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SCREENING OF MARDI RICE ACCESSIONS FOR SALINITY TOLERANCE AT SEEDLING STAGE BASED ON GROWTH PERFORMANCE AND MOLECULAR ANALYSIS

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Abstract

Salinity is one of the important abiotic factors that can interfere with rice production in many rice-growing areas. In Malaysia, many major rice growing areas are located near to coastal saline land. Therefore, identification of potential rice accessions for future breeding program for salinity tolerance is crucial. A total of 28 rice accessions were selected for this study. The screening was performed at EC=12dSm^{-1} under controlled environment at seedling stage. Shoot length, root length, biomass, percentage of surviving seedlings, and leaf injury score were used to assess seedling growth performance. Whereas, the molecular analysis was performed using eight Simple Sequence Repeat (SSR) markers for salinity tolerance. Significant variations were shown for all the recorded traits among the rice accessions, treatments, and environments. Lui Oui, Boro Shunga, and Khao Tah Haeng showed the highest shoot length under salinity condition with the readings at 34.61 cm, 36.47 cm, 35.45 cm, respectively. The rice accessions also showed higher biomass production and lower leaf injury score. The molecular analysis observed the average PIC value was 0.741, with values ranging from 0.545 to 0.907. The SSR marker, RM10720 was found to be superior for analysis of genetic diversity as shown by the highest PIC value. By comparing the two dendrograms, four rice varieties namely Lui Oui, Sinandomeng, Khao Tah Haeng, and Kao Saard 108 were clustered with Pokkali in both dendrograms. Considering the performance under salinity condition and confirmation with molecular analysis, Lui Oui, Sinandomeng, Khao Tah Haeng, and Kao Saard 108 are considered potential donors that can be used in breeding programmes for the development of salinity tolerant rice cultivars. The SSR markers can be used in screening and identification of salinity tolerance genotypes.

Key words: Rice, Salinity tolerance, Seedling stage, Growth performance, SSR markers.

Introduction

Salinity is one of the key limitations to plant productivity and a severe concern in many agricultural areas, particularly in hot and dry climates (Pattanagul & Thitisaksakul, 2008). Salinity has been reported to cause serious problems in arable soils due to the accumulation of trace amounts of sodium chloride from irrigation water (Tester & Davenport, 2003), whereas salinity in rainfed areas near coastal areas is caused by seawater intrusion during the high tide phenomenon (Bharathkumar *et al*., 2016). In Malaysia, saline soils cover 186,523 hectares in Peninsular Malaysia, while 358,434 and 571,078 hectares in Sabah and Sarawak, respectively. In this regard, many major rice-growing areas in Malaysia, notably Muda areas in the north-west, Seberang Perai and the Kerian, and Barat Laut Selangor districts further south, are located near coastal saline land (Hashim, 2003).

Salinity has been found to negatively affect the yield components of rice including reduced tillering, spikelet filling, number of spikelets per panicle, 1000 grain weight, grain yield, harvest index, shoot and root biomass (De Leon *et al*., 2015) and delayed heading (Lang *et al*., 2017). Salinity also has a significant impact on rice cell division and cell elongation, resulting in reduced root, leaf growth, and yield (Munns, 2002). Previous studies have indicated that every unit increase in EC (electrical conductivity in solution) above 3.0 dSm^{-1} reduces rice yield by 12% (Grattan *et al*., 2002; Hanson *et al*., 1999).

In general, rice can withstand a small amount of saltwater without affecting its growth development or yield. However, it is highly dependent on the types and species of rice as well as their stage of development (Rahman *et al*., 2017). Recently, several methods for screening rice for salt tolerance have been developed, including on-field mass screening and controlled environment screening by using hydroponics or other artificial media (Ismail & Horie, 2017). However, most of salinity screening particularly is done in the laboratory or greenhouse under controlled conditions at seedling stage (Flowers & Yeo, 1988). Salinity screening at the seedling stage is widely accepted since it is based on a simple selection criterion and offers a rapid screening procedure (Gregorio *et al*., 1997). Many studies have found that rice genotypes show different phenotypic responses to salt stress during the seedling stage, indicating varied genotypic responses (Arzani *et al*., 2008; Kakar *et al*., 2019). In recent year, DNA based molecular markers have been widely used to assess genetic diversity in most plant species. Microsatellites or Simple Sequence Repeat (SSR) markers are the most popular DNA markers because of their efficiency, reproducibility, co-dominance, and high degree of polymorphism. Besides, several SSR markers have been shown to be effective in identifying salt tolerance rice genotypes (Bhowmik *et al*., 2009; Arzani *et al*., 2008).

In this study, a total of 28 rice accessions were selected for salinity screening at the seedling stage using readily acceptable procedures (Gregorio *et al*., 1997) with minor modifications. The screening was carried out under controlled environment. The objectives of this study were to screen 28 rice accessions selected from the MARDI

Rice genebank for salinity tolerance based on growth performance at seedling stage, to evaluate the genetic diversity of rice accessions based on eight SSR markers for salinity tolerance, to identify the potential donors of salinity tolerance among the rice accessions for future use in breeding program.

Materials and Methods

Selection of rice accessions for salinity screening: A total of 28 accessions of rice germplasm were selected from the MARDI Rice Genebank for salinity screening (Table 1). The selection of rice accessions was based on their country of origin, which have faced with sea water intrusions during high tide phenomenon. These countries are Bangladesh, India, Indonesia, Philippines, Vietnam, and Thailand. Two control varieties were included in the study, Pokkali I (MRGB02491) and Pokkali II (MRGB11679) as salinity tolerance control varieties and IR64 (MRGB07854) as salinity susceptible control variety.

Experimental design: The experiment was conducted under controlled conditions, in a glasshouse at MARDI Seberang Perai, Pulau Pinang. MARDI Seberang Perai is located at the north part of Peninsular Malaysia with the latitude at 05°25' N and longitude 100°15' E, respectively. The experiment was laid out in a Completely Randomized Design (Gomez & Gomez, 1984) and consisted of four replications with twelve plants for each rice accession. The rice seedlings were evaluated under two growth conditions namely salinity and control conditions.

