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PHENOTYPIC AND GENOTYPIC SCREENING OF RICE GENOTYPES AT REPRODUCTIVE STAGE FOR SALT TOLERANCE

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ABSTRACT

Salinity screening of 24 rice genotypes was performed at the reproductive stage for evaluating their salt tolerance level. On the basis of yield and yield components, genotypes were categorized as tolerant, susceptible and moderately tolerant. PBRC-30, Ashfal, Horkuch, STL-20 and Pokkali were found as tolerant while Binadhan-7, S-39 L-11, S-37 L-27, S-37 L-36 and S-37 L-39 were found as susceptible. Selected three SSR markers viz. RM336, RM21 and RM510 were used to determine salinity tolerance. The genetic diversity was ranges from 0.8194 to 0.8854 with an average of 0.8530. The highest PCI value was 0.8742 and the lowest was 0.8004 from RM510 and RM21, respectively. The UPGMA clustering system generated six genetic clusters. The highest genetically dissimilarity of (Cluster 1) vs (Cluster 2 sub-cluster A) and the crossing would be helpful for salt tolerant rice development. Thus, selected SSR primers and genotypes would be useful in marker assisted breeding, quantitative trait loci (QTL) mapping and gene pyramiding in breeding programmed for improvement of rice for salt tolerance.

Keywords: Rice, salinity tolerance, SSR markers, reproductive stage

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food for one third of the world's population and occupies almost one-fifth of the total land area covered under cereals (Chakravarthi and Naravaneni, 2006). This staple food ranked first position by production (130 Lac Metric Tons) during the year 2013-14 among all cereals in Bangladesh (BBS, 2013). Over 800 million hectares of land throughout the world are salt affected, either by salinity (397 million ha) or the associated condition of sodicity (434 million ha). Out of 2.85 million hectares of coastal and offshore land of Bangladesh, about 1.0 million hectares are affected by varying degrees of salinity. The coastal saline soils are

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distributed unevenly in 64 upazillas of 13 districts, covering portions of eight agro-ecological zones (AEZ) of the country (Seraj and Salam, 2000). Salinity is one of the major constraints for rice production worldwide. Hence, adoption of salt tolerant rice varieties has been considered as one of the strategies to increase rice production in salinity areas. Although soil salinity affects all stages of growth and development of rice plant, but salinity at the reproductive stage depresses grain yield much more than salinity at the vegetative stages. Therefore, screening for salt tolerance at reproductive stages has been considered to be more useful. The use of physiological characters as selection criteria in salt tolerance breeding requires the identification of the contribution of each individual character to salt tolerance (Sabouri et al., 2009). Panicle weight, tiller numbers per plant and harvest index are important agronomic characters for the prediction of rice yield. These yield components are severely affected by salinity (Mojakkir et al., 2015). Breeding for salinity tolerance in rice requires suitable screening techniques and appropriate molecular marker technology (Gregorio et al., 2002). SSR or microsatellite markers are proved to be an ideal method for making genetic maps (Islam, 2004; Niones, 2004), assisting selection procedure (Bhuiyan, 2005) and studying genetic diversity of rice germplasms. Microsatellite marker analysis is promising to identify major gene locus for salt tolerance that can be helpful for plant breeders developing new cultivars. The objective of this study was assessing phenotypic variability of rice genotypes under salt stress at reproductive stage and identification of salt tolerant rice genotypes.

MATERIALS AND METHODS

This study was conducted at the glasshouse and laboratory of Department of Biotechnology, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. A total of 24 traditional and improved rice genotypes were used in the study.

Phenotypic screening

The genotypes were evaluated for their tolerance to salinity under sustained water bath using IRRI standard protocol (Gregorio et al., 1997). At reproductive stage, seedlings were grown in perforated pots which were served as a water tank. Rice yield and yield component data were recorded from the reproductive stage in both normal and salinized conditions. Data were recorded on plant height (cm), days to flowering, days to maturity, number of effective tillers/plant, number of field grains, reduction of number of field grains, number of unfilled grains, total dry mater(g), reduction of total dry matter (g), percent fertility and grain yield (g). These data were recorded following the standard evaluation system of IRRI (IRRI, 1997). MSTATC software was used to perform data analysis on yield and yield components for normal and salinized environments.

