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Research Article

Morphological and Molecular Screening of Rice (*Oryza sativa L*) for Salinity Tolerance at Seedling Stage

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Abstract

Saline agriculture encourages the use of salt-tolerant rice to maintain rice productivity, which is one of the solutions to the saline soil problem. In this study, the screening of salinity tolerance at the seedling stage of rice genotypes was carried out on the basis of morphological and molecular characterization. 21 rice genotypes along with the tolerant check (Pokkali), were screened using Peter nutrient solution with salinized (6 dSm^{-1} NaCl), and non-salinized (control) conditions. For phenotypic observation, four parameters such as shoot length, root length, shoot fresh weight and root fresh weight were used for salinized and non-salinized conditions in hydroponic system. The International Rice Research Institute's modified standard evaluation score (SES) was used to assess the visual symptoms of salt toxicity. A wide range of salt injury was observed in response to 10 days of salt stress, resulting in a range of salt tolerance scores from 3 to 9. Based on the SES scores, percent reduction and stress tolerance index at 6 dSm^{-1}, 8 genotypes were screened as tolerant, 10 genotypes as moderately tolerant and 3 genotypes as susceptible. Twelve SSR markers linked to salt-tolerance QTL were used to evaluate the salinity of genotypes. Across all loci, a total of 31 alleles were observed. Four markers (RM336, RM7075, RM10793 and RM3412b), could differentiate genotypes based on their PIC value and MI index. Only the PSBK rice genotype was genetically related to Pokkali, according to cluster analysis and haplotype analysis. After phenotypic and molecular assessment, 6 genotypes were identified as tolerant, 11 genotypes as moderately tolerant and 5 genotypes as susceptible.

INTRODUCTION

Rice is a tropical diploid (2n=2x=24), glycophyte that is currently the model crop for cereals [1]. Asia grows almost 90 percent of the world's rice (roughly 640 million tons), with 85 percent destined for human consumption [2]. Various abiotic stresses have a significant impact on rice productivity, and salinity is the second most widespread soil problem in rice growing countries around the world and it is seen as a serious threat to increased rice production globally [3].

A soil is considered saline if the electrical conductivity (EC) of its saturated extract is above 4 dSm^{-1} [4]. Under salt stress, various biochemical and physiological processes in plant cells are altered, resulting in growth inhibition and significant yield reduction [5]. Rice plants were indeed impacted by excessive soil salinity in two ways: osmotic stress and ion toxicity [6]. Firstly, salinity causes water and nutrient deficiencies in the root zone resulting in plant metabolic alterations [7]. Secondly, excess ion accumulation in plant cells causes a variety of metabolic changes in rice including excessive reactive oxygen species (ROS) accumulation and cell membrane damage [8]. Salt tolerant plants evolve several mechanisms for salt tolerance including the modification of membrane characteristics involved in ion absorption, translocation, compartmentation and excretion of salt [9].

Previous studies have suggested that rice is vulnerable to salt stress during seedling and reproductive stages [10]. Screening under controlled conditions has the advantage of reducing environmental impact and the hydroponic system is exempt from soil related stress factors. Because of the enormous impact of the environment and the narrow-sense heredity of salt tolerance, traditional methods of plant selection for salt tolerance are difficult [3]. Traditional methods inhibit the development of a screening method, whereas the hydroponic system is accurate, quick and reliable. Screening for salt tolerant rice genotypes based on phenotypic performance alone is not reliable and will delay progress in breeding.

Thus, searching for DNA markers that are strongly connected to salt tolerance features has become a significant goal in most breeding programs, and it is expected that molecular markers will allow for the rapid and cost-effective screening of large populations [7]. Simple sequence repeats (SSRs) are the genetic markers that have been extensively used in rice diversity studies [11,12].

Therefore, the current study was conducted to document salinity tolerance at the seedling stage of some populations of rice using IRRI screening techniques. The objective of this study is to screen 22 rice genotypes for salinity response and to assess SSR markers for the identification of salt tolerant genotypes at the seedling stage.

MATERIALS AND METHODS

Rice Seeds Collection and Experimental Design

The plant material consisted of 21 rice genotypes, including seeds of 7 rice genotypes kindly provided by Seed Bank Section, Department of Agricultural Research (DAR); Nay Pyi Taw, 6 rice genotypes from Department of Agricultural Research (DAR); Kyaukse and seeds of 8 different rice varieties obtained from Plant and Agricultural Biotechnology Research Department, Department of Biotechnology Research (DBR); Kyaukse for assessment of their salt tolerance at seedling stage. In this study, the salt tolerant Indian variety "Pokkali" was employed as a check cultivar as shown in (Table 1). This experiment was carried out by following Randomized Complete Block Design (RCBD), in two treatments (EC 0 $dSm^{-1}$and EC 6 dSm^{-1}NaCl), with three replications (each replication had six rice seedlings).

Surface Sterilization and Seedling Cultivation

The selected seeds were those uniform in size. All the seeds were soaked in water overnight. Soaked seeds were disinfected with fungicide (10mL/L), for one hour and then thoroughly washed with distilled water. Under aseptic condition, a final treatment with 70% ethanol was given for five minutes after which the seeds were thoroughly washed several times with distilled water. Sterilized seeds for each cultivar were placed in each petri dish on moist filter papers and incubated at 30˚C for 48 hours in dark condition to provide favourable condition for germination.

Pregerminated seeds including the tolerant check were sown in each hole of Styrofoam seedling float and placed in a 8 L plastic tray filled with distilled water. After 4 days, the distilled water was replaced with Peter nutrient solution to allow sufficient growth and maintained for 10 days. After three days, the initial salinity (EC 4dSm^{-1}), was increased to EC 6 dSm^{-1} by adding NaCl to the nutrient solution and maintained for the next seven days. The solution was renewed every eight days and the pH was adjusted to 5.0 as well as the EC with 6dSm^{-1} synchronizing with the Peter solution. The volume of the Peter solution was adjusted to the level of touching the seedling float at two days interval and this test is conducted in ordinary green house.

The EC of the saline solution was measured by Lovibond (Senso Direct), EC-meter and the pH of the nutrient solution was adjusted to 5.0 throughout the growth period by Mettler Toledo (Switzerland) pH meter.