Salinity stress composition: The salinity screening was conducted according to Gregorio *et al*., (1997) with minor modifications. In this experiment, the salinity condition was created by using seawater. The seawater was diluted with tap water and maintained the salinity condition at EC=12dSm^{-1}. Saline condition is categorized as EC (electrical conductivity) >4 dSm^{-1}, ESP (exchangeable sodium percentage) <15, and SAR (sodium absorption ration) <15 (Sajid *et al*., 2017). The salinity condition was monitored daily by using an Electrical Conductivity (EC) meter.

The seeds of each rice accessions were sown in petri dish until the rice seedling reached an age of 7 days after sowing (DAS). Then, the seedlings were transferred to plastic planting trays containing paddy field soil. One seedling was sown in each hole. All seedlings were left in tap water for 3 days to allow the seedlings to recover from injuries caused by the transplanting process (Gregorio *et al*., 1997). At 10 DAS, the seedlings on the plastic planting trays were exposed to salt stress by soaking the seedlings in saline water. The seedlings were left in the salinity condition for 20 days until the second to third leaf stages (Lee *at al*., 2007). Meanwhile, the seedlings for control condition were maintained in normal tap water throughout the experiment.

Table 1. List of selected rice accessions for salinity screening.

No.	Accession number	Variety name	Country of origin	No.	Accession number	Variety name	Country of origin
1.	MRGB00660	Kataktara	Bangladesh	15.	MRGB00674	Kaedinga A	Malaysia
2.	MRGB04270	Boro Shunga	Bangladesh	16.	MRGB03625	Pulut Laut	Malaysia
3.	MRGB04272	Deshi Boro	Bangladesh	17.	MRGB05141	Palawan	Philippines
4.	MRGB06705	Naria Bachi	Bangladesh	18.	MRGB04259	Kinandang Patong	Philippines
5.	MRGB05092	Ratnagire	India	19.	MRGB00209	Bicol	Philippines
6.	MRGB06766	Karekagga 78	India	20.	MRGB10832	Sinandomeng	Philippines
7.	MRGB02104	Jayanti	India	21.	MRGB00477	Gion Chem 351	Vietnam
8.	MRGB04288	Pankhari 203	India	22.	MRGB10788	Tetep	Vietnam
9.	MRGB00202	Benong 130	Indonesia	23.	MRGB01112	Nep Trung Vit	Vietnam
10.	MRGB00693	Ketian Gadjih	Indonesia	24.	MRGB00818	Lui Uoi	Vietnam
11.	MRGB08517	Rojolele	Indonesia	25.	MRGB00171	Bang Pra 2169	Thailand
12.	MRGB07013	Utri Merah	Indonesia	26.	MRGB01235	Pin Khao 2051	Thailand
13.	MRGB03780	Subang Intan	Malaysia	27.	MRGB00640	Kao Saard 108	Thailand
14.	MRGB04195	Lakatan Pasir	Malaysia	28.	MRGB00700	Khao Tah Haeng	Thailand

Table 2. Modified standard evaluation system (SES) of visual salt injury at seedling stage.

Score	Observation	Tolerance
1.	Normal growth, no leaf symptoms	Highly tolerant
3.	Nearly normal growth, but leaf tips of few leaves whitish and rolled	Tolerant
5.	Growth severely retarded, most leaves rolled	Moderately tolerant
7.	Complete cessation of growth, most leaves dry, some plant dying	Susceptible
9.	Almost all plant dead or dying	Highly susceptible

Morphological characterization for seedling growth performance under salinity condition: Morphological data as determinants of seedling growth performance under salinity condition were collected after 20 days of treatment. The data on shoot length, root length, and biomass were collected from three seedlings of each rice accession. The biomass at the seedling stage was measured by determining the dry weight of the shoots and roots. An assessment on salinity symptoms was scored using a modified standard evaluation system (SES) (Table 2). The symptoms of salinity were assessed by visual observation of the leaf injury at seedling stage according

to the method proposed by Gregorio *et al*., (1997). The percentage of surviving seedlings was determined by counting the number of surviving seedlings of each rice accession in all replications.

Statistical analysis

The data were analysed statistically using SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA) to test the significance of variance sources (ANOVA). Mean separation between the two experimental conditions for all traits were estimated using Tukey's Studentized Range (HSD) Test at 5% significance level using SAS software. Principal Component Analysis was performed using PAST software (version 4.06) to determine the pattern of correlation within studied traits of rice accessions. Cluster analysis was performed using the UPGMA (unweighted pair group method with arithmetic mean) based on Euclidean distance using PAST software (version 4.06).

Molecular Analysis with SSR markers for salinity tolerance

SSR markers selection: In this study, eight SSR markers for salinity tolerance were chosen from GRAMENE (https://archive.gramene.org/markers/). The features of each marker, including repeat motif, forward and reverse primers are shown in Table 3.

Genomic DNA extraction: Young leaves of each rice accession, including control varieties, were collected and dried using silica gel before being preserved in the freezer at -20°C. Then, the DNA of each rice accession was isolated using the protocol of Mace *et al*., (2003) with some modifications in term of using semi-robotic equipment for high throughput DNA extraction. The frozen tissue was ground using Tissue Lyser (Qiagen, Germany) and immediately added with the extraction buffer. The DNA concentration and integrity were measured using 0.8% agarose gels and Fluoroskan Ascent (Thermo Fisher Scientific, United States), respectively.

PCR amplification and gel electrophoresis: The amplification through PCR was performed according to the protocol by Schuelke (2000), the primers (either forward or reverse) were ligated with a non-fluorescent labelled M13 sequence tail (TGTAAAACGACGGCCAGT). Whereas, the sequence adapter was complimented and ligated with fluorescent label M13 sequence adapter (FAM, NED, PET, or VIC). The PCR cocktails with a final volume of 10 µL containing 1x buffer (Invitrogen, United States), 10 µM of each forward and reverse primer, 5 µM fluorescence-labeled M13 primer, 2 µM of each dNTP (Invitrogen, United States) and 1U of Taq polymerase (Invitrogen, United States) were prepared. The PCR reaction was performed using GeneAmp® PCR System 9700 (Applied Biosystems, United States) with the profile set as follows: (1) initial denaturation at 94°C for 2 min, (2) followed by 36 cycles of 94°C for 30 sec, 41–65°C for 45 sec, and 72°C for 45 sec, and (3) followed by final extension at 72°C for 7 min. The PCR product were then multiplexed up to four different fluorescent dye. Finally, the PCR products were resolved using ABI 3730 xl and GeneScan 500 LIZ as a standard ladder.