Genotypic screening

DNA was extracted using the mini preparation CTAB method (Bhowmik et al., 2009) from 21 days age seedling. Out of 21 microsatellite primers tested three primers (RM510, RM336 and RM21) were selected for polymorphisms and clear band. These three selected primers were used in final polymerase chain reaction (PCR) amplification for this study.

SSR data analysis

The size of most intensely amplified fragments was determined by comparing the migration distance of amplified fragments relative to the molecular weight of known size markers, 100 base pairs (bp) DNA ladder using Alpha-Ease FC 5.0 software. The number of alleles per locus, major allele frequency, gene diversity and PIC values were calculated using Power Marker version 3.25 (Liu and Muse, 2005). NTSYS-pc was used to construct a UPGMA (unweighted pair group method with arithmetic averages) dendrogram showing the distance-based interrelationship among the genotypes.

RESULTS AND DISCUSSION

Reproductive stage is one of the most sensitive growth stages rice under the saline conditions. Considerable effect due to salinity was observed for most of the traits evaluated during the reproductive stage.

Phenotypic screening

The percent reduction of plant height, total dry matter and number of filled grains of 24 genotypes differed from each other. The per cent reduction in plant height of Pokkali, PBRC-30, PBSAL-656, STL- 15, STL-20, Ashfal, Horkuch, S-39 L-32 and S-39 L-34 were lower (0.8, 6.3, 5.4, 5.0, 3.0, 3.6, 2.8, 4.8 and 2. 7, respectively). On the other hand, Binadhan-7, S-39 L-11, S-39 L-27, S-37 L-14, S-37 L-27, S-37 L-36 and PBSAL-730 showed higher reduction (41.5, 17.2, 33.3, 13.6, 17.9, 13.8 and 13.33, respectively) (Table 1). Salinity stress might inhibit cell division or cell enlargement so that plant height was reduced. Similar result also found by Mojakkir et al. (2015).

Reduction (%) of total dry matter was higher in PBSAL-656 (61.3), Binadhan-7 (75.5), S-39 L-11 (35.8), S-39 L-16 (37.1), S-39 L-27 (57.8), S-37 L-19 (29.8), S-37 L-23 (34.7) and S-37 L-36 (38.3) (Table 1). Similarly per cent reduction of number of filled grains was lower in PBRC-30, PBRC-37, STL-15, STL-20, Ashfal, Horkuch and Pokkali (28.8, 29.9, 16.8, 14.8, 20, 27.3 and 17.7, respectively). Binadhan-7, S-39 L-11, S-39 L-27, S-37 L-19, S-37 L-24, S-37 L-27, S-37 L-36 and S-37 L-39 had higher reduction (81.5, 80.6, 78.4, 71.6, 66.6, 75.1, 63.3, and 65.2, respectively). This is because of loss of biomass production was lower in tolerant genotypes which increased the assimilation and ultimately produced the higher number of grains. Tolerant genotypes showed lower reduction than the susceptible. This result was consistent with the result observed by Islam (2004) who worked with 80 RILs from Pokkali X IR29 cross. He reported that total biomass of tolerant lines was reduced by 49.5% in salinized condition whereas those of susceptible lines were reduced by 64.0%. Most of the genotypes showed higher number of unfilled grains in salinized condition than the normal condition. According to the performance

Table 1. Means of studied traits of 24 rice genotypes under salinized and non salinized conditions NS= Non salinized; S= Salinized; R= Reduction

