IRRI	66	Pokkali	IRRI
17	IR78933-B-24-B-B-3	IRRI	67	OM71L	CLRRI
18	IR78933-B-24-B-B-4	IRRI	68	OM72L	CLRRI
19	WAB326-B-B-7-H1	IRRI	69	OM5681	CLRRI
20	IR79008-B-11-B-B-1	IRRI	70	OM6730	CLRRI
21	IR75499-38-1-B	IRRI	71	OM6014	CLRRI
22	V3M-92-1	IRRI	72	OM6778	CLRRI
23	IR75499-21-1-B	IRRI	73	OM6033	CLRRI
24	V3M-109-2	IRRI	74	OM6032	CLRRI
25	WAB272-B-B-8-H1	IRRI	75	OM6613	CLRRI
26	WAB340-B-B-2-H2	IRRI	76	OM6729	CLRRI
27	WAB176-42-HB	IRRI	77	OM62L	CLRRI
28	IR78937-B-20-B-B-1	IRRI	78	OM10279	CLRRI
29	WAB880-1-38-18-20-P_1-HB	IRRI	79	OM10280	CLRRI
30	WAB881SG9	IRRI	80	OM10418-1	CLRRI
31	IR78997-B-16-B-B-B-SB2	IRRI	81	OM10704	CLRRI
32	IR78966-B-10-B-B-B-SB1	IRRI	82	OM3673	CLRRI
33	IR78944-B-8-B-B-B	IRRI	83	OM4249	CLRRI
34	IR78941-B-16-B-B-B	IRRI	84	OM4693	CLRRI
35	IR78948-B-21-B-B-B	IRRI	85	OM4726	CLRRI
36	IR78942-B-2-B-B-2	IRRI	86	OM4796	CLRRI
37	IR78937-B-20-B-B-3	IRRI	87	OM6379	CLRRI
38	IR78985-B-13-B-B-B	IRRI	88	OM5629	CLRRI
39	IR78933-B-24-B-B-1	IRRI	89	OM6387	CLRRI
40	WABC165	IRRI	90	OM6426	CLRRI
41	IR80315-49-B-B-4-B-B-B	IRRI	91	HATRI15	HATRI
42	IR78966-B-16-B-B-B	IRRI	92	HATRI16	HATRI
43	IR78913-B-22-B-B-B	IRRI	93	HATRI33	HATRI
44	OMCS2000	CLRRI	94	HATRI20	HATRI
45	OM6161	CLRRI	95	HATRI35	HATRI
46	OM10405	CLRRI	96	HATRI28	HATRI
47	HATRI144	HATRI	97	HATRI50	HATRI
48	HATRI 1	HATRI	98	HATRI192	HATRI
49	OM10408	CLRRI	99	HATRI603	HATRI
50	IR29	IRRI	100	HATRI608	HATRI

Table 2. The primers used to study.

Primer	PCR product size (bp)	Sequence	
		Forward	Reverse
RM223	200 bp-220	GAGTGAGCTTGGGCTGAAAC	GAAGGCAAGTCTTGGCACTG
RM3252	220 bp-230	GGTAACTTTGTTCCCATGCC	GGTCAATCATGCATGCAAGC

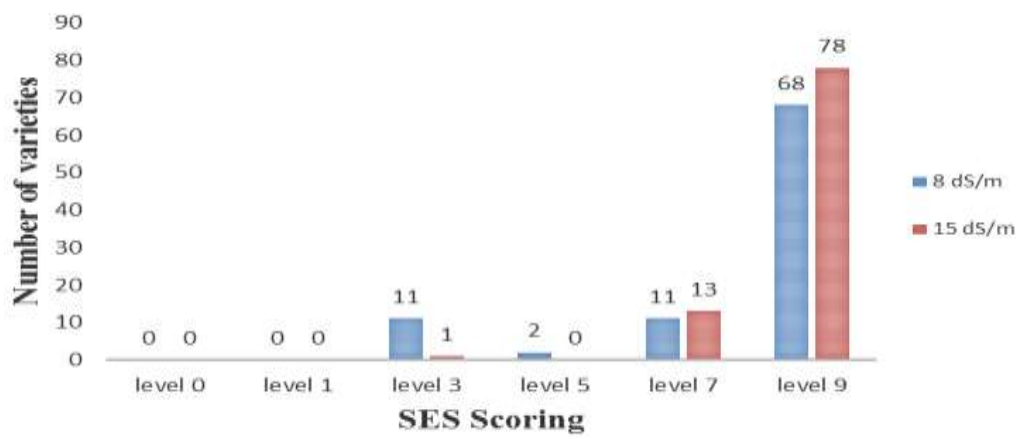

Figure 1. Performance of SES scoring of rice germplasms under salt stress.