Evaluation of Salt Tolerance Seedling Scores and Data Analysis

The evaluation was done using modified Standard Evaluation System (SES) in rating the visual salt injury at seedling stage following the method proposed by Gregoria et al. [3] (Table 2). Data regarding different growth parameters such as shoot length, root length, shoot fresh weight and root fresh weight were recorded. Changes in shoot and root length on morphological response of seedlings due to saline exposure were collected on the 10th day of salt treatment in 26 days old seedlings grown in non-salinized (0 dSm^{-1}), and salinized (6 dSm^{-1}) conditions, while shoot and root fresh weight were measured after 10 days of salt stress using an analytical weighting balance. The experimental data were subjected to analysis of variance and Duncan's Multiple Range Test (DMRT) for comparing population means. Mean values were compared by one-way ANOVA using SPSS V16 software (IBM Corporation SPSS, North America).

The stress tolerance index (STI), is calculated using the following formula [13]:

$$STI = Yp \times Ys/\bar{Y}p2$$

where Yp is the character response under normal environment (0 dSm-1), Ys is the character response in saline environment (6 dSm-1), and $\bar{Y}p$ is the average genotype response to characters in normal environment.

Percent reduction (%R) of plant growth parameters such as shoot length percent reduction (%RSL), root length percent reduction (%RRL), shoot fresh weight percent reduction (%RSFW), and root fresh weight percent reduction (%RRFW) were calculated from the control by the following formula [14].

Table 1: List of rice genotypes used in this study.

Sr. No.	Genotype	Accession No.	Sr. No.	Genotype	Accession No.
1	Pokkali (Check Variety)	Pokkali	12	Shwe Thwe Yin	STY
2	Sitt Pwar	SP	13	Shwe Pyi Mhwe	SPM
3	Mee Kauk	MK	14	Lone Thwe Mhwe	LTM
4	Kar Le Latt Yone	KLLY	15	Thee Thet Yin	THY
5	YaeNatt Ngar	YNN	16	Sin Akari-3	SAKR-3
6	ManawThukha	MNTK	17	Pyi Myanmar Sein	PMYMS
7	Yadanar Win	YDNW	18	Yae Anae Lo	YANL
8	NgaChate	NC	19	Pyi Taw Yin	PTY
9	Paw San Bay Kyar	PSBK	20	Yadanar Toe	YDNT
10	Thiri Thuka	TRTK	21	Yae Sin Lone Thwe	YSLT
11	SalT- Sinn Thwe Latt	ST-STL	22	Shwe Ma Naw	SMN

Table 2: Scoring criteria for salt tolerance [3].

Score	Description	Remark
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, only the tip of few leaves whitish and rolled	Tolerant
5	Growth severely retarded, most leaves rolled, the two youngest leaves were still elongating	Moderately tolerant
7	Complete cessation of growth, all lower leaves dried out, the two youngest leaves started to wilt	Susceptible
9	The whole plant dried out and dead	Highly susceptible

Table 3: Information on selected SSR markers for 22 rice genotypes.

Sr. No.	Primers	Chromosome	Sequences	Annealing Temperature (°C)	Expected Size (bp)
1	RM490	1	F: ATCTGCACACTGCAAACACC	55	110
			R: AGCAAGCAGTGCTTTCAGAG		
2	RM493	1	F: TAGCTCCAACAGGATCGACC	55	211
			R: GTACGTAAACGCGGAAGGTG		
3	RM7075	1	F: GCGTTGCAGCGGAATTTGTAGG	55	155
			R: CCCTGCTTCTCTCGTGCAGTCG		
4	RM562	1	F: CACAACCCACAAACAGCAAG	55	243
			R: CTTCCCCCAAAGTTTTAGCC		
5	SalT1	1	F: GATGGTATTCATCGGCTACG	55	159
			R: AGTCCAAGAATGTCGTTTCG		
6	RM8094	1	F: AAGTTTGTACACATCGTATACA	55	209
			R: CGCGACCAGTACTACTACTA		
7	RM10694	1	F: TTTCCCTGGTTTCAAGCTTACG	55	194
			R: TACGGTACCTTGATGGTAGAAAGG		
8	RM3412b	1	F: AAAGCAGGTTTTCCTCCTCC	55	110
			R: CCCATGTGCAATGTGTCTTC		
9	RM10793	1	F: GACTTGCCAACTCCTTCAATTCG	55	124
			R: TCGTCGAGTAGCTTCCCTCTCTACC		
10	AP3206f	3	F: GGAGGAGGAGAGGAAGAAG	55	167
			R: GCAAGAATTAATCCATGTGAAAGA		
11	RM253	6	F: TCCTTCAAGAGTGCAAAACC	60	141
			R: GCATTGTCATGTCGAAGCC		
12	RM336	7	F: CTTACAGAGAAACGGCATCG	55	154
			R: GCTGGTTTGTTTCAGGTTCG		

$$Percent\ reduction\ (\%) = \frac{value\ of\ control\ plant - value\ of\ stress\ plant}{value\ of\ control\ plant} \times 100$$

DNA Extraction and SSR Genotyping

Genomic DNA isolation was extracted from leaf tissue of 14 days old seedling using CTAB method with a few modifications [15]. Its quantity was estimated spectrophotometrically using Nanodrop (ND 1000, Thermo Scientific, Madison USA) and quality was checked on 0.8% agarose gel electrophoresis stained with ethidium bromide in 0.5X TBE buffer. The resolved bands were documented using the Alpha Imager system (Fisher Scientific).

A total of 12 primers were chosen from those previously reported by several researchers for salt tolerance (Table 3). Information about all the markers was obtained from the Gramene database (http://www. Gramene.org).

The PCR reactions were carried out in a Proflex Thermal Cycler (Applied Biosystems, USA) with the total reaction volume of 10 µl, containing 5µl of 2^x Taq DNA Polymerase Master Mix Kit (VWR, Denmark), 0.5 µl forward primer, 0.5 µl reverse primer, 3.5 µl ddH_2O and 0.5 µl of each template DNA (200 ng/µl). PCR conditions were initial denaturation at 95˚C for 4 min, followed by 30 cycles of denaturation at 95˚C for 30 sec, annealing at appropriate temperature (55˚C and 60˚C depending on the primer) for 30 sec and extension at 72˚C for 1 min and final

extension at 72˚C for 5 min. The amplification products along with DNA ladder were mixed with loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol and 40% sucrose) and resolved on 3% agarose gel in 0.5^{x} TBE buffer at a constant voltage of 120 V for 30 min, and detected by ethidium bromide staining. The size of the PCR products was compared to the molecular size standard of 100 bp DNA ladder.

Scoring of Amplified Fragments

The well-separated and consistently reproducible, amplified DNA bands were scored in a binary matrix based on the presence (1) or absence (0) of the particular band across the 22 rice genotypes keeping Pokkali as a tolerant genotype. For a set of accessions, genetic diversity parameters such as the number of alleles per locus, allele frequency, heterozygosity and PIC values were estimated using the Power Marker version 3.25 software [16]. Genetic relatedness among the genotypes was calculated with the Unweighted Pair Group Method Arithmetic Average (UPGMA) cluster analysis by using NTSYSpc version 2.0 software [17].