Sl. No	Variety	Plant height (cm)			Total dry matter (g)			Number of filled grain			No. of unfilled grain		% fertility		Yield/Plant (gm)		1000 seed weight	
		NS	S	% R	NS	S	% R	NS	S	% R	NS	S	NS	S	NS	S	NS	S
1	PBRC – 30	95	89	6.3	14.1	12.2	13.4	288	205	28.8	54	63	84.2	76.6	4.3	1.3	14.8	6.1
2	PBRC – 37	92	84	8.7	8.4	6.8	19.0	300	210	29.9	48	87	86.2	67.6	5.2	3.2	17.3	14.9
3	PBSAL - 656	93	88	5.4	10.8	4.2	61.3	255	165	35.2	30	63	89.5	72.5	4.1	2.2	15.8	13.1
4	PBSAL - 730	90	78	13.3	6.2	5.1	19.0	198	98	50.2	24	73	89.2	57.6	3.1	1.4	15.5	13.9
5	STL – 15	100	95	5.0	9.3	8.1	23.3	260	216	16.8	20	32	92.9	87.0	5.2	3.2	20.0	14.9
6	STL – 20	100	97	3.0	12.2	10.1	17.2	270	230	14.8	48	87	84.9	72.6	6.3	3.9	23.4	17.1
7	Ashfal	110	106	3.6	18.1	16.2	10.5	318	254	20	24	66	92.9	79.3	5.4	4.3	16.9	16.9
8	Horkuch	112	109	2.7	10.3	8.6	16.1	290	211	27.3	26	50	91.8	80.8	5.8	3.8	20.0	17.9
9	Pokkali	125	124	0.8	11.4	9.8	13.8	444	365	17.7	24	45	94.9	89.0	7.5	6.1	16.9	16.6
10	BD – 7	135	79	41.5	23.3	5.7	75.5	660	122	81.5	66	241	90.9	33.6	8.5	1.6	13.1	13.4
11	S-39, L-11	99	82	17.2	10.9	7.0	35.8	495	96	80.6	90	148	84.6	39.2	9.9	1.2	20.1	12.9
12	S-39, L-16	97	90	7.2	9.7	6.1	37.1	360	167	53.6	96	132	78.9	55.8	6.1	2.8	17.1	16.9
13	S-39, L-27	105	70	33.3	13.3	5.6	57.8	480	103	78.4	30	199	94.1	34.2	12.4	2.1	25.8	19.9
14	S-39, L-31	88	81	7.9	12.3	10.9	11.4	270	126	53.3	66	106	80.4	54.3	4.1	1.8	15.0	13.9
15	S-39, L-32	105	100	4.8	9.7	8.3	15.9	288	143	50.5	55	141	83.9	50.2	4.6	2.1	16.0	14.7
16	S-39, L-34	112	109	2.7	13.6	11.2	17.6	280	114	59.3	84	162	76.9	41.3	4.7	1.6	17.1	13.9
17	S-37, L-14	88	76	13.6	7.7	6.1	20.7	195	105	45.9	65	91	75.0	53.8	2.9	1.3	14.9	11.9
18	S-37, L-18	85	78	8.2	6.6	5.6	15.2	270	128	52.5	20	76	93.1	62.8	6.4	1.8	23.5	13.9
19	S-37, L-19	93	81	12.9	13.1	9.2	29.8	550	156	71.6	77	263	87.7	37.2	11.6	3.1	21.0	20.1
20	S-37, L-23	86	78	9.3	9.8	6.4	34.7	336	217	35.2	81	123	80.6	63.9	6.7	4.1	20.1	18.9
21	S-37, L-24	100	88	12.0	7.9	6.2	21.5	294	98	66.6	64	167	82.1	37.0	4.4	1.1	15.1	11.4
22	S-37, L-27	95	78	17.9	10.8	8.3	23.2	320	8o	75.1	65	271	83.1	22.8	7.3	1.6	23.1	19.9
23	S-37, L-36	80	69	13.8	6.3	3.9	38.3	252	93	63.3	90	180	76.7	33.9	4.1	1.3	15.9	14.3
24	S-37, L-39	97	89	8.2	15.8	12.4	21.5	313	110	65.2	91	189	77.6	36.8	5.4	1.5	16.9	13.9
$LSD_{0.05}$		2.114	3.470		1.052	1.189		5.921	4.998		3.917	2.221	1.927	0.933	0.817	0.543	0.744	0.832

of yield/plant in salinized condition, Pokkali, Horkuch, Ashfal, STL-15, STL-20, PBRC-37, S-37 L-19 and S-37 L-23 showed as tolerant (> 3.00) and PBRC-30, PBSAL-730, Binadhan-7, S-39 L-11, S-39 L-34, S-37 L-14, S-37 L-24, S-37 L-27, S-37 L-36 and S-37 L-39 showed as susceptible genotype (≤1.6) (Table 1). Asch et al. (1998) also found that cultivars differed in their salt uptake and the tolerant cultivars had lower salt effect on yield and yield components than the susceptible.

