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Research Article
Screening of Rice Landraces of Coastal Areas for Salt Tolerance at Seedling Stage Using Molecular Markers

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Abstract

Background and Objective: Salinity is becoming a serious problem in the world and a widespread soil problem in rice growing countries. The saline area is 3 times larger than land used for agriculture. The conventional methods of plant selection for salt tolerance are difficult because of the large effects of the environment. The main objective of this study was to develop salt-tolerant rice varieties by identifying suitable parents and genetic diversity analysis. **Materials and Methods:** The study was conducted under Biotechnology Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh. Initially 80 germplasms were used to evaluate the salinity tolerance at seedling stage at glass-house following IRRI standard protocol. Among them, 12 were found as salt tolerant, 13 were found as moderately tolerant, 29 were highly susceptible and 26 were susceptible by phenotypic analysis. Among them, 25 germplasms were used for molecular study, which carry all tolerant variety found in phenotypic study (Hogla, Jamai Naru, Dakhsail, Patnai, Kute Patnai, Holde Gotal, Bazra Muri, Ghunshi, Tal Mugur, Nona Bokhra, Kashrail and FL378), 7 were moderately tolerant, 5 were highly susceptible and 1 was susceptible. These germplasms were characterized by 3 SSR markers which are RM510, RM585 and RM336. Data were analyzed by POPGENE (version 1.31), Power Marker (version 3.25) and NTSYS-PC (version 2.2). **Results:** The number of alleles/locus ranged from 10-12, with an average number of alleles of 11/locus and PIC values ranged from a low of 0.8533 (RM336) to a high of 0.8940 (RM585). The average gene diversity of overall SSR loci for the 25 genotypes was 0.8885, ranged from 0.9024-0.8672. Unweighted pair group method of arithmetic means (UPGMA) dendrogram constructed from Nei's (1972) genetic distance produced five distinct clusters of 25 rice genotypes. FL378 of IRRI was used as check variety. It is confirmed that Holde Gotal, Bazra Muri and Hamai were salt tolerant compared to FL378. **Conclusion:** This scientific information could be used for solution of suitable parents, development of salt tolerant rice varieties, gene identification for salt tolerance and genetic diversity analysis.

Key words: *Oryza sativa*, germplasms, tolerance, SSR marker, polymorphism, genotyping

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rice, *Oryza sativa* (2n = 24) belonging to the family *Graminae* and subfamily *Oryzoidea* is the staple food of more than 50% of the world's population[1]. This crop is being cultivated in at least 95 countries throughout the world and is the second agricultural crop plant in the world[2]. Rice is the staple food of about 149 million people of Bangladesh and is the 4th largest rice producer in the world. Bangladesh has been increasing rice production over many years and is now relatively self-sufficient in rice production. In Bangladesh, rice provides nearly 48% of rural employment, about two-third of total calorie supply and about one-half of the total protein intakes of an average person in the country and rice sector contributes one-half of the agricultural GDP and one-sixth of the national income[3]. Moreover, traditional landraces are important reservoirs of valuable trait and need attention for conservation and improvement[4].

Salinity is becoming a serious problem of several parts of the world and a widespread soil problem of rice growing countries. The saline area are 3 times larger than land used for agriculture[5]. It has been reported that 20% areas of Bangladeshis covered by coastal areas which actually cover over 30% of the net cultivable area. About 1 million hectares of cultivable land in the coastal regions are affected by varying degrees of salinity[6,7]. Satkhira is one of these coastal areas, where 70% is highly saline with soil conductivity levels ranging from 4-16 dS m^{-1}. Salt stress is a major constraint across many rice production areas because of the high sensitivity of modern rice varieties[7,8]. There are two major effects of high salt toxicity in crop plants, i.e. the osmotic and the ionic effects. So, development of salt tolerant varieties has been considered as one of the strategies to increase rice production of saline prone coastal areas. Several studies also indicated that rice is salt tolerant during germination, becomes very sensitive during early seedling stage (2-3 leaf stage), gains tolerance during vegetative growth stage becomes sensitive during pollination and fertilization and then become increasingly more tolerant at maturity[6,9,10].