Marker index (MI) is calculated using the following formulation:

MI = PIC x EMR

where PIC is the value of the polymorphism information content and EMR is the effective multiplex ratio [18]. Haplotype diversity was studied according to Wilson and Gregorio [19].

RESULTS AND DISCUSSION

The ability of seedlings to grow in salinized culture solution was the basic principle in screening for salinity tolerance in rice at the seedling stage [3]. In the present study, different morphological and molecular characters were assessed in 22 rice genotypes at the seedling stage to evaluate their relative salt tolerance abilities under 6 dSm^{-1}. The salinity intensity 6 dSm^{-1} was chosen because rice is very sensitive to salinity at seedling stage. Its height, root length, emergence of new roots, and dry matter decrease significantly at EC of 5-6 dSm^{-1} [20,21].

Morphological Behaviour of Rice Seedlings in Response to Saline Stress

Each genotype was scored for vigour after 10 days of salinization. Using modified standard evaluation system (SES) of IRRI [3], phenotypic scoring and observation were recorded on the 26th day of crop life span in both non-salinized (control) and salinized (6 dSm-1) conditions. Salt stress symptoms start with reduction in leaf area and the oldest leaves start to roll and die, followed by the next older ones and so on. All screened rice genotypes were divided into three groups, i.e; tolerant (T), moderately tolerant (MT) and sensitive (S) depending on the visual symptoms of leaves under saline condition. For the evaluation of salt treated rice leaves, Pokkali was used as a salt tolerant check variety since it has previously been employed as a check variety in salt-tolerant rice studies [22,23]. Among 22 genotypes, ten were identified as tolerant, seven as moderately

Table 4: Means of different morphological parameters of rice seedlings under control and stress conditions.

Sr. No.	Rice Varieties	Control (0 dSm^{-1})				Treatment (6 dSm^{-1})			
		SL (cm)	RL (cm)	SFW (mg)	RFW (mg)	SL (cm)	RL (cm)	SFW (mg)	RFW (mg)
1	Pokkali	38.07	9.32	705.07	97.2	34.62	8.52	323.32	54.8
2	SP	36.85	9.08	561.1	161.8	28.5	8.12	447.67	81.82
3	MK	31.47	8.58	363.37	115.83	25.33	6.25	202.5	20.63
4	KLLY	38.17	11.58	560.3	201.48	32.25	8.42	474.3	65.85
5	YNN	32.33	8.67	462.75	104.17	21.33	7.25	236.35	36.4
6	MNTK	21.83	9.25	225.45	69.68	16.08	6.67	87.38	20.7
7	YDNW	35.42	8.17	438.93	163.47	28.42	5.67	359.28	37.25
8	NC	31.08	9.67	467.62	177.77	15.58	6.67	79.2	21.37
9	PSBK	40.45	6.68	706.23	95.53	34.2	6.45	312.65	50.47
10	TRTK	30.92	10.33	386.8	121.05	23.83	9.5	304.97	46.45
11	ST-STL	24.58	10.92	297.65	68.17	17.5	8.17	197.08	33.02
12	STY	22.08	10	258.38	78.32	18	8.33	217.62	44.65
13	SPM	24.42	10.42	359.68	128.28	17.58	7.42	205.82	48.52
14	LTM	29	9.75	244.88	70.45	24.5	8.75	191.45	30.75
15	THY	24.5	11.67	309.08	115.53	18.67	6.5	177.5	41.3
16	SAKR-3	26.25	11.67	391.33	134	20.58	9.17	288.17	63.07
17	PMYMS	23.45	11	288.82	80.37	19.85	9.2	283.17	41.15
18	YANL	27.75	11.83	375.87	131.18	21.4	9.77	301.78	78.43
19	PTY	28.37	15.42	827.23	173.37	21.42	11.38	414.45	59.25
20	YDNT	26.58	14.83	468.85	122.6	21.12	12.83	362.3	42.98
21	YSLT	29.08	12.92	490	68.15	19.75	9.33	217.15	10.78
22	SMN	19.08	9.33	164.25	14.97	15.42	6.35	70.03	5.05

Abbreviations: SL: Shoot Length; RL: Root Length; SFW: Shoot Fresh Weight; RFW: Root Fresh Weight.

tolerant and five as sensitive. Ten rice genotypes, i.e. Pokkali, KLLY, PSBK, TRTK, ST-STL, LTM, PMYMS, YANL, PTY, YDNT were identified as salt tolerant (Table 5). This SES scoring distinguishes the susceptible from the tolerant and moderately tolerant genotypes.

ANOVA is the initial basis for determining the character of selection in tolerance screening [24,25]. When grown in hydroponic solution in the absence of salt stress, rice seedling of all genotypes developed normally and displayed 100% survival. Seedling exposed to salt stress for ten days showed a wide range of morphological characters as shown in (Table 4). The mean values of shoot and root length showed the substantial difference between tolerant, moderately tolerant, and sensitive genotypes (Figure 1, Figure 2 and Figure 3). When compared to the other rice varieties, Pokkali shows the highest mean in shoot lengths at EC value of $6dSm^{-1}$. Because of their shorter shoot and root lengths, MK, MNTK, YDNW, NC, and SMN genotypes can be considered salt susceptible. Tolerant and moderately tolerant genotypes mostly maintained their normal growth under saline condition. In compatible with our results, the shoot length of rice, which is susceptible to salinity, decreased after 7 days of salt stress in a study conducted by Liu et al. [26].

The result of shoot and root fresh weight of genotypes was significantly affected under salt stress as shown in (Table 4). Tolerant genotypes, Sitt Pwar (SP), had the highest seedling shoot fresh weight (447.67mg), and root fresh weight (81.82mg), respectively, whereas the lowest seedling shoot fresh weight (81.82mg), and root fresh weight (5.05mg), were found in sensitive genotypes, Shwe Ma Naw (SMN), at $6dSm^{-1}$ NaCl stress. In our results, plant growth and biomass of susceptible genotypes showed higher percent reduction (%R) than tolerant genotypes (Table 5). Similar to this, [27], found that root and shoot length of susceptible rice were reduced more than salt tolerant genotypes. Lower percent reduction of shoot length was recorded in genotypes Pokkali (9.06), PMYMS (15.35), PSBK (15.45), KLLY (15.50), and LTM (15.52) and STY (18.49). On the other hand, higher percent reduction of shoot length was shown by genotypes NC (49.87), YNN (34.02) and YSLT (32.09). The maximum curtailment of root length (44.29-30.61%), was observed in THY, SMN, NC and YDNW whereas PSBK, TRTK, Pokkali, LTM and SP genotypes were found at minimum percent of reduction. The significant reduction in seedling growth induced by salinity may be related to the toxic effects of NaCl and imbalance nutrient uptake. These negative effects of salinity may result in a significant decrease in photosynthesis rate and an

Table 5: Percent Reduction (%R), Stress Tolerance Index (STI) and salt tolerance level of rice genotypes.