Molecular data analysis: GeneMapper Version 5 (Thermo Fisher Scientific, United States) was used to score the raw data generated by the ABI 3730 xl to determine the allele size. Major allele frequency, number of genotypes, number of alleles, availability, gene diversity, heterozygosity, and polymorphic information content (PIC) were estimated using PowerMarker (Liu & Muse., 2005). Cluster analysis was performed using the UPGMA (unweighted pair group method with arithmetic mean) based on distance matrix obtained from PowerMarker and the dendrogram was visualized using MEGA7 (Kumar *et al*., 2016).

Results

Salinity effect on seedlings growth performance: The study observed highly significant variations for all traits under salinity stress (Table 4). Meanwhile, Table 5 showed a highly significant differences for all the studied traits based on variety (rice accessions) and environments (Table 5).

Based on shoot length, Lui Oui, Boro Shunga, and Khao Tah Haeng showed the highest shoot length under salinity condition at 38.89 cm, 36.47 cm, 35.45 cm, respectively (Table 6). Whereas, the salinity tolerant varieties, Pokkali I and Pokkali II showed vigorous growth performance under salinity condition as having higher shoot length than the susceptible control variety, IR64. Moreover, the two salinity tolerance control varieties also showed less shoot length reduction between the two tested conditions compared to susceptible control variety, IR64 with the percentage of reduction of 19.85%, 18.72% and 41.70%, respectively. Lui Oui, Boro Shunga, and Khao Tah Haeng also showed less shoot length reduction with the percentage were 18.35%, 20.23%, and 19.99%, respectively, which were not markedly different from salinity tolerant control varieties.

The lower reduction in root length were observed in Rojolele (7.94%), Khao Tah Haeng (8.10%), Bang Pra 2169 (8.76%), and Kinandang Patong (8.99%). The tolerance control varieties, Pokkali I and Pokkali II showed reduction in root length with the readings of 20.01% and 12.00%, respectively. Whereas, the susceptible control variety, IR64 showed higher root length reduction with the percentage of reduction was 44.48%.

This study observed a significant highest reduction of biomass in most rice accessions except for Pokkali I (11.11%), Pokkali II (10.53%), Sinandomeng (10.53%), Bicol (11.76%), and Khao Tah Haeng (11.76%). In addition, Sinandomeng, Bicol, and Khao Tah Haeng also showed higher biomass production under salinity condition with the value of 0.17 g, 0.15 g, and 0.15 g, respectively.

The salinity tolerance control varieties showed the highest percentage of surviving seedlings compared to the susceptible control variety (Fig. 1). Lui Oui, Sinandomeng, and Khao Tah Haeng showed a higher percentage of surviving seedlings at 93.75%, 93.56%, and 91.67%, respectively. Meanwhile, Subang Intan (41.67%), Ketian Gadjih (37.50%), and Lakatan Pasir (35.42%) showed the lower percentage of surviving seedlings in salinity conditions. Whereas, IR64 showed the lowest percentage of surviving seedlings at 25.69%.

Leaf injury scores indicate the tolerance level of the rice accessions (Fig. 2). The lowest score as highly tolerant was observed in salinity tolerance control varieties. Whereas, the lowest score of leaf injury among the rice accessions was tolerant. The varieties were Bicol, Lui Oui, and Sinandomeng. On the other hand, eight rice accessions were ranked to be susceptible to salinity condition, the varieties were Deshi Boro, Kaedinga A, Karekagga 78, Ketian Gadjih, Lakatan Pasir, Subang Intan, Boro Shunga, and Kataktara. Whereas, IR64 showed the highest leaf injury score and was scored as highly susceptible. The remaining rice accessions, consisted of 17 rice accessions were scored as moderately tolerant to salinity condition at seedling stage.

Principal component analysis (PCA) to determine the correlation between traits: The component score coefficients, Eigenvalues, Eigenvalues at 97.5%, and individual percentage of variance are presented in (Table 7). According to principal component analysis, PC1 and PC2 accounted for 59.37 % and 21.89 % of the variance, respectively. The first component was most influenced by biomass and leaf injury score, and both are considered as the most important traits in salinity tolerance. Biomass (0.53%) and leaf injury score (0.52%) had positive and negative effect, respectively. Meanwhile, shoot length and root length explained most of the variation in the second and third factor. The second factor revealed that increasing shoot and root length had a positive effect on rice salinity tolerance. The third component identified was the negative and positive effect on salinity tolerance respectively in the shoot length and root length. Biomass and the percentage of surviving seedlings had the greatest influence on the fourth and fifth factors, respectively. The biplot of PC1 versus PC2 is presented in (Fig. 3). Based on all traits studied, the biplot clearly differentiated the genotypes into different salinity resistance categories. The salinity tolerant control varieties and several rice accessions with lower leaf injury score were located on the right side of the biplot; these varieties were salinity tolerant genotypes. On the other hand, rice accessions located far to the left side of the graph, were salinity susceptible.

Assessment on salinity tolerance using SSR markers: Eight SSR markers were used to determine the major allele frequency, genotype number, allele number, gene diversity, heterozygosity and polymorphic information content (PIC) values among 28 rice accessions for salinity tolerance (Table 8). The markers were RM140, RM562, RM1287, RM7075, RM10720, RM10825, RM10843, and RM10852. The results showed that 71 reproducible alleles were detected using these eight markers. The number of alleles produced ranged from 3 (RM10852) to 15 (RM10720). The average gene diversity over all SSR loci was 0.78, with the highest was 0.913 (RM10720) and the lowest was 0.86 (RM1287). Polymorphic Information Content (PIC) values varied from 0.545 to 0.907, the highest value belonged to RM10720, while RM10852 showed the lowest PIC value.

Table 3. List of eight SSR markers.

SSR marker	Repeat motif	Forward primer	Reverse primer
RM140	(CT)12	TGCCTCTTCCCTGGCTCCCCTG	GGCATGCCGAATGAAATGCATG
RM562	(AAG)13	CACAACCCACAAACAGCAAG	CTTCCCCCAAAGTTTTAGCC
RM1287	(AG)17	GTGAAGAAAGCATGGTAAATG	CTCAGCTTGCTTGTGGTTAG
RM7075	(ACAT)13	TATGGACTGGAGCAAACCTC	GGCACAGCACCAATGTCTC
RM10720	(TA)34	GCAAACGTCTACGTGAGAAACAAGC	GCATGTGGTGCCTTAACATTTGG
RM10825	(AAG)10	GGACACAAGTCCATGATCCTATCC	GTTTCCTTTCCATCCTTGTTGC
RM10843	(TC)10	CACCTCTTCTGCCTCCTATCATGC	GTTTCTTCGCGAAATCGTGTGG
RM10852	(ATAG)5	GAATTTCTAGGCCATGAGAGC	AACGGAGGGAGTATATGTTAGCC

Table 4. ANOVA Table shows the performance of the traits under salinity stress.