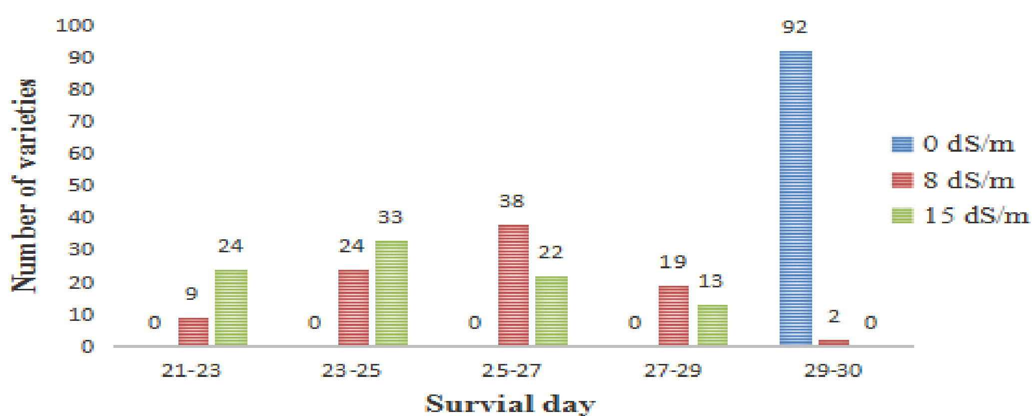

Figure 2. Performance of survival day at the seedling stage of rice germplasms under salt stress.

varieties at level 7. Only the Pokkali variety was expressed at level 3. The results in these experiments showed that the few tolerant varieties based on phenotype analysis exist.

The expression of salt tolerance also included the correlation of survival time and reduction of height seedling (Figures 2 and 3). In the saline environment with EC = 0 dS/m, all varieties survived 30 days of purification. The highest survival time in the 8 dS/m medium was 29.5 days while in the saline environment EC = 15 dS/m (28.8 days). The lowest survival time in a saline environment with EC = 8 dS/m was 22.1 days and in the environment 21 days. Saline medium with EC = 8 dS/m survived 21-23 days with 9 varieties, 23 - 25 days with 24 varieties, 25 - 27 days with 38 varieties, 27 - 29 days with 19 varieties, 29 - 30 days. The saline environment with EC = 15 dS/m survived from 21-23 days with 24 varieties, 23 - 25 days with 33 varieties, 25 - 27 days with 22 varieties, and 27 - 29 days with 13 varieties. In general, the varieties survived in EC = 0 dS/m more than in saline environments with EC = 8 dS/m, and in saline environments with EC = 15 dS/m were the most dead varieties over 30 days of salt purification in the nutrient environment.

The largest plant height reduction (from 8 to 20 cm) was observed in the three rice germplasms at EC = 8 dS/m. On the other hand, the lowest plant height (from 6 to 8