Sr. No.	Rice Varieties	Percent Reduction (%R)				Stress Tolerance Index (STI)				SES Score
		SL (cm)	RL (cm)	SFW (mg)	RFW (mg)	SL (cm)	RL (cm)	SFW (mg)	RFW (mg)	
1	Pokkali	9.06	8.59	54.14	43.62	0.91	0.91	0.46	0.56	T
2	SP	22.66	10.64	20.22	49.43	0.77	0.89	0.8	0.51	MT
3	MK	19.49	27.18	44.27	82.19	0.81	0.73	0.56	0.18	MT
4	KLLY	15.5	27.34	15.35	67.32	0.84	0.73	0.85	0.33	T
5	YNN	34.02	16.35	48.92	65.06	0.66	0.84	0.51	0.35	MT
6	MNTK	26.34	27.93	61.24	70.29	0.74	0.72	0.39	0.3	S
7	YDNW	19.76	30.61	18.15	77.21	0.8	0.69	0.82	0.23	MT
8	NC	49.87	31.03	83.06	87.98	0.5	0.69	0.17	0.12	S
9	PSBK	15.45	3.49	55.73	47.17	0.85	0.97	0.44	0.53	T
10	TRTK	22.91	8.06	21.16	61.63	0.77	0.92	0.79	0.38	T
11	ST-STL	28.81	25.19	33.79	51.56	0.71	0.75	0.66	0.48	T
12	STY	18.49	16.67	15.78	42.99	0.82	0.83	0.84	0.57	MT
13	SPM	27.99	28.8	42.78	62.18	0.72	0.71	0.57	0.38	S
14	LTM	15.52	10.26	21.82	56.35	0.84	0.9	0.78	0.44	T
15	THY	23.81	44.29	42.57	64.25	0.76	0.56	0.57	0.36	S
16	SAKR3	21.59	21.43	26.36	52.94	0.78	0.79	0.74	0.47	MT
17	PMYMS	15.35	16.36	1.96	48.8	0.85	0.84	0.98	0.51	T
18	YANL	22.88	17.46	19.71	40.21	0.77	0.83	0.8	0.6	T
19	PTY	24.5	26.16	49.9	65.82	0.75	0.74	0.5	0.34	T
20	YDNT	20.56	13.48	22.73	64.94	0.79	0.87	0.77	0.35	T
21	YSLT	32.09	27.74	55.68	84.18	0.68	0.72	0.44	0.16	MT
22	SMN	19.21	31.96	57.36	66.26	0.81	0.68	0.43	0.34	S

Abbreviations: SL: Shoot Length; RL: Root Length; SFW: Shoot Fresh Weight; RFW: Root Fresh Weight; T: Tolerant; MT: Moderately Tolerant; S: Susceptible; SES: standard evaluation score.

Figure 1 Comparison of rice genotypes grown under salinized: 6 dSm^{-1} and non-salinized conditions after 10 days of salinization.

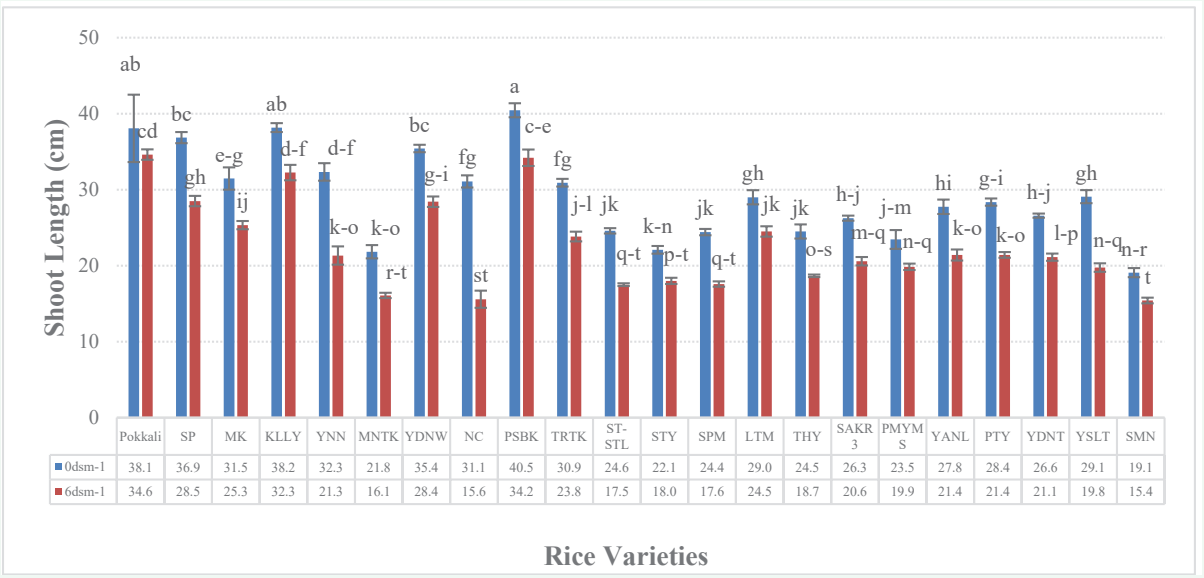

Figure 2 Shoot length (cm) of 22 rice genotypes grown under salinized: 6 dSm-1 and non-salinized conditions after 10 days of salinization. In each bar, values with a common letter are not significantly different at p ≤0.05 by Duncan's Multiple Range Test (DMRT).

increase in respiration rate of seedlings that lead to a shortage of assimilate to the developing organs and may slow down or stop growth entirely [28].