Variable	Mean	Maximum	Minimum	DF	Sum of squares	Mean square	F Value	Pr>F
Shoot length	35.00	52.17	13.00	154	12067.98	78.36	7.94	<.0001**
Root length	12.64	39.27	3.60	154	2555.33	16.59	1.85	0.0007**
Biomass	0.10	0.39	0.02	154	0.50	0.00	1.44	0.0277*
Percentage of survival	82.28	100	0	154	160690.32	1043.44	3.87	<.0001**
Leaf injury score	3.48	9	1	154	1927.35	12.52	13.77	<.0001**

** Highly significant at 0.01; * Significant at 0.05

Table 5. Analysis of variance of the traits among the rice accessions.

Sum of squares	DF	Shoot length	Root length	Biomass	Percentage of surviving seedling	Leaf injury score
Model	154	12067.98**	2555.33**	0.50**	160690.31**	1927.35**
Error	93	917.41	835.82	0.21	25098.63	84.50
Variety	30	5891.09**	708.98**	0.21**	28842.67**	161.35**
Replications	3	82.93*	28.08	0.02	12999.59**	38.21**
Environments	1	4860.42**	725.25**	0.01*	77906.34**	1520.15**

** Highly significant at 0.01; * Significant at 0.05

Table 6. The mean of shoot length, root length, and biomass between two environments of rice accessions.

Variety name	Shoot length (cm)			Root length (cm)			Biomass		
	Control condition	Saline condition	Reduction (%)	Control condition	Saline condition	Reduction (%)	Control condition	Saline condition	Reduction (%)
Pokkali I	47.31	37.92	19.85	14.34	11.47	20.01	0.18	0.16	11.11
Pokkali II	48.46	39.39	18.72	14.75	12.98	12.00	0.19	0.17	10.53
Lui Uoi	47.63	38.89	18.35	12.02	10.47	12.90	0.12	0.10	16.67
Boro Shunga	45.72	36.47	20.23	15.80	9.72	38.48	0.12	0.08	33.33
Khao Tah Haeng	44.31	35.45	19.99	12.60	11.58	8.10	0.17	0.15	11.76
Deshi Boro	43.94	34.58	21.30	15.99	9.40	41.21	0.15	0.05	66.67
Karekagga 78	43.85	32.42	26.07	16.55	11.20	32.33	0.09	0.07	22.22
Gion Chem 351	43.60	29.24	32.94	15.99	13.14	17.82	0.14	0.10	28.57
Ketian Gadjih	43.22	34.91	19.23	14.22	9.18	35.44	0.09	0.07	22.22
Naria Bachi	42.49	33.34	21.53	14.29	8.36	41.50	0.10	0.06	40.00
Kaedinga A	41.79	33.09	20.82	14.38	9.88	31.29	0.10	0.07	30.00
Tetep	41.59	31.80	23.54	15.70	13.96	11.08	0.16	0.11	31.25
Kao Saard 108	41.12	31.67	22.98	15.28	11.49	24.80	0.15	0.12	20.00
Subang Intan	40.98	30.04	26.70	15.18	11.51	24.18	0.09	0.06	33.33
Benong 130	40.59	33.57	17.29	19.15	15.03	21.51	0.10	0.08	20.00
Pulut Laut	40.51	32.56	19.62	15.20	12.14	20.13	0.10	0.07	30.00
Pin Khao 2051	40.32	30.55	24.23	14.32	12.71	11.24	0.12	0.09	25.00
Bang Pra 2169	40.29	28.55	29.14	13.59	12.40	8.76	0.11	0.07	36.36
Rojolele	39.52	31.39	20.57	14.48	13.33	7.94	0.12	0.08	33.33
Lakatan Pasir	37.35	32.70	12.45	14.01	9.58	31.62	0.10	0.06	40.00
Kataktara	36.48	28.40	22.15	14.60	7.75	46.92	0.09	0.05	41.67
Palawan	35.72	29.35	17.83	17.20	13.06	24.07	0.10	0.08	20.00
Kinandang Patong	35.52	26.59	25.14	13.23	12.04	8.99	0.10	0.08	20.00
Utri Merah	35.32	27.80	21.29	11.33	9.21	18.71	0.08	0.06	25.00
Sinandomeng	34.87	29.03	16.75	13.62	10.53	22.69	0.19	0.17	10.53
Pankhari 203	34.19	27.53	19.48	13.03	8.95	31.31	0.09	0.06	33.33
Bicol	33.62	28.82	14.28	15.30	11.73	23.33	0.17	0.15	11.76
Ratnagire	32.66	25.65	21.46	15.11	6.28	58.44	0.09	0.05	44.44
Nep Trung Vit	31.04	22.33	28.06	13.87	8.68	37.42	0.11	0.07	36.36
IR64	30.96	18.05	41.70	11.23	6.23	44.52	0.18	0.07	61.11
Jayanti	27.13	19.83	26.91	12.86	7.30	43.23	0.07	0.05	28.57

Table 7. Component matrix with five principal components of rice accessions.