At the reproductive stage, highly significant and positive correlation found between plant height and total dry matter; total dry matter and number of filled grain; plant height and number of filled grain at both salinized and non salinized condition. Grain yield per plant had also the highly significant and positive correlation with plant height, number of filled grains, per cent of fertility and 1000-seed weight. Percent fertility also showed significant and positive correlation with number of filled grain and plant height (Table 2). These results revealed that the higher values increase of such traits have the significant role in the increase of other traits. There was no significant correlation of total dry matter with percent fertility and number of filled grain with 1000-seed weight. Peng et al. (1999) reported that increasing plant height would allow greater biomass production. Zhang et al. (2004) found that increase of plant height was responsible for increase in biomass; so as to increase yield potential. It is crucial to note that Pokkali, PBRC-30, Ashfal, Horkuch and STL-20 genotypes showed higher plant height and total dry matter and also performed as salt tolerant.

Table 2. Correlation of different traits at reproductive stage under salinized and non salinized condition

Trait	Plant height		Total dry matter		No. of filled grain		Fertility (%)		Grain yield/ Plant	
	S	NS	S	NS	S	NS	S	NS	S	NS
Total dry matter	0.572**	0.679**								
No. of filled grain	0.701**	0.578**	0.399**	0.626**						
Fertility (%)	0.583*	0.444**	0.271	0.195	0.835**	0.337*				
Grain yield/ Plant	0.628**	0.305*	0.310*	0.362*	0.915**	0.838**	0.672**	0.407**		
1000-seed wt	0.0337	-0.146	-0.0201	-0.152	0.164	0.134	-0.111	0.332*	0.528**	0.620**

* Significant at 5% level of probability, ** Significant at 1% level of probability

S = Significant and NS = Non-Significant

Genotypic screening

Out of 21 primer pairs tested, three primers were identified as polymorphic. A total of 30 alleles were detected at the loci of three microsatellite markers across 24 rice genotypes. On average, 25% of the 24 rice genotypes shared a common major allele at any given locus. Diversity exists among three loci tested across 24 rice genotypes, ranged from 0.8194 to 0.8854 with an average of 0.8530. The polymorphism information content (PIC) value is a measure of polymorphism among the genotypes for a marker locus used in linkage analysis. The PIC value of each marker, which can be evaluated on the basis of its alleles, varied for all tested SSR loci (Table 3). The highest PIC value 0.8742 was obtained from RM510 followed by RM336 (0.8390), RM21 (0.8004), respectively. Nejada et al (2010) also found that PIC value varied from 0.56 to 0.88, the highest value belonged to RM8094, while RM8095 showed the lowest PIC value (0.56). The SSR marker RM8094 was found to be superior for analysis of genetic diversity among the markers in the region.

Table 3. Number of genotype, alleles number, gene diversity and polymorphism information content (PIC) value found among 24 rice genotypes for three SSR markers

Primers	Major Allele Frequency	Genotype no.	Allele no.	Gene Diversity	PIC
RM336	0.2500	11.0000	11.0000	0.8542	0.8390
RM510	0.1667	10.0000	10.0000	0.8854	0.8742
RM21	0.3333	9.0000	9.0000	0.8194	0.8004
Mean	0.2500	10.0000	10.0000	0.8530	0.8379

The values of pair-wise comparisons of Nei’s (1973) genetic distance (D) between genotypes were computed from combined data for the three primers, ranged from 0.0000 to 1.000 (Table 4). Comparatively higher genetic distance (1.000) was observed between a number of germplasm or germplasm pair. Among them G1/ G 8/ G 9/ G 7 vs G 10/G 11/G 22/ G 23/ G24 were important. The higher genetic distance between them indicates that genetically they are diverse compare to lower genetic distance value. Basically this value is an indication of their genetic dissimilarity. Variety pair with higher value is more dissimilar than a pair with a lower value. The lowest genetic distance (0.0000) was found in G1 vs.G8, G1 vs. G9, G8 vs.G9 and G10 vs. G11 etc. Genotypic pair indicating that they are genetically much closer among the genotypes tested. However, potential hybrid line can be produced by inter-varietal crossing based on the genetic dissimilarity value since the more the genetic dissimilarity value the more chance of getting vigorous heterosis in the progeny. Hence microsatellite marker based molecular fingerprinting could serve as a potential basis for the identification of genetically distance genotypes as well as sorting of duplication for morphologically closer genotype.