The percent reduction of shoot fresh weight ranged from 1.96 to 83.06. Lower percent reduction of shoot fresh weight was found in genotypes PMYMS, KLLY and STY. In contrast, NC, MNTK and SMN genotypes showed higher percent reduction of shoot fresh weight. Tolerant genotypes showed a significant reduction in the root fresh weight ranging from 40.21% to 67.32%. Reduction percent of sensitive genotypes under salt stress were NC (87.98), MK (82.19) and YSLT (84.18) in the case of root fresh weight. The significant reductions in shoot fresh weight and root fresh weight were mainly observed in most of the rice genotypes under salt condition (6dSm^{-1}). The root system of plants is damaged when it comes into direct contact with saline solution [29,30]. When root parts become seriously damaged, shoot growth is hindered due to the inhibition of vital nutrient uptake through symplastic xylem loading in the root [31].

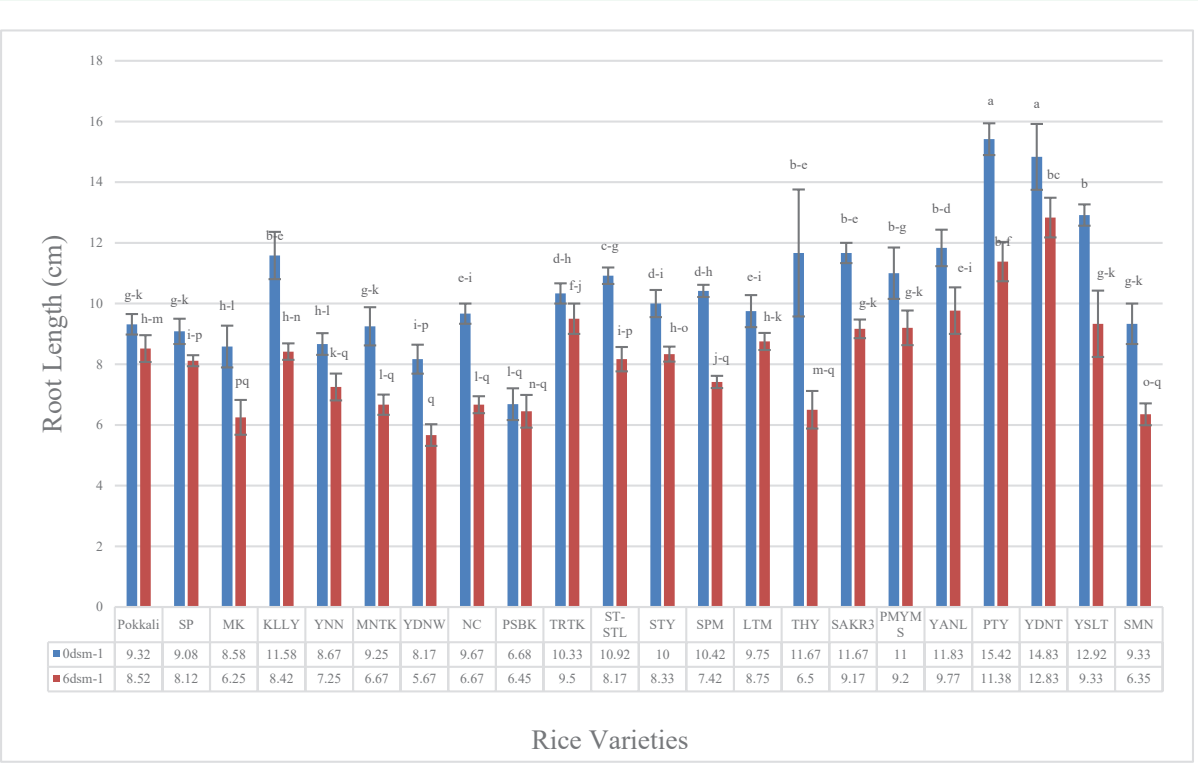

Figure 3 Root length (cm) of 22 rice genotypes grown under salinized: 6 dSm-1 and non-salinized conditions after 10 days of salinization. In each bar, values with a common letter are not significantly different at $p \leq 0.05$ by Duncan's Multiple Range Test (DMRT).

The results of the stress tolerant index (STI) on morphological characters of rice seedlings are shown in (Table 5). STI analysis showed that shoot length, root length, shoot fresh weight and root fresh weight of susceptible genotypes had lower stress tolerant index (STI), values than tolerant genotypes. Variety of Pokkali (0.91, 0.91), showed the maximum stress tolerant index (STI), for shoot and root length, followed by varieties, PSBK (0.85, 0.97), PMYMS (0.85, 0.84), STY (0.82, 0.83) and LTM (0.84,0.90).

Salt sensitive genotypes, NC and MNTK, exhibited the lowest STI value of shoot fresh weight at 0.17 and 0.39, respectively, whereas the highest STI value for shoot fresh weight was displayed by genotypes, namely PMYMS (0.98) and KLLY (0.85). The lowest STI value for root fresh weight was recorded in genotype MK (0.18) and followed by YSLT (0.16) and NC (0.12), whereas tolerant cultivars, namely YANL (0.60), STY (0.57), Pokkali (0.56) and SP (0.51) indicated higher STI value for root fresh weight.

Salt tolerant check "Pokkali" had a better STI value than "PSBK" as a positive control for all plant parameters except root length growth under EC 6dSm^{-1} NaCl. Among the genotypes, Pokkali, SP, KLLY, PSBK, STY, PMYMS and YANL exhibited the least reduction in growth traits as well as the highest STI value under saline condition. Therefore, these genotypes could be identified as salt tolerant, while rest of the genotypes namely MK, YNN, MNTK, YDNW, TRTK, ST-STL, SPM, LTM, SAKR-3, PTY, YDNT and YSLT were preliminary screened as moderately tolerant genotypes under salt-stressed situation. Some genotypes, namely NC, THY, SMN, showed a greater reduction of morphological traits and these were regarded as salt-susceptible genotypes.

At 6 dSm^{-1} salinity stress, eight genotypes were screened as tolerant, ten genotypes as moderately tolerant, and three genotypes as sensitive based on SES scores, percent reduction, and stress tolerance index. None of them was highly susceptible at 6dSm^{-1}.

Molecular Characterization and SSR Polymorphisms

DNA markers are now recognized as a rather convenient and high-quality tool for assessing genetic diversity at the molecular level [19]. Most of the genetic variations against environmental stress are regulated by a large number of genes, each with small effects, that is spread throughout the genome [32].

In this study, twelve SSR markers tightly linked with salt tolerance QTLs present on chromosome numbers 1, 3, 6 and 7 were used for screening 22 genotypes of rice. The information of all SSR markers such as chromosome number, sequences, annealing temperature and expected size (bp), were shown in (Table 3). For each marker, the genotype number, major allele frequency, allele number, genetic diversity, observed heterozygosity, PIC and marker index were obtained for each locus (Table 6). For the studied 22 rice genotypes, a total of 31 alleles were recorded in all SSR markers. The major allele frequencies of each marker were observed to range from 0.5 (RM7075, RM10793) to 0.954 (SalT1). The allele number ranged from 2 (RM490, RM493, RM562, SalT1, RM8094, RM10694 and AP3206f) to 4 (RM336) with an average of 2.583 alleles per locus.