Traits	Factor				
	1	2	3	4	5
Shoot length	0.30488	0.65521	-0.65276	-0.10969	0.19907
Root length	0.32504	0.58077	0.73639	-0.10867	-0.054551
Biomass	0.52673	-0.10923	-0.025291	0.84224	-0.061464
Percentage of surviving seedling	0.49993	-0.3976	0.099069	-0.30853	0.69784
Leaf injury score	-0.52347	0.25178	0.14554	0.41425	0.68311
Eigenvalue	2.96824	1.09433	0.579205	0.242438	0.115782
% Variance	59.365	21.887	11.584	4.8488	2.3156
Eig 97.5%	66.523	28.775	14.06	6.1338	2.5635

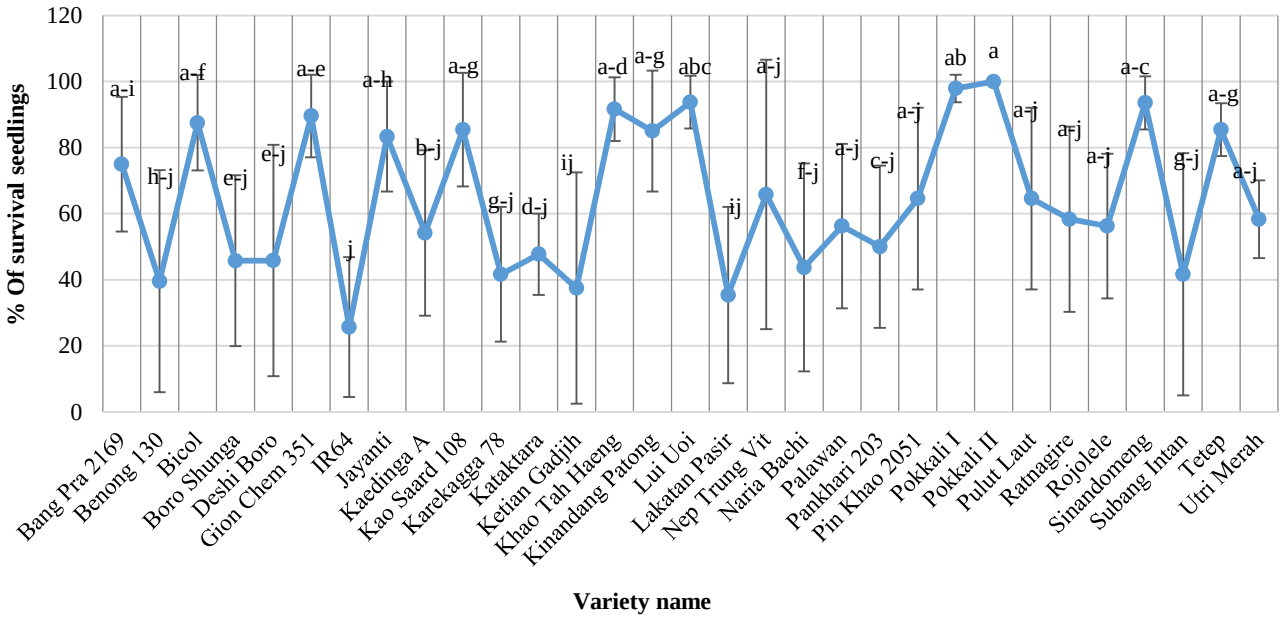
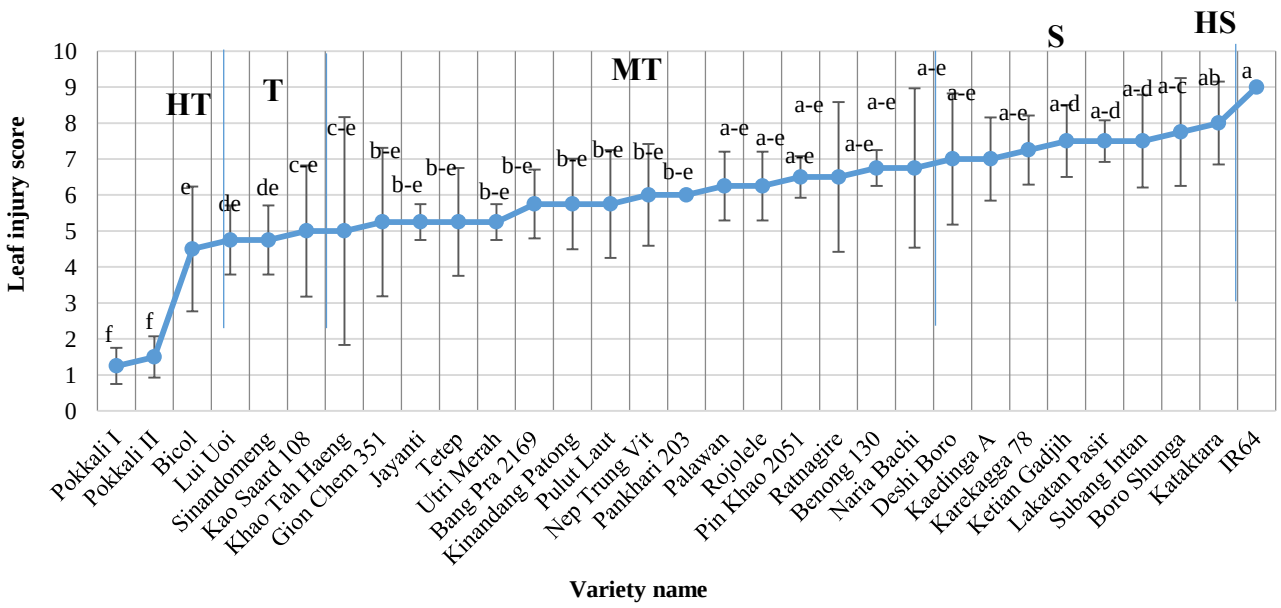


Fig. 1. Percentage of surviving seedlings with standard error bars under salinity condition.



HT = Highly tolerant, T = Tolerant, MT = Moderately tolerant, S = Susceptible, HS = Highly susceptible

Fig. 2. Leaf injury score of rice accessions with standard error bars under salinity condition.

Table 8. Major allele frequency, genotype number, allele number, gene diversity, heterozygosity and Polymorphic Information Content (PIC) using eight SSR markers.