The conventional methods of plant selection of salt tolerance are not easy because of the large effects of the environment and low narrow sense heritability of salt tolerance. On the other hand, DNA markers seem to be the best option for efficient evaluation and selection of plant material[11].

SSRs (Simple Sequence Repeat) or microsatellite markers have been proved to be ideal for making genetic maps[12], assisting selection and studying genetic diversity in germplasms[13].

SSR markers are playing an important role to identify gene for salt tolerance that can be helpful for plant breeders to develop new cultivars. The present study were made to evaluate 80 rice germplasms for salinity tolerance at seedling stage at glasshouse following IRRI standard protocol and among them only 25 germplasms were salt tolerant and moderately tolerant and was used for further molecular analysis by using microsatellite markers.

MATERIALS AND METHODS

The whole experiment were done at glasshouse and Biotechnology division of Bangladesh Institute of Nuclear Agriculture (BINA) and Department of Biotechnology, Bangladesh Agricultural University (BAU), 2200 Mymensingh. The experiments were performed during July, 2011-March, 2013.

Collection of germplasms: Initially a total number of 80 rice germplasms were collected from coastal areas of Bangladesh (Mainly from Satkhira, Patuakhali, Noakhali) and IRRI (FL 378 and FL 478). Phenotypic analysis was done by using SES scoring and calculation on the reduction of plant height, root length and total dry matter weight with field performance study. On the basis of phenotypic results, 25 germplasms were taken for molecular study, in which 12 were tolerant varieties, 7 were moderately tolerant, 5 were highly susceptible and 1 was susceptible (from phenotypic result). FL378 was a tolerant variety of IRRI and showed tolerance in initial phenotypic study. As a result, this variety was used as a check variety of this study.

Isolation of rice genomic DNA: For molecular analysis, healthy and vigorously growing fresh leaf samples were collected from 25 days old seedlings of selected rice germplasms. About 7-8 cm long leaf tip was cut apart with sterilized scissors and washed in distilled water and ethanol and dried on fresh tissue paper to remove spore of microorganisms and any other source of foreign DNA. DNA was extracted by PCI (phenol, chloroform, isoamyl alcohol) purification and ethanol precipitation method. The extracted genomic DNA samples were evaluated both qualitatively and quantitatively using agarose gel electrophoresis (0.8%) and spectrophotometer, respectively. After electrophoresis, DNA samples were documented through Image Documentation System.

Quantification of DNA concentration: For estimating concentration, DNA was quantified through spectrophotometer. The absorbance of each sample was

Table 1: Sequences of microsatellite markers used for this study (Brand:Invitrogen)

Primer name	Chromosome position	Repeat motif		Primer sequence	PIC	Annealing temperature (°C)
RM 585*	6	(TC)45	Fwd	CAGTCTTGCTCCGTTTGTTG	0.84	55
			Rev	CTGTGACTGACTTGGTCATAGG		
RM 510*	6	(GA)15	Fwd	AACCGGATTAGTTTCTCGCC	0.67	55
			Rev	TGAGGACGACGAGCAGATTC		
RM 336*	7	(CTT)18	Fwd	CTTACAGAGAAACGGCATCG	0.79	55
			Rev	GCTGGTTTGTTTCAGGTTCG		
RM 515	8	(GA)11	Fwd	TAGGACGACCAAAGGGTGAG	0.79	55
			Rev	TGGCCTGCTCTCTCTCTCTC		
RM 351	7	(CCG)9 (CGAAG)4	Fwd	CCATCCTCCACCGCCTCTCG	0.50	55
			Rev	TGGAGGAAGGAAAGGGGACG		
RM 234	7	(CT)25	Fwd	ACAGTATCCAAGGCCCTGG	0.828	55
			Rev	CACGTGAGACAAAGACGGAG		

measured at 260 nm and absorbance reading was recorded. Using the absorbance readings, the original sample concentrations were determined according to the following Eq.[10,13,14]:

$$\text{DNA concentration (ng L}^{-1}) = \text{Absorbance} \times \frac{\text{Volume of distilled water (µL)}}{\text{Amount of DNA sample (µL)}} \times \text{C.F (0.05)} \times 1000 \quad (1)$$