Table 4. Pair-wise comparisons of Nei's (1973) genetic distance between 24 rice genotypes

OTU	G1	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G2	G20	G21	G22	G23	G24	G3	G4	G5	G6	G7	G8	G9
G1	0.0000																							
G10	1.0000	0.0000																						
G11	1.0000	0.0000	0.0000																					
G12	1.0000	0.6667	0.6667	0.0000																				
G13	1.0000	0.6667	0.6667	1.0000	0.0000																			
G14	0.6667	0.6667	0.6667	1.0000	0.3333	0.0000																		
G15	1.0000	0.6667	0.6667	1.0000	0.6667	0.6667	0.0000																	
G16	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	0.0000																
G17	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	0.0000															
G18	1.0000	1.0000	1.0000	0.6667	0.6667	1.0000	1.0000	1.0000	1.0000	0.0000														
G19	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	1.0000	1.0000	1.0000	0.0000													
G2	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	1.0000	1.0000	1.0000	0.6667	0.0000												
G20	1.0000	0.6667	0.6667	0.6667	0.3333	0.3333	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000											
G21	1.0000	1.0000	1.0000	0.6667	1.0000	1.0000	0.6667	0.6667	1.0000	0.6667	1.0000	1.0000	1.0000	0.0000										
G22	1.0000	0.3333	0.3333	1.0000	0.6667	0.6667	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	0.6667	0.0000									
G23	1.0000	0.6667	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	0.3333	0.0000								
G24	1.0000	0.3333	0.3333	1.0000	0.6667	0.6667	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	1.0000	0.3333	0.6667	0.0000							
G3	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000						
G4	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000					
G5	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.3333	1.0000	0.0000				
G6	0.6667	1.0000	1.0000	1.0000	0.6667	0.3333	1.0000	0.6667	1.0000	1.0000	1.0000	1.0000	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000			
G7	0.3333	1.0000	1.0000	1.0000	1.0000	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	1.0000	0.6667	0.0000		
G8	0.0000	1.0000	1.0000	1.0000	1.0000	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	0.3333	0.0000	
G9	0.0000	1.0000	1.0000	1.0000	1.0000	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	0.3333	0.0000	0.0000

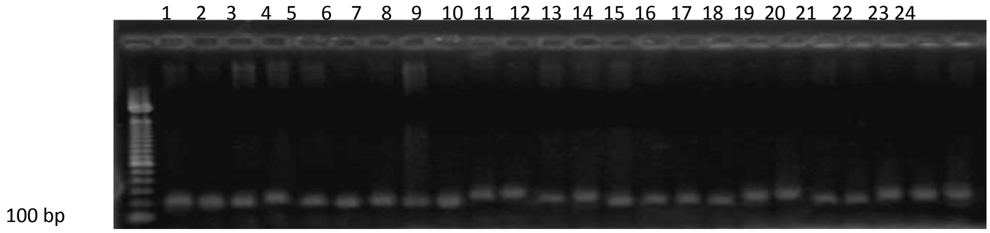

Figure 1. Amplification of DNA segments of tested genotypes using RM336: (Lane 1 to 25 respectively, lKb+; PBRC-30; PBRC-37; PBSAL-656; PBSAL-730; STL-15; STL-20; Ashfal; Horkuch; Pokkali; Binadhan-7, S-39 L-11; S-39 L-16; S-39 L-27; S-39 L-31; S-39 L-32; S-39 L-34; S-37 L-14; S-37 L-18; S-37 L-19; S-37 L-23; S-37 L-24; S-37 L-27; S-37 L-36 and S-37 L-39)