SSR marker	Major Allele frequency	Genotype no.	Sample size	No. of observation	Allele no.	Availability	Gene diversity	Heterozygosity	PIC
RM140	0.414	7	90	87	7	0.967	0.713	0.000	0.670
RM562	0.333	11	90	90	12	1.000	0.809	0.033	0.789
RM1287	0.300	12	90	90	13	1.000	0.858	0.033	0.847
RM7075	0.300	9	90	90	9	1.000	0.811	0.000	0.787
RM10720	0.138	15	90	87	15	0.967	0.913	0.000	0.907
RM10825	0.467	5	90	90	5	1.000	0.669	0.000	0.613
RM10843	0.267	8	90	90	7	1.000	0.801	0.033	0.771
RM10852	0.433	3	90	90	3	1.000	0.624	0.000	0.545
Mean	0.331	8.750	90.000	89.250	8.875	0.992	0.775	0.013	0.741

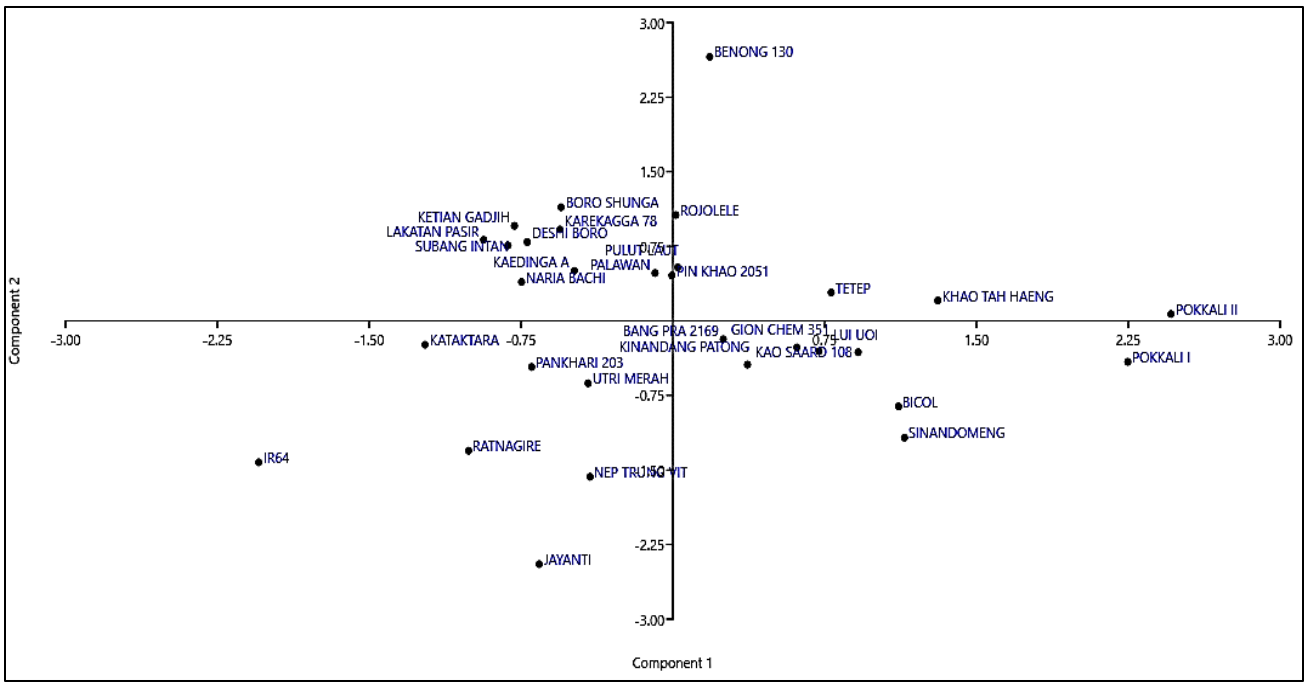


Fig. 3. Principal component analysis (PCA) of rice accessions based on two principal components scores, PCA1 vs PC2.

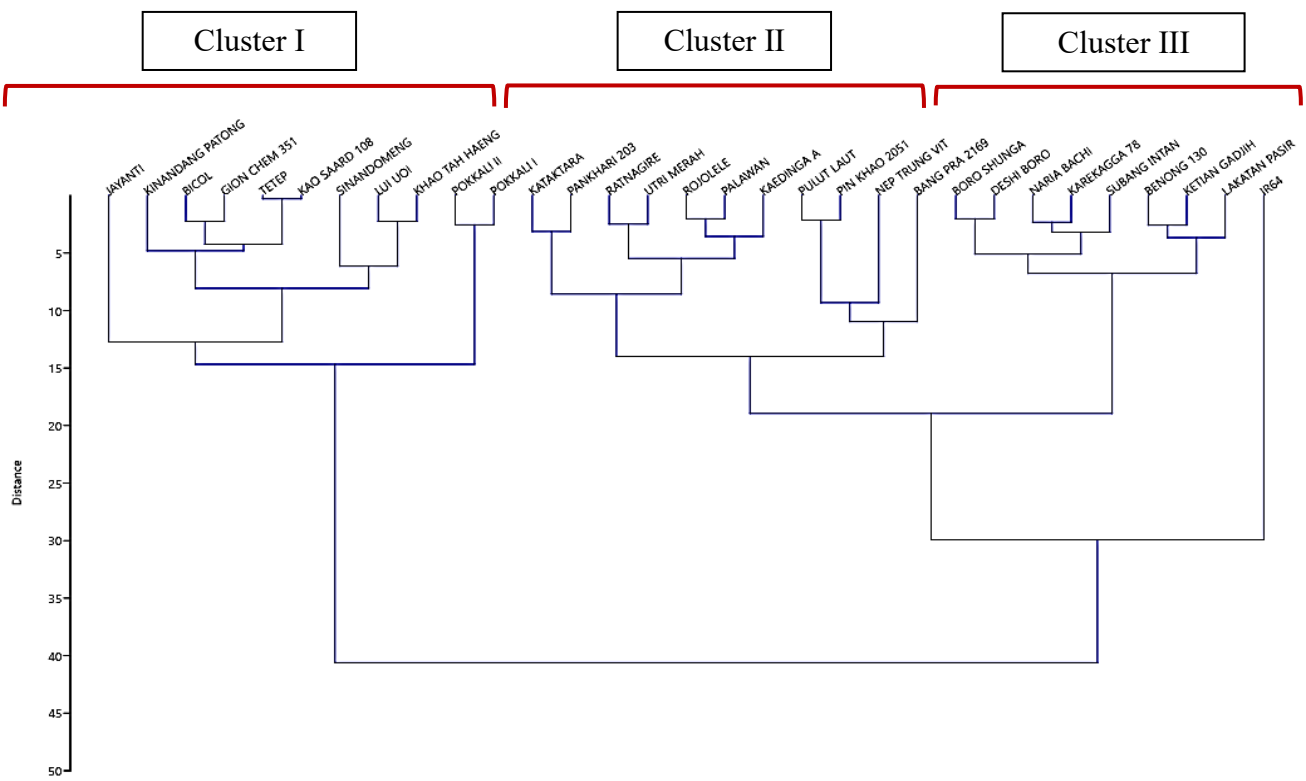


Fig. 4. Dendrogram based on seedlings growth performance under salinity condition.