Table 6: Summary statistics for 12 SSR Markers used to screen the selected rice genotypes.

Sr. No.	Marker	MAF	GN	AN	GD	H	PIC	MI
1	RM490	0.7045	3	2	0.4163	0.1364	0.3297	0.659
2	RM493	0.7273	2	2	0.3967	0	0.318	0.636
3	RM7075	0.5	4	4	0.5785	0	0.4914	1.966
4	RM562	0.5909	2	2	0.4835	0	0.3666	0.733
5	SalT1	0.9545	2	2	0.0868	0	0.083	0.166
6	RM8094	0.6818	2	2	0.4339	0	0.3398	0.68
7	RM10694	0.5909	2	2	0.4835	0	0.3666	0.733
8	RM3412b	0.7273	3	3	0.4298	0	0.3855	1.156
9	RM10793	0.5	3	3	0.6157	0	0.5419	1.626
10	RM3206f	0.5455	2	2	0.4959	0	0.3729	0.746
11	RM253	0.8636	3	3	0.2438	0	0.2284	0.685
12	RM336	0.3182	4	4	0.7314	0	0.6809	2.724
Mean		0.6420	2.6667	2.5833	0.4496	0.0114	0.3754	1.042

MAF: Major Allele Frequency; GN: Genotype Number; AN: Allele Number; GD: Gene Diversity; H: Observed Heterozygosity; PIC: Polymorphism Information Content; MI: Marker Index.

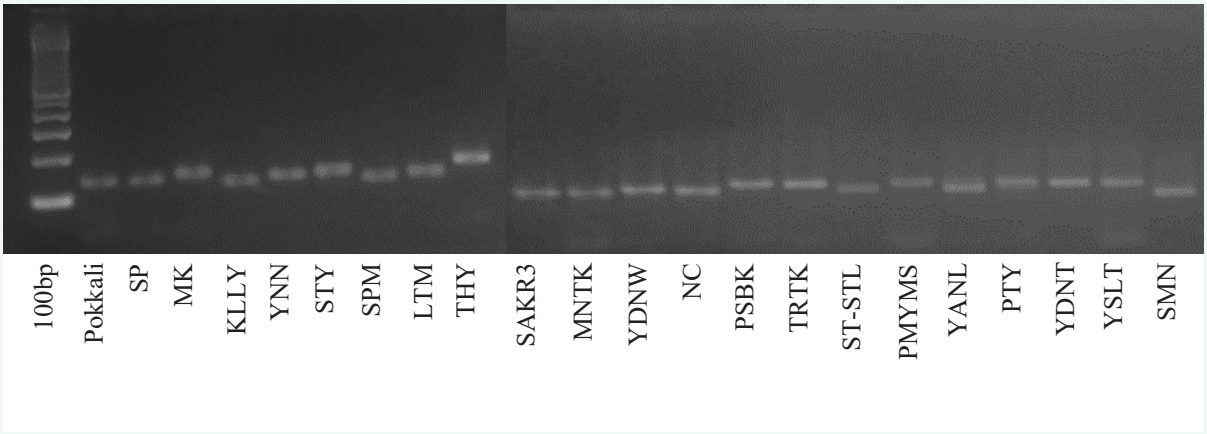

Figure 4 Polymorphic pattern of the 22 rice genotypes with the microsatellite marker RM7075.

The allele size varied from 80 bp (RM490) to 250 bp (RM562). Figure 4 shows the polymorphic pattern of RM7075 in all the 22 rice genotypes. Expected gene diversity was detected ranging from 0.2438 (RM253) to 0.7314 (RM336) with a mean value of 0.4496. Heterozygous genotypes were observed only using RM490. Polymorphism information content value was used to indicate the ability of a primer combination to distinguish between genotypes [16]. PIC values ranged from 0.0830 (SalT1) to 0.6809 (RM336) with a mean value of 0.3754. RM336 has the highest PIC value whereas SalT1 has the lowest PIC value. The Marker Index (MI) ranged from 0.166 (SalT1) to 2.724 (RM336). Based on PIC and MI value, RM336 showed the highest PIC and MI value followed by three markers; RM7075, RM10793 and RM3412b.

Previous researches used RM8094, RM3412 and RM493 for discriminating ability in salt tolerance [33,34]. Ganie et al. [35] reported in 2016 that RM8094 was a good marker for distinguishing salt-tolerant genotypes from susceptible ones because it has the most alleles and the highest PIC value. However, our study revealed that the SSR marker RM336 was the most informative marker to discriminate salt tolerance lines among 22 rice varieties.

The UPGMA-generated dendrogram divides all genotypes into three major clusters (Figure 5). Four genotypes in cluster I, seven genotypes in cluster II and ten genotypes in cluster III are grouped. In cluster I, the genotypes (PSBK, ST-STL, STY are grouped with Pokkali (tolerant check), in the same cluster and thus this may help them to be considered as salt tolerant genotypes. In cluster II, MK, MNTK and YDNW which are susceptible to salinity are grouped into sub-cluster within cluster II. TRTK and SPM were found together under cluster III. PMYMS, PTY, YDNT and YSLT, which are moderately tolerant to salinity, are grouped close to each other forming one sub-cluster in III. Among these genotypes, it was found that PMYMS which has superior RSFW value, did not fall into the same cluster with Pokkali. SMN formed a separate clade from the rest of the 9 genotypes within cluster III. The haplotype analysis of genotype was also carried out in Figure 6. PSBK exhibited the greatest number of common alleles (9/12),

while MK had the lowest number of common alleles (3/12).