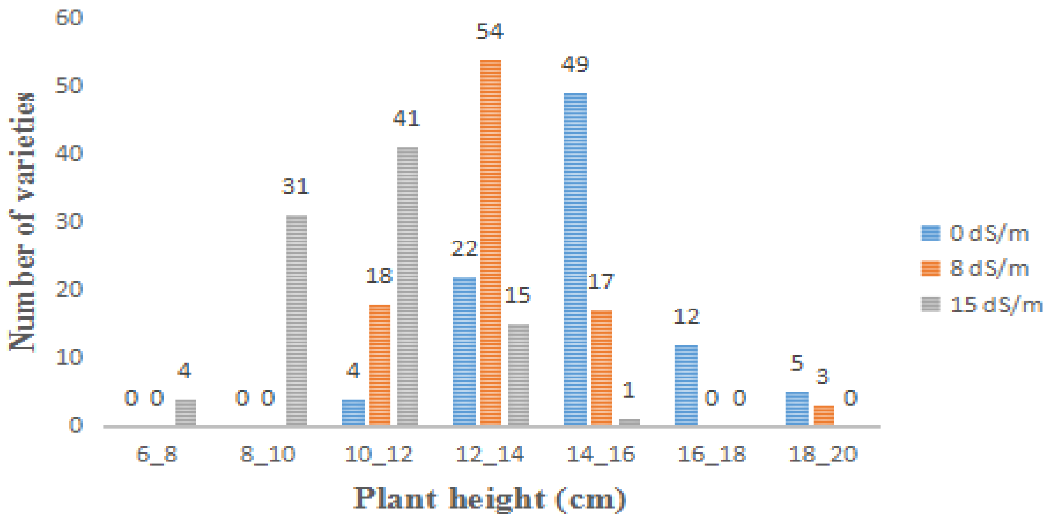

Figure 3. Performance of plant height (cm) at the seedling stage of rice germplasms under salt stress.

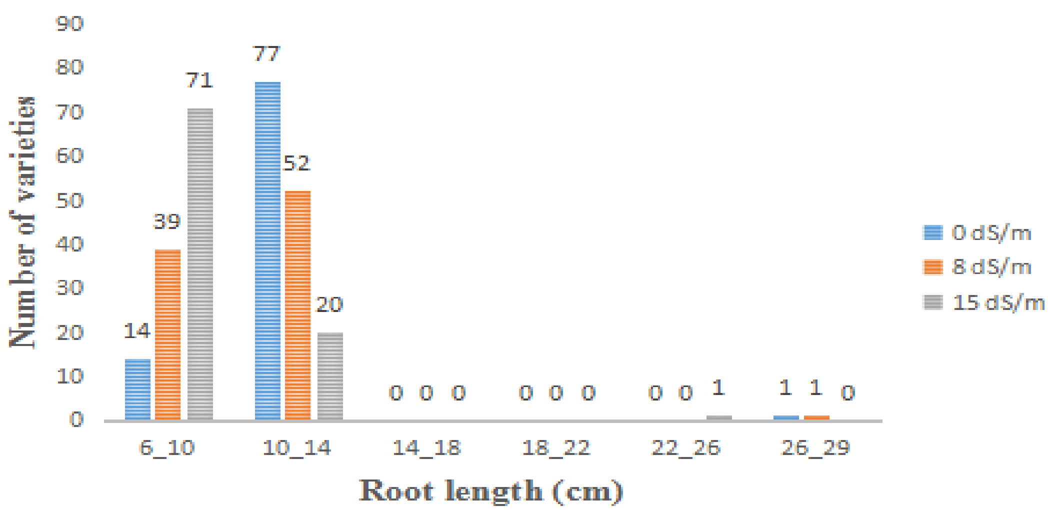

Figure 4. Performance of plant root length (cm) at the seedling stage of rice germplasms under salt stress.

cm) was observed in the five rice varieties at EC = 15 dS/m. The highest number of varieties (54 rice varieties) was found by plant height from 12-14 cm at 8 dS/m followed by 41 rice varieties from 10-12 cm at 15 dS/m. There was only one variety (16 cm) which was observed at 15 dS/m. The previous studies showed that the increase in salinity level reduced the seedling height (Javed et al., 2006; Maiti et al., 2006). These results suggested that the plant height was reduced under higher concentration of salt stress. To adapt to saline conditions, stress-tolerant rice plants will grow very quickly to get out of the sensitive phase with salt and adapt to acclimatization to harsh environments.

The roots and biomass traits were reduced in the rice seedling stage in different salt concentrations (Figures 4 to 6). Varieties have different root lengths and vary between concentrations. In this study, several rice germplasms showed higher root length reduction at 8 dS/m only one line (26 cm). At 8 dS/m, it showed the highest number of rice germplasms in 54 varieties which had roots lengths ranging from 11 to 14 cm. Almost all rice varieties also showed lower root length reduction (6-8 cm) at 15 dS/m (Figure 4). The result showed that root length decreased under salinity stress. Root length is very sensitive to salinity, so the fluctuation is complex, especially in the nutrient environment. Roy et al. (2002) reported that the number of roots per plant decreased with increasing levels of salinity. Rodrigues et al. (2002) reported that root length was reduced due to the effect of salinity, which coincided with the present study.