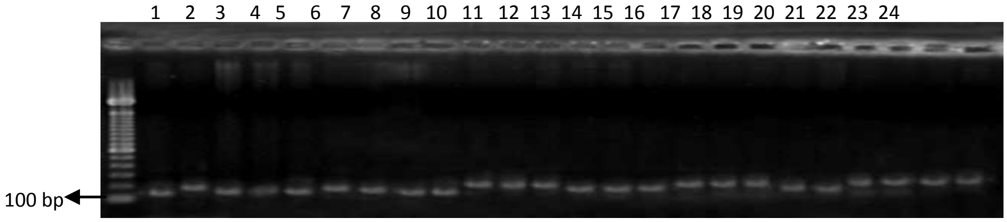

Figure 2. Amplification of DNA segments of tested genotypes using RM510

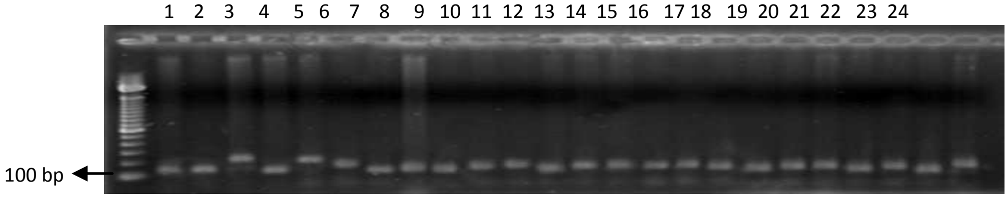

Figure 3. Amplification of DNA segment of tested genotypes using RM21

UPGMA Dendogram

A cluster analysis using UPGMA based on similarity coefficients was done to resolve the phylogenetic relationships among the 24 rice genotypes tested. The UPGMA clustering system generated six genetic clusters with similarity coefficient 11% (Figure 4). Cluster 2 was the biggest group which contained nine genotypes viz. G06, G10, G11, G13, G 14, G20, G22, G23, and G24. This cluster had two separate additional sub-clusters within it, where G10, G11, G22, G23 and G24 were in sub cluster A and G6, G20, G13 and G14 formed sub cluster B. The sub-cluster A were mainly salt susceptible. The cluster analysis revealed that G10, G11, G22 and G24 are closer than G23 while G10, G11 are closer than G22 and G24.

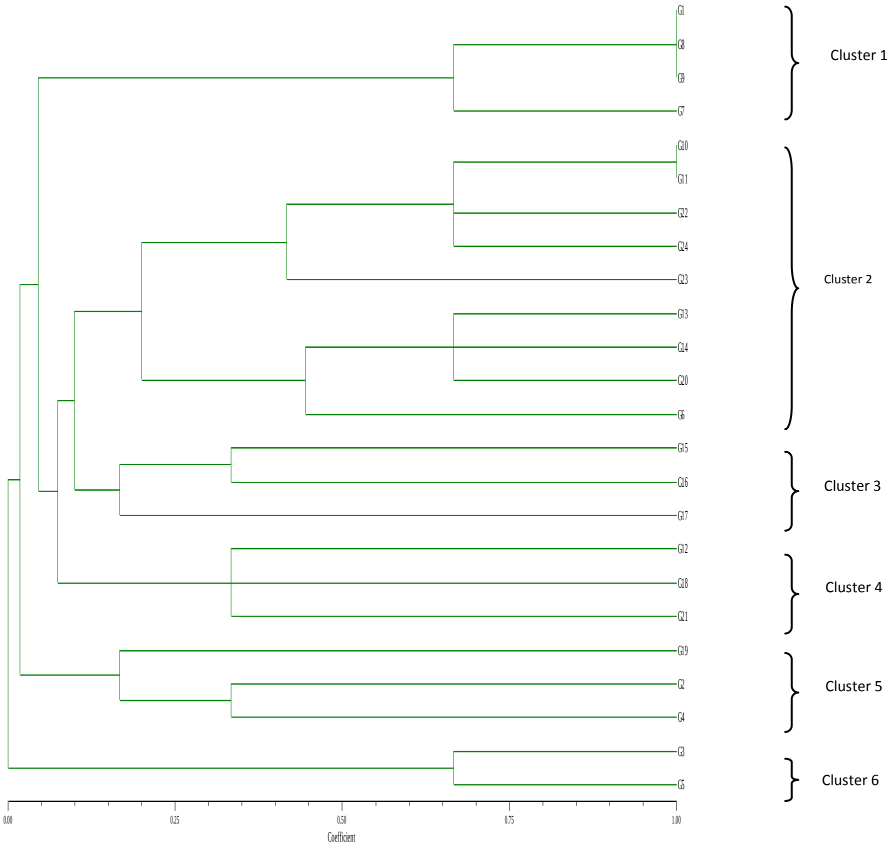

Figure 4. A UPGMA clustering dendogram showing the genetic relationship among 24 rice genotypes based on three SSR markers.