Cluster analysis of selected rice accessions: Two dendrograms were constructed based on seedlings growth performance under salinity condition (Fig. 4) and genotypic traits using eight SSR markers for salinity tolerance (Fig. 5). Based on seedling growth performance, the 28 rice accessions were grouped into three main clusters. Nine rice accessions were grouped into sub-cluster along with salinity tolerance control variety, Pokkali in Cluster I. These varieties were Jayanti, Kinandang Patong, Bicol, Gion Chem 351, Tetep, Kao Saard 108, Sinandomeng, Lui Oui, and Khao Tah Haeng. Among the varieties mentioned, three varieties namely Bicol, Sinandomeng, and Lui Oui were found to be tolerant to salinity, while others were moderately tolerant to salinity.

Whereas, based on the genotypic traits, rice accessions were clearly grouped into four main clusters. Ten rice accessions were grouped into sub-cluster along with salinity tolerance control variety, Pokkali in Cluster III. The

sub-cluster consisted of Lui Oui, Sinandomeng, Khao Tah Haeng, Kao Saard 108, Ratnagire, Utri Merah, Rojolele, Nep Trung Vit, Karekangga 78, and Ketian Gadjih. Most of these varieties were scored as moderately tolerant to salinity stress, only two varieties namely Karekagga 78 and Ketian Gadjih were scored as susceptible varieties.

Interestingly, Sinandomeng, Lui Oui, Khao Tah Haeng and Kao Saard 108 were found to have been grouped into the same cluster with tolerance control variety, Pokkali in both dendrograms. This revealed that the rice accessions had similar seedling growth performance under salinity condition and share similar salinity tolerance genes with salinity-tolerant control varieties in this study. Meanwhile, for Bicol, which demonstrated great growth performance under salinity stress but falls into the same sub-cluster as the susceptible salinity control variety, IR64. As a result, there is a need to discover other Saltol genes in this variety.

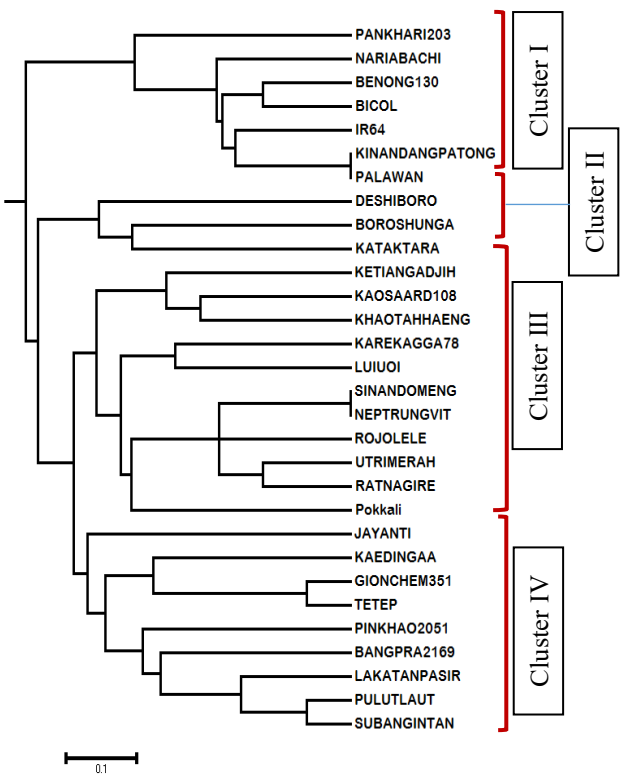


Fig. 5. Dendrogram based on eight SSR markers linked to salinity tolerance.

Discussion

Salinity can cause severe effects that result in decreased growth of rice plants by reducing shoot length, root length, and plant biomass (Ali *et al*., 2014). Similarly in this study, the rice accessions showed a reduction for all traits under salinity condition, however, some of the rice accessions showed a minimal reduction. Based on shoot length, Lui Oui, Boro Shunga, and Khao Tah Haeng showed less reduction and performed similarly to salinity tolerance control varieties. Under salinity condition, the most harmful effect of salt stress in rice is due to accumulation of Sodium (Na^+) and Chloride (Cl^-) in plant tissue and soil around the roots (Nishimura *et al*., 2011) The addition of Na^+ and Cl^- causes serious damage to the chlorophyll contents in rice leaves (Munns *et al*., 2006), as reported by Cha-umi *et al*., (2009), the content of chlorophyll and carotenoids in rice leaves decreased significantly under salinity conditions. This situation leads to a decrease in photosynthetic efficiency by the complex of photosystem II (PSII) (Sajid *et al*., 2017). A decrease in the rate of photosynthesis, along with an increase in the rate of respiration can triggers a lack of assimilation in the developing organs, thus slowing the growth rate and ultimately contributing to plant death (El-Hendawy *et al*., 2005). Approximately 300% of leaf mortality in salt stress have been reported after one week of exposure (Sajid *et al*., 2017). On the other hand, rice accessions with superior performance under salinity condition is normally due to its ability to maintain the rate of leaf appearance, photosynthesis and transpiration efficiency (Harris *et al*., 2010). Thus, higher photosynthesis rate (Chen *et al*. 2013) and integration with the satisfactory transpiration rate commonly observed in genotypes with less growth reduction are indicators as superior traits in tolerant genotypes (Shereen *et al*., 2016). Meanwhile, Mansuri *et al*., (2012) discovered that genotypes with the maximum plant height and the minimum height reduction were more tolerant to salinity than other genotypes.

Root length is also strongly influenced by salt stress. Sensitive genotypes commonly showed highest shoot reduction (Lang *et al*., 2017) and root restriction at higher salt concentrations (Evers *et al*., 1997). Many studies have reported a significant reduction in root length and number of roots per plant under salinity stress (Jamil *et al*., 2006; Jiang *et al*., 2010). Rice root reduction occurs mainly due to disruption of cell division and cell elongation (Munns 2002). In this study, three rice accessions that showed the lowest reduction in root length under salinity condition were Khao Tah Haeng, Rojolele, and Kinandang Patong. These varieties were ranked as moderately tolerant genotypes based on leaf injury score. In contrast, tolerance control varieties (Pokkali I and Pokkali II) showed a greater decrease in root length. This finding is consistent with Safitri *et al*., (2017) as they found that moderate tolerance genotype showed the lowest reduction in root length than the highly sensitive genotype and the tolerant genotype. Whereas, other study has found that reduced root and enhanced shoot growth are major factors that may contribute to salinity tolerance in salinity tolerant rice cultivars by limiting harmful ion flux to the shoot and so delaying the beginning of the tolerance threshold (Pattanagul & Thitisaksakul, 2008). In addition, Kakar *et al*., (2019) stated that increased root biomass is essential for tolerant genotypes to maintain vigorous shoot growth.