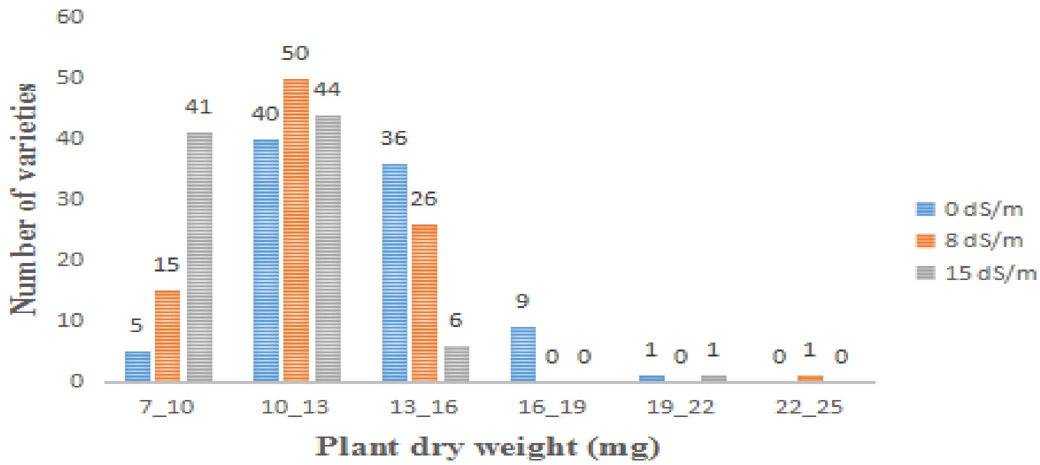

Figure 5. Performance of plant dry weight at the seedling stage of rice germplasms under salt stress.

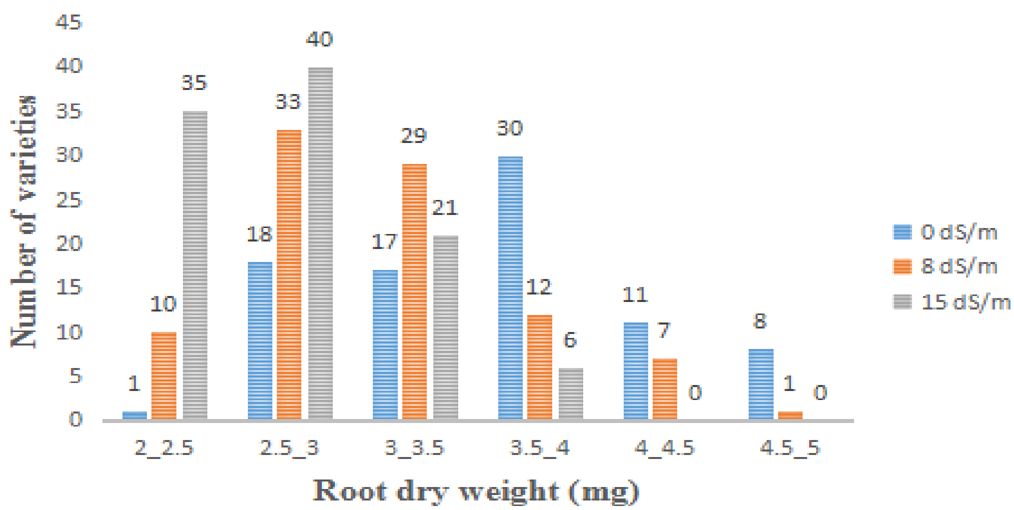

Figure 6. Performance of root dry weight (mg) at the seedling stage of rice germplasms under salt stress.

The biomass traits as plant dry weight and root dry weight are important traits to evaluate the performance of rice varieties for salt tolerance. The salinity caused a significant reduction in plant dry weight of the rice germplasms, compared with control EC = 0 dS/m (Figure. 5). The highest of plant dry weight reduction was found in one variety (26 mg) at 8 dS/m followed by one variety (26 mg) at 15 dS/m and 6 varieties (from 13 to 16 mg) at 15 dS/m after 30 days of stress (Figure 5). The lowest plant dry weight reduction was found in 41 varieties (7-10 mg).
Similar to the plant height and root length, the weight of dry shoots was greatly influenced by the saline environment. In addition, a lesser amount of dry mass in rice has been produced under salt stress (Sakina et al., 2016). Salinity reduces the ability to accumulate dry matter in rice due to the slowing down of nutrient transfer, the ability to form and accumulate dry matter in reducing the dry weight of plants. Salinity caused a significant reduction in the root dry weight compared with the control (Figure 6). The maximum number of rice germplasms recorded at 15 dS/m (40 varieties) by root dry weight varied from 2.5 to 3 mg, whereas, the minimum in one variety at 8 dS/m by root dry weight was about 4.5 mg. Morphological parameters such as plant dry weight and root dry weight are well correlated with crop salt tolerance at early growth stages and can be used as an indicator for salt tolerance. Under salt stress, reduction in biomass production is a common feature in crop plants. In this study, salinity caused a significant reduction in shoot and root dry weights of the varieties compared with control (Shereen et al., 2005); a significant reduction in seedling growth under salinity was also observed.