Legend: G1= PBRC-30; G2= PBRC-37; G3= PBSAL-656; G4= PBSAL-730; G5= STL-15; G6= STL-20; G7= Ashfal; G8= Horkuch; G9= Pokkali; G10= Binadhan-7; G11= S-39 L-11; G12= S-39 L-16; G13= S-39 L-27; G14= S-39 L-31; G15= S-39 L-32; G16= S-39 L-34; G17= S-37 L-14; G18= S-37 L-18; G19= S-37 L-19; G20= S-37 L-23; G21= S-37 L-24; G22= S-37 L-27; G23= S-37 L-36; G24= S-37 L-39

In sub-cluster B G13, G14 and G20 are closer than G6. In cluster 1 G1, G8 and G9 formed a sub-cluster and only G7 formed another sub-cluster and also revealed that G1, G8 and G9 are very closer than G7. The genotypes under Cluster 1 were salt tolerant Cluster 3, 4 and 5 contained three genotypes in each cluster. G15 and G16 are closer than G17 (Cluster 3). The three genotypes G12, G18 and G21 were clustered in the same group (Cluster 4). Cluster 5 contains G19, G2 and G4 where G2 and G4 are closer than G19. Cluster 6 was the smallest group which contains G3 and G5. This clustering analysis agreed with the allelic diversity observed among Basmati and Non-basmati long grain indica rice varieties using microsatellite markers (Siwach et al., 2004). DNA fingerprinting and phylogenic analysis of Indian aromatic high quality rice germplasms also showed similar trend (Jain et al., 2004).

CONCLUSION

Salinity screening was done using IRRI standard protocol under non salinized and salinized conditions. Yield and yield components of the germplasms were reduced in saline condition. Pokkali, PBRC-30, Ashfal, Horkuch and STL-20 were found tolerant, on the other hand Binadhan-7, S-39 L-11, S-37 L-27, S-37 L-36 and S-37 L-39 were found susceptible for all the traits. Tolerant genotypes showed higher number of effective tillers/plant, per cent fertility, yield/plant and 1000-seed weight than the susceptible genotypes. Grain yield/plant had the positive and significant correlation with total dry matter, number of filled grains and per cent of fertility.

Out of 21 primers tested three primers (RM336, RM21 and RM510) were selected for genotyping across the 24 rice genotypes tested in this study. Diversity exists among three loci ranged from 0.8194 to 0.8854 with an average of 0.8530. The polymorphism information content (PIC) value was evaluated on the basis of its alleles. The highest PCI value was obtained from RM510 and lowest RM21 respectively, 0.8742 and 0.8004. The values of Nei's (1973) genetic distance between genotypes were calculated from combined data of three primers. The higher genetic distance between them indicates that genetically they are diverse compare to lower genetic distance value. This value is an indication of their genetic dissimilarity. Variety pair with higher value is more dissimilar than a pair with a lower value. The lowest genetic distance (0.0000) was found in G1 vs.G8, G1 vs. G9, G8 vs.G9 and G10 vs. G11 etc. indicating that they are genetically much closer among the genotype. The UPGMA clustering system generated six genetic clusters. The biggest cluster 2 was contained nine genotypes with two sub-clusters, viz. sub-cluster A and sub-cluster B. The sub-cluster A contained G10, G11, G22, G23 and G24 on the other hand G6, G20, G13 and G14 formed sub-cluster B. The sub-cluster A were mainly salt susceptible. The salt tolerant genotypes were under Cluster 1 and this contained G1, G8, G7 and G9. The crossing between Cluster 1 vs Cluster 2 sub-cluster A for salt tolerant rice development will be successful due the highest genetically dissimilarity. The genotypes G15, G16 and G17 were under (Cluster 3). The three genotypes G12, G18 and G21 were clustered in the same group (Cluster 4).

Cluster 5 contains G19, G2 and G4 and last clastar (Cluster 6) was contained G3 and G5. Considering both phenotypic and genotypic observations, five genotypes viz. Pokkali, PBRC 30, Ashfal, Horkuch and STL-20 were identified as salt tolerant on the other hand Binadhan-7, S39 L11, S37 L27, S37 L36 and S37 L39 were identified as salt susceptible. The markers (RM336, RM21 and RM510) that were used in this study showed polymorphism, these markers could be proficiently used in tagging salt tolerant genes, in marker-assisted selection and quantitative trait loci (QTL) mapping; and identified salt tolerant rice genotypes could be used in the improvement of salt tolerant rice genotypes.

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