The results of this study found that Lui Oui, Sinandomeng, Bicol, and Khao Tah Haeng showed higher biomass production with lower shoot length reduction under salinity stress. According to Peng *et al*., (1999), increasing plant height would result in greater biomass production. Similarly, Bhowmik *et al*., (2009) have reported that the salt tolerance genotypes with lower salt tolerance scores had higher plant height and total dry matter. Previous studies have also found highly significant and positive correlations between biomass and seedling height in salinity conditions (Bhowmik *et al*., 2009; Ali *et al*., 2014). As a result, this finding agrees with Ali *et al*., (2014), who suggested that biomass and shoot parameters could be better descriptors for identifying salinity tolerance genotypes.

Rice genotypes having lowest salt injury scores were regarded to be salinity tolerant (Abu Sayed *et al*., 2018). The salinity level can be easily classified using the modified SES scoring system at seedling stage by observing the indications of leaf damage. Three rice accessions were scored as tolerant namely Bicol, Lui Oui, and Sinandomeng, whereas 17 rice accessions were scored as moderately tolerant to salinity stress. The tolerant and moderately tolerant rice accessions developed more vigorously under salinity condition, with new leaf development and less brown and whitish leaf tips. This is because resistant genotypes are able to develop faster as they get past the salt-sensitive stage, allowing them to gradually adapt to severe surroundings (Lang *et al*., 2017). Whereas, visualization symptoms for sensitive genotypes include leaf rolling, leaf blade browning and necrosis, leaf tip whitish, stunted growth, complete cessation and seedling death (Bhowmik *et al*., 2009).

Principal component analysis (PCA) was used to identify key component traits that contribute to salinity tolerance in germplasm and environments (Negrão *et al*., 2017). In this study, PCA analysis revealed that biomass and shoot length showed the highest influenced in factor one and two, indicating the traits are the most important traits contributing to salinity tolerance. According to Kakar *et al*., (2019) shoot parameters and biomass were also found to be key traits for indices of salinity tolerance. Ali *et al*., (2014) have also discovered a similar finding. As a result, PCA analysis successfully classified the rice accessions in agreement with the results obtained from the salt injury score, with the tolerant rice accessions along with salinity tolerant control varieties located on the right side of the biplot and the susceptible rice accessions together with IR64 located on the other side of the biplot.

Molecular study for salinity tolerance were performed using eight SSR markers. The average polymorphic information content (PIC) value in this study was 0.741, with values ranging from 0.545 to 0.907. Similarly, study by Arzani *et al*., (2008) showed that PIC value ranged from 0.28 to 0.88 with an average of 0.73, while Ali *et al*., (2014) observed PIC value varied from 0.55 to 0.84. A high PIC value (more than 0.50) indicated that the markers have a higher possibility to detect more alleles among genotypes and exhibited high degree of polymorphism (Anyomi *et al*., 2018). Moreover, RM10720 had the highest PIC value (0.907) and gene diversity (0.913) indicating that it was the best marker for analysing salinity tolerance genetic diversity. Simple sequence repeat (SSR) or microsatellites markers have been widely used in rice genetic studies because they are relatively abundant, co-dominant, highly reproducible, and interspersed across the genome (Anyomi *et al*., 2018), and they can describe the structure, degree of genetic variability, and reveal many traits differences at the DNA level (Prabakaran *et al*., 2010).

Two dendrograms were generated based on seedlings growth performance under salinity stress and eight SSR markers for salinity tolerance. Based on seedlings growth performance under salinity stress, Jayanti, Kinandang Patong, Bicol, Gion Chem 351, Tetep, Kao Saard 108, Sinandomeng, Lui Oui, and Khao Tah Haeng were grouped with Pokkali. Meanwhile, ten rice accessions, namely Lui Oui, Sinandomeng, Khao Tah Haeng, Kao Saard 108, Ratnagire, Utri Merah, Rojolele, Nep Trung Vit, Karekangga 78, and Ketian Gadjih were grouped with Pokkali based on genotypic data. By comparing the two dendrograms, Lui Oui, Sinandomeng, Khao Tah Haeng, and Kao Saard 108 were clustered with Pokkali in both dendrograms. According to Anyomi *et al*., (2018), genotypes that clustered closely to check varieties often show similarities with them. Similarly in this study, Lui Oui, Sinandomeng, and Khao Tah Haeng showed vigorous growth performance under salinity stress similar to Pokkali, with less shoot reduction, higher biomass production, and lower leaf injury score. In addition, Lui Oui and Sinandomeng were identified as tolerant varieties, whereas Khao Tah Haeng and Kao Saard 108 were performed as moderate tolerant varieties based on leaf injury score. Therefore, these varieties are potential novel donor and can be utilized in breeding for development the salinity tolerant rice cultivars. Meanwhile, discovery for new salinity tolerant genes in Bicol should be emphasized in future study.

Conclusion

This study successfully identified the potential rice accessions for salinity tolerant based on seedlings growth performance and molecular analysis using eight SSR markers for salinity tolerance. In this study, PCA analysis revealed that biomass and shoot length are the most important descriptors for determining salinity tolerance, and the tolerant and susceptible rice accessions were successfully distinguished. Four rice accessions viz. Lui Oui, Sinandomeng, Khao Tah Haeng, and Kao Saard 108 showed great growth seedling performance under salinity conditions, similar to Pokkali, in terms of higher shoot length; less reduction in shoot length, root length, and biomass; lower leaf injury score, and a higher percentage of surviving seedlings under salinity stress. Assessment using eight SSR markers showed that these varieties belong to the tolerant group because they are included in the sub-grouped along with the salinity tolerance rice varieties. Meanwhile, the discovery of new salinity tolerant genes for Bicol that also demonstrated vigorous growth performance under salinity stress should be emphasized. The SSR markers used in this study showed high polymorphism in the rice accessions, thus these markers can be proficiently used in screening and identification of salinity tolerance genotypes.

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