Table 3. The coefficient of correlation among different traits from salt stress in the seedling stage (30 days).

Trait	Survival day	Plant height (cm)	Root length (cm)	Plant dry weight (mg)	Root dry weight (mg)
EC= 0 dS/m					
Survival day	1				
Plant height (cm)	-	1			
Root length (cm)	-	0.704**	1		
Plant dry weight (mg)	-	0.968**	0.839**	1	
Root dry weight (mg)	-	0.987**	0.701**	0.971**	1
EC = 8 dS/m					
Survival day	1				
Plant height (cm)	0.960**	1			
Root length (cm)	0.681**	0.842**	1		
Plant dry weight (mg)	0.900**	0.977**	0.917**	1	
Root dry weight (mg)	0.973**	0.961**	0.727**	0.928**	1
EC = 15 dS/m					
Survival day	1				
Plant height (cm)	0.953**	1			
Root length (cm)	0.708**	0.857**	1		
Plant dry weight (mg)	0.946**	0.981**	0.884**	1	
Root dry weight (mg)	0.995**	0.962**	0.734**	0.958**	1

**Significant at the 0.01 level.

The correlation among different traits under two salinized concentrations (8 and 15 dS/m) are shown in Table 3. For salt stress, rice plants respond very differently depending on the characteristics of each variety. However, the correlation of the salt tolerant characteristics showed a significant positive correlation with each other (Table 3). A similar finding was reported by Lang et al. (2017) and Zang et al. (2004) regarding larger biomass production. In this study, the results implied that the salt tolerance germplasms had lower salt tolerance score, higher traits as plant height, root length, and dry total mater. On the other hand, the relative importance of the attributes must be decided on based on the higher condition among the traits.

Screening rice germplasms by using SSR markers

In this study, two primers RM233 and RM 3252 were used for the polymorphism survey of 100 rice germplasms. The bands were obtained from the Pokkali; a cultivar was used as salt tolerant genotype in genotype banding. With respect to RM233, eight varieties (Pokkali, OM4900, HATRI144, HATRI60, OM5704, HATR162, HATRI131 and HATRI132) were found tolerant of salt stress in Figure 7. A similar result was found by Lang et al. (2000) who found that RM233 was closely linked to the salt tolerant gene in chromosome 8. The SSR marker RM223 also was tested by Lang et al. (2017), with an accuracy of 82% between genotype and phenotype at the sexually productive stage and 92% at the seedling stage. In the case of RM3252, five rice germplasms were identified tolerant for salt stress compared to the tolerant variety Pokkali in Figure 8. These two markers showed polymorphisms in the same of four varieties (OM5704, HATR162, HATRI131 and HATRI132). Finally, salt tolerant rice germplasms were identified in both phenotype and genotype using eight varieties. The tested marker RM233 could be used efficiently to identify the study for salt tolerance in the rice breeding program.

Conclusion

Based on the results, it can be concluded that the experiment was performed to classify 100 rice germplasms into groups of tolerance, moderately susceptible and susceptible. The salinity tolerance showed a significant positive correlation to those traits (survival days, plant height, root length, plant dry weight, and root dry weight). Genotype analysis recorded the polymorphism on both markers (RM233 and RM3252). Among rice germplasms, Pokkali, OM4900, HATRI144, HATRI60, OM5704, HATR162, HATRI131 and HATRI132