Combining phenotypic and molecular assessment, 6 genotypes were screened as tolerant, 11 genotypes as moderately tolerant and 5 genotypes as susceptible. Our findings were found to be useful to plant breeders and farmers working in saline soils in general. According to Reddy et al. [36], salinity tolerance is a complex phenomenon as it requires the integration of different traits and needs to focus on studying each trait independently. Some studies have also cautioned against assuming that marker-QTL linkage will persist across genetic backgrounds or testing environment, particularly for complex traits [17]. Even when

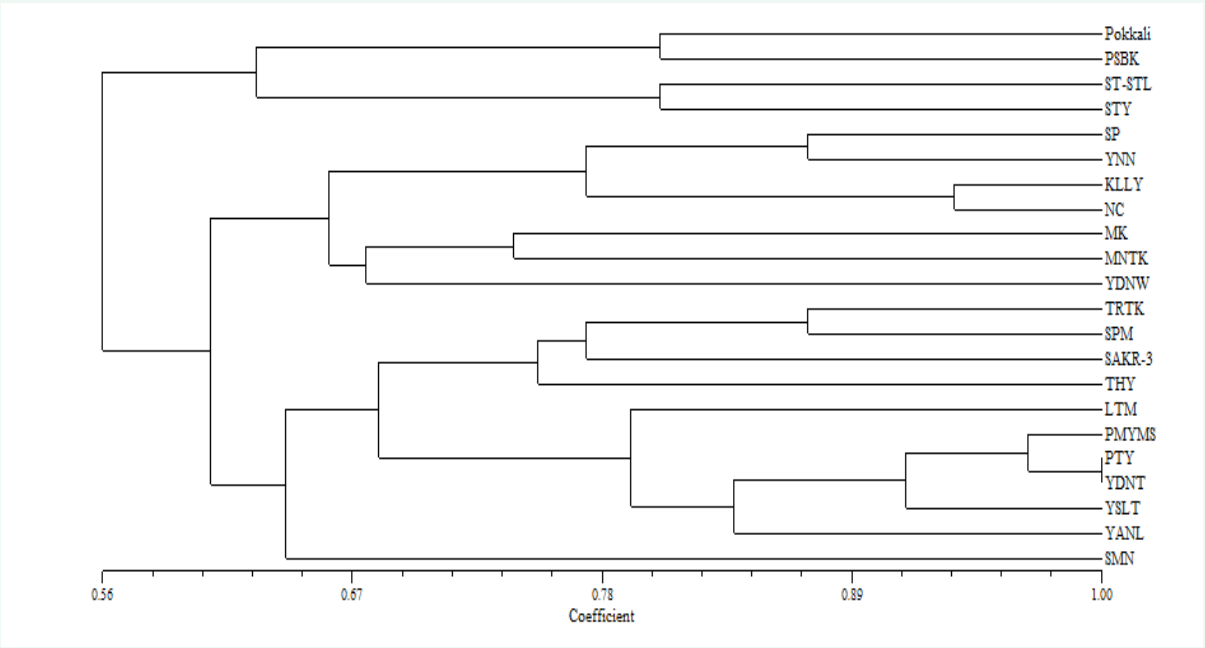

Figure 5 Dendrogram for 22 rice genotypes based on unweighted pair group method with arithmetic average (UPGMA) cluster analysis using 12 SSR markers.

Primer	Pokkali	SP	MK	KLLY	YNN	MNTK	YDNW	NC	PSBK	TRTK	ST-STL	STY	SPM	LTM	THY	SAKR-3	PMYMS	YANL	PTY	YDNT	YSLT	SMN
RM 490	■	■		■	■	■		■	■	■			■	■	■	■	■	■	■	■	■	
RM 493	■								■		■	■		■				■				
RM 7075	■	■		■	■		■	■		■	■	■	■			■						
RM562	■	■	■		■			■	■		■	■		■								
Sal T1	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■
RM8094	■	■					■		■	■	■		■	■	■	■	■	■	■	■	■	
RM10694	■					■			■	■	■	■	■		■	■						
RM3412B	■									■			■		■							
RM 10793	■								■							■		■				
AP 3206F	■	■	■	■	■	■	■	■	■	■												
RM253	■	■		■	■			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
RM336	■			■	■	■		■				■										
Total	12	7	3	6	7	5	4	7	9	8	6	7	7	6	6	7	4	6	4	4	4	2

Black boxes represent similarity to Pokkali allele.

Figure 6 Haplotype analysis for the 22 rice genotypes using Pokkali as the reference genotype.

a single gene regulates a specific trait, there is no guarantee that DNA markers identified in one population will be useful in another, especially if the populations are descended from distinctly related germplasm [17].

CONCLUSION

According to SES scores, percent reduction, and stress tolerance index, eight genotypes were screened as tolerant, ten genotypes as moderately tolerant, and three genotypes as sensitive at 6 dSm^{-1} saline condition. Our result implies that out of twelve SSR markers; RM336, RM7075, RM10793 and RM3412b could differentiate genotypes based on genetically analysis. Through phenotypic and genotypic studies, Pokkali, Sitt Pwar, Paw San Bay Kyar, Thiri Thuka, SalT Sinn Thwe Latt, Yae Anae Lo were screened as salt tolerant genotypes. In our studies, some salt tolerant genotypes were documented and also observed to have relatedness between phenotype and genotype at the seedling stage for salt tolerance of selected rice varieties. Besides, the identified tolerant genotypes should be further tested at higher salinity and biochemical studies should be conducted to determine their ability and relationship between salt tolerance and physiological characters at the seedling stage.

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CONFLICT OF INTEREST

All the authors have declared no conflict of interest.