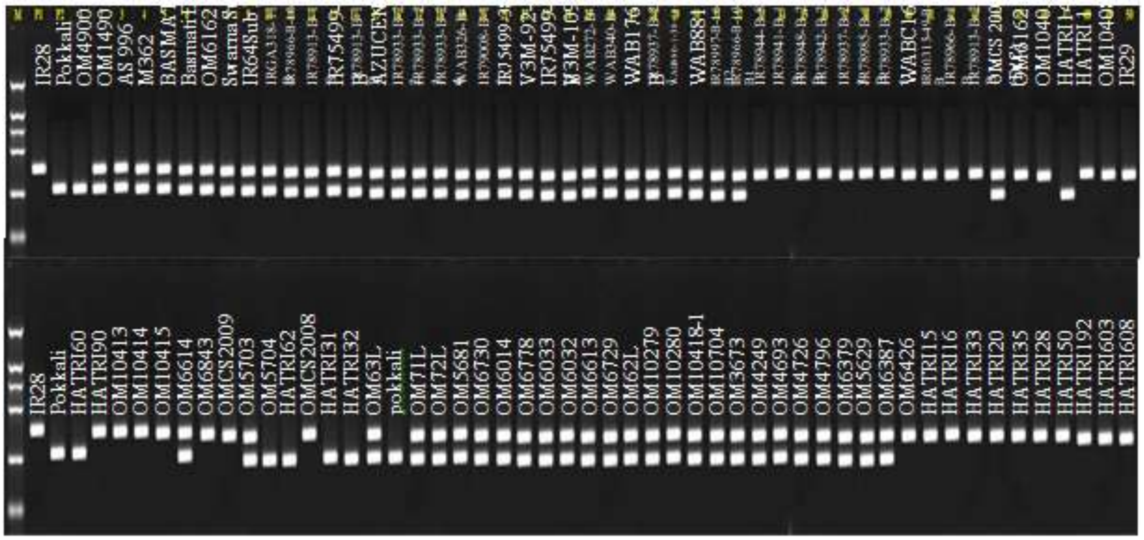

Figure 7. PCR profiles of 100 rice germplasms using RM223.

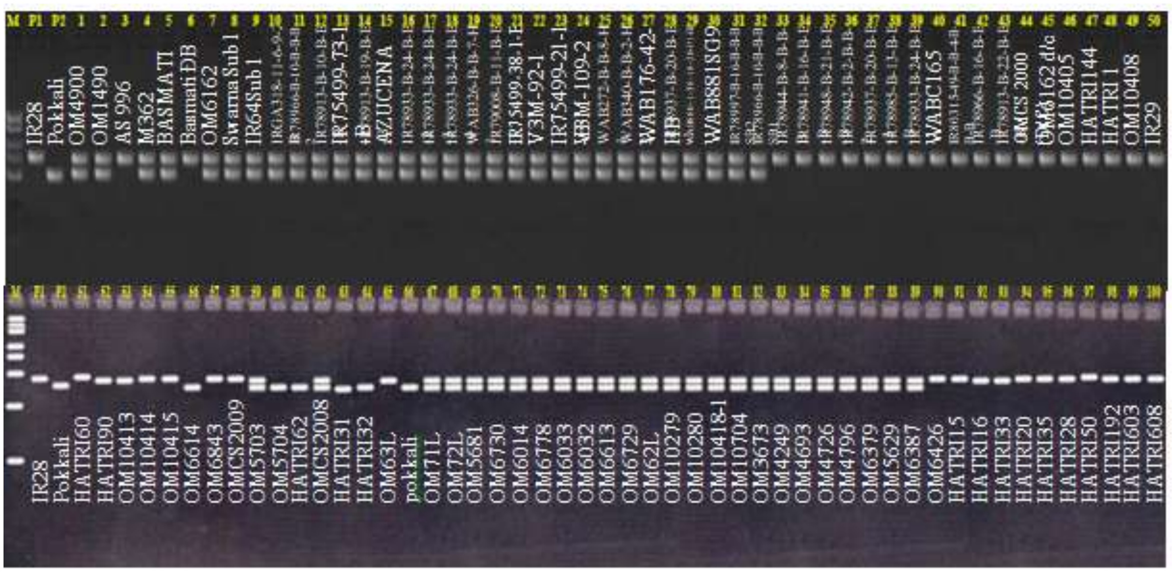

Figure 8. PCR profiles of 100 rice germplasms using RM3252.

were found to be salt tolerant at the seedling stage, which can be used in breeding rice for tolerance to salinity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Ali MN, Yeasmin L, Gantait S, Goswami R, Chakraborty S (2014). Screening of rice landraces for salinity tolerance at seedling stage through morphological and molecular markers. Physiology and Molecular Biology of Plants 20:411-423.

Buu BC, Lang NT (1995). Application of biotechnology in rice variety improvement. Agriculture Publishing House, Hanoi.

De Leon TB, Linscombe S, Subudhi PK (2016). Molecular Dissection of seedling salinity tolerance in rice (*Oryza sativa* L.) sing a high-density GBS-Based SNP Linkage Map. Rice 9:52.

Gale M (2002). Applications of molecular biology and genomics to genetic enhancement of crop tolerance to abiotic stress – a discussion document. FAO – Consultative Group on International Agricultural Research Interim Science Council. Rome, Italy.

Gregorio TL, Dharmawansa S, Mendoza RD (1997). Screening rice for salinity tolerance. IRRI, Discussion Paper Series No.22, International Rice Research Institute. Manila, The Philippines, pp. 1-30.

Hariadi YC, Nurhayati AY, Soeparjono S, Arif I (2015). Screening six varieties of rice (*Oryza sativa*) for salinity tolerance. Procedia Environmental Sciences 28:78-87.

Islam MM (2004). Mapping salinity tolerance genes rice (*Oryza sativa* L.) at productive stage. PhD dissertation university of the Philippine Los Banos College, Laguna, Philippine P. 149.

Javed MA, Ishii T, Kamijima O, Misoo S (2006). Discrepancy of two ecotypes of *Oryza Sativa* L. to salinity at germination and seedling stages. Annals of Biology 22:201-211.

Lang NT, Phuoc NT, Ha PTT, Buu BC (2017). Identifying QTLs associated and marker-assisted selection for salinity tolerance at the seedling, vegetative and reproductive stages in rice (*Oryza sativa* L.). International Journal of Environment, Agriculture and Biotechnology. 2:2927-2935.

Lang NT, Yanagihara S, Buu BC (2000). Quantitative trait loci for salt tolerance in rice via molecular markers. Omonrice 8:37-48.

Lang NT, Yanagihara S, Buu BC (2001). A microsatellite marker for a gene conferring salt tolerance on rice at the vegetative and reproductive stages. Sabrao 33:1-10.

Maiti RK, Vidyasagar P, Banejee PD (2006). Salinity tolerance in rice (*Oryza sativa* L.) hybrids and their parents at emergence and seedling stage. Crop Research Hisar 31:427-433.

Mondal TK, Ganie SA (2014). Identification and characterization of salt responsive miRNA- SR markers in rice (*Oryza sativa*) Gene 535(2):204-209.

Munns R (2002). Comparative physiology of salt and water stress. Plant Cell and Environment 25:239-250.

Naresh BN, Vinod KK, Gopala KS, Bhowmick PK, Vanaja T, Krishnamurthy SL, Nagarajan M, Singh NK, Prabhu KV, Singh AK (2014). Marker based haplotype diversity of saltol QTL in relation to seedling stage salinity tolerance in selected genotypes of rice. Indian Journal of Genetics 74(1):16–25.

Niones JM (2004). Fine mapping of the salinity tolerance gene on chromosome 1 of rice *(Oryzasativa* L.) using near isogenic lines, M. S. dissertation, College, Laguna, Philippines: University of the Philippines Los Baños, Laguna

Rodrigues LN, Ferandes PD, Gheyi HR, Viana SBA (2002). Germination and formation of rice seedlings under saline stress. Revista Brasileira de Engenharia Agrícola e Ambiental 6(3):397-403.

Roy SK, Patra SK, Sarkar KK (2002). Studies on the effects salinity stress on rice (*Oryza sativa* L.) at seedling stage. Journal of Interacademicia 6:254-259.

Sakina A, Ahmed I, Shahza A, Iqbal M, Asif M (2016). Genetic variation for salinity tolerance in Paksistan rice (*Oryza sativa* L.) germplasm. Journal of Agronomy and Crop Science 202(1):25-36.

Shereen A, Muntaz S, Raza S, Khan MA, Solangi S (2005). Salinity effects on seedling growth and yield components of different inbred rice lines. Pakistan Journal of Botany 37:131-139.

Wassmann R, Jagadish SVK, Heuer S, Ismail A, Redona E, Serraj R, Singh RK, Howell G, Pathak H, Sumfleth K (2009). Climate change affecting rice production: the physiological and agronomic basis for possible adaptation strategies. Advances in Agronomy 101:59-122.

Yoshida S, Forno DA, Cock JH, Gomez KA (1976). Laboratory manual for physiologycal studies of rice. IRRI, Los Banos, Lauguna, Philippines, pp. 61-66.

Zang ZH, Li P, Wang LX, Hu ZL, Zhu LH, Zhu YG (2004). Genetic dissection the relationships of biomass production and partitioning with yield and yield related traits in rice. Plant Science 167:1-8.