REFERENCES

1. Jenkins G, Phillips D, Mikhailova EI, Timofejeva L, Jones RN. Meiotic Genes and Proteins in Cereals. Cytogenet Genome Res. 2008; 120: 291-301.
2. IRRI. Annual Report for 1997. IRRI, Los Banos, Philippines. 1997; 308.
3. Gregoria GB, Senadhira D, Mendoza RD. Screening Rice for Salinity Tolerance. IRRI Discussion Paper Series no 22. International Rice Research Institute, Manila, Philippines. 1997; 1-30.
4. Allison L, Richards L. Diagnosis and improvement of saline and alkali soils. Soil and Water Conservative Research Branch, Agricultural Research Service, US Government Printer, Washington, DC. 1954.
5. Ghosh B, Md NA, Gantait S. Response of Rice under Salinity Stress: A Review Update. Rice Res. 2016; 4: 167.
6. Vaid N, Pandey P, Srivastava V, Tuteja N. Pea Lectin Receptor-Like Kinase Functions in Salinity Adaptation without Yield Penalty, by Alleviating Osmotic and Ionic Stresses and Upregulating Stress-Responsive Genes. Plant Mol Biol. 2015; 88: 193-206.
7. Munns R, James RA. Screening Methods for Salinity Tolerance: A Case Study with Tetraploid Wheat. Plant and Soil. 2003; 253: 201-218.
8. Sahi C, Singh A, Kumar K, Blumwald E, Grover A. Salt Stress Response in Rice: Genetics, Molecular Biology, and Comparative Genomics. Funct Integr Genomics. 2006; 6:263-284.
9. Li N, Chen S, Zhou X, Li C, Shao J, Wang R, et al. Effect of NaCl on Photosynthesis, Salt Accumulation and Ion Compartmentation in Two Mangrove Species, Kandelia candel and Bruguiera gymnorhiza. Aquatic Botany. 2008; 88:303-310.
10. Zeng L, Shannon MC, Lesch SM. Timing of Salinity Stress Affects Rice Growth and Yield Components. Agricultural Water Management. 2001; 48:191-206.
11. Bhowmik SK, Titov S, Islam MM, Siddika A, Sultana S, Haque MS. Phenotypic and Genotypic Screening of Rice Genotypes at Seedling Stage for Salt Tolerance. Afr j biotechnol. 2009; 8:6490-6494.
12. Senguttuvel P, Raveendran M, Vijayalakshmi C, Thiyagarajan K, Bapu JK, Viraktamath B. Molecular Mechanism of Salt Tolerance for Genetic Diversity Analysed in Association with Na+/K+ Ratio through SSR Markers in Rice (Oryza Sativa L.). Int. J. Agric. Res. 2010; 5:708-719.
13. Singh S, Sengar R, Kulshreshtha N, Datta D, Tomar R, Rao V, Garg D, Ojha A. Assessment of Multiple Tolerance Indices for Salinity Stress in Bread Wheat (Triticum Aestivum L.). J. Agric. Sci. 2015; 7:49.
14. Omisun T, Sahoo S, Saha B, Panda SK. Relative Salinity Tolerance of Rice Cultivars Native to North East India: A Physiological, Biochemical and Molecular Perspective. Protoplasma. 2018; 255:193-202.
15. Doyle J, Doyle J. Isolation of DNA from Small Amounts of Plant Tissues. BRL Focus. 1990; 12: V15.
16. Liu K, Muse SV. PowerMarker: An Integrated Analysis Environment for Genetic Marker Analysis. Bioinformatics. 2005; 21:2128-2129.
17. Rohlf F. NTSYS-pc Numerical Taxonomy And Multivariate Analysis System, Exeter Software. Applied Biostatistics Inc., Setauket, New York. 1998.
18. Chesnokov YV, Artemyeva A. Evaluation of the Measure of Polymorphism Information of Genetic Diversity. Agricultural biology. 2015; 50:571-578.
19. Wilson AJ, F, Gregorio GB. Morphological and Molecular Characterization at Novel Salt-Tolerant Rice Germplasms from The Philippines and Bangladesh. Rice Sci. 2019; 26:178-188.
20. Pearson GA, Ayers AD, Eberhard DL. Relative Salt Tolerance of Rice during Germination and Early Seedling Development. 1966; 102:151-156.
21. Akbar M, Yabuno T. Breeding for saline-resistant varieties of rice: II. Comparative performance of some rice varieties to salinity during early development stages. Japanese Journal of Breeding. 1974; 24:176-181.
22. Rahman MA, Thomson MJ, Shah-E-Alam M, de Ocampo M, Egdane J, Ismail AM. Exploring Novel Genetic Sources of Salinity Tolerance in Rice through Molecular and Physiological Characterization. Ann Bot. 2016; 117:1083-1097.
23. Xie JH, Zapata-Arias FJ, Shen M, Afza R. Salinity Tolerant Performance and Genetic Diversity of Four Rice Varieties. Euphytica. 2000; 116:105-110.
24. Anshori MF, Purwoko BS, Dewi IS, Ardie SW, Suwarno WB. A New Approach to Select Doubled Haploid Rice Lines under Salinity Stress Using Indirect Selection Index. Rice Sci. 2021; 28:368-378.
25. Abdel-Farid IB, Marghany MR, Rowezek MM, Sheded MG. Effect of Salinity Stress on Growth and Metabolomic Profiling of Cucumis sativus and Solanum lycopersicum. Plants (Basel, Switzerland). 2020; 9: 1626.

26. Liu Y, Wang B, Li J, Song Z, Lu B, Chi M, Yang B, Liu J, Lam Y-W, Li J. Salt-response Analysis in Two Rice Cultivars at Seedling Stage. Acta Physiol Plant. 2017; 39:1-9.

27. Krishnamurthy S, Sharma P, Sharma S, Batra V, Kumar V, Rao L. Effect of Salinity and Use of Stress Indices of Morphological and Physiological Traits at the Seedling Stage in Rice. Indian J Exp Biol. 2016; 54:843-850.

28. El-Hendawy S, Hu Y, Yakout G, Awad A, Hafiz S, Schmidhalter U. Evaluating Salt Tolerance of Wheat Genotypes Using Multiple Parameters. Eur J Agron. 2005; 22:243-253.

29. Gholizadeh F, Navabpour S. Effect of Salinity on Morphological and Physiological Characteristics in Correlation to Selection of Salt Tolerance in Rice (Oryza sativa L.). Int. j. agric. 2011; 6:780-788.

30. Alam MA, Juraimi A, Rafii M, Hamid A, Aslani F, Alam MJFC. Effects of salinity and salinity-induced augmented bioactive compounds in purslane (Portulaca oleracea L.) for possible economical use. Food Chem. 2015; 169:439-447.

31. Lauchli A, Grattan S. Plant Growth and Development under Salinity Stress. Advances in Molecular Breeding toward Drought and Salt Tolerant Crops. Springer. 2007; pp 1-32.

32. Lutts S, Kinet J, Bouharmont J. Changes in Plant Response to NaCl during Development of Rice (Oryza sativa L.) Varieties Differing in Salinity Resistance. J. Exp. Bot. 1995; 46:1843-1852.

33. Thomson M, De Ocampo M, Egdane J, Rahman M, Sajise A, Adorada D, Tumimbang-Raiz E, Blumwald E, Seraj Z, Singh R. Characterizing the Saltol quantitative trait locus for salinity tolerance in rice. 2010; Rice 3: 148-160.

34. Chowdhury A, Haritha G, Sunitha T, Krishnamurthy S, Divya B, Padmavathi G, Ram T, Sarla N. Haplotyping of Rice Genotypes Using Simple Sequence Repeat Markers associated with Salt Tolerance. Rice Sci. 2016; 23:317-325.

35. Ganie S A, Borgohain M J, Kritika K, Talukdar A, Pani D R,Mondal T K. Assessment of genetic diversity of Saltol QTL among the rice (Oryza sativa L.) genotypes. Physiol Mol Biol Plants. 2016; 22: 107–114.

36. Reddy INBL, Kim SK, Kim BK, Yoon IS, Kwon TR. Identification of Rice Accessions Associated With K+/Na+ Ratio and Salt Tolerance Based on Physiological and Molecular Responses. Rice Sci. 2017; 24: 360-364.

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