Before PCR, DNA concentrations were adjusted to 25 ng µL^{-1} using the following Eq.[6,7,9]:

$$S1 \times V1 = S2 \times V2$$

Where:
S1 = Initial strength (ng µL^{-1})
S2 = Final strength (ng µL^{-1})
V1 = Initial volume (µL)
V2 = Final volume (µL)

PCR analysis for microsatellite markers: In this experiment six random primers viz. RM585, RM234, RM336, RM510, RM515 and RM351 were used for parental surveys and four randomly selected germplasms were used for this. The details of the primers are given in Table 1. Among them five showed polymorphism and three (RM585*, RM510* and RM336*) were selected to evaluate germplasms for salt tolerance.

Amplification of SSR markers by PCR: The PCR cocktail had total volume of 10 µL reaction mixture including 2 µL DNA based on salinity protocol, was placed in the PCR tubes and run in the DNA thermal cycler. The total volume of PCR cocktail for this study was 10 µL per sample. It contained 1 µL 10x PCR buffer, 1 µL dNTPs, 1 µL primer forward (Bio-rad), 1 µL primer reverse (Bio-rad), 0.12 µL *Taq* polymerase (in vitrogen) and 4.78 µL sterile dH_2O. Two microliters genomic DNA was added with 8 µL PCR cocktails. Template DNA was initially denatured at 94°C for 3 min followed by 34 cycles of PCR amplification with the following thermal profile; the 1 min of denaturation at 94°C, 1 min of primer annealing at 55°C and 2 min of primers extension at 72°C. Finally, 7 min incubations at 72°C was allowed for completion of primer extension. For checking amplification, gel electrophoresis was done by 1.5% agarose gel in 0.5X TBE.

Polyacrylamide gel electrophoresis for microsatellite analysis: Before sample loading, both the gel and PCR products were preheated. During preheating of gel, the comb was again placed on the gel allowing sample to be loaded easily and 1200 mL 1×TBE buffer was added to the vertical chamber. The gel was pre-run for 30 min at 120 W to raise the temperature up to 50°C. Meanwhile, PCR-products and 5 µL 100 bp DNA ladder were preheated at 95°C for 5 min. After electrophoresis the gel was stained with ethidium bromide. The gels were viewed by the GEL Doc.

Analysis of SSR data: Only clear and unambiguous SSR markers were scored to the presence and absence of the corresponding band among the genotypes. All the genotypes were scored for the presence (score '1') and absence (score '0') of the SSR bands. Polymorphism information content (PIC) was calculated, according to the method of Anderson[17]. The PIC or expected heterozygosity for each SSR marker was calculated based on the formula $Hn = 1-\Sigma p_i^2$, where p_i is the allele frequency for the i-th allele.

Molecular weight for each amplified allele was measured in base pair using Alpha Ease FC 4.0 software. Based on SSR markers data were subjected to cluster analysis using the microsoft software POPGENE (version 1.31). The allele frequency data from Power Marker Version 3.25 was used to export the data in binary format (Allele presence =1 and allele absence = 0) for analysis with NTSYS-PC version 2.2[15]. The

unweighted pair-group method with arithmetic means (UPGMA) dendrogram was drawn by using the Java software TREEVIEW. Nei[16] genetic distance values was computed using the formula as described in the POPGENE (version 1.31) microsoft software user manual.

RESULTS AND DISCUSSION

Genotyping performance of 25 rice germplasms: Three microsatellite primers were employed to provide genetic diversity among 25 rice germplasms. The primers showed polymorphism in 25 rice germplasms. The microsatellite profiles of loci RM585, RM510 and RM336 are shown in Fig. 1-3.

The number of alleles ranged from 10-12/locus (Table 2) with an average number of alleles was 11. The study revealed that the primer RM510 had the highest number of alleles (12) compared to primers RM336 and RM585. Nearly similar observation was found by RM 152, RM 7075, RM10701 with an average number of allele/locus was 10 among the 26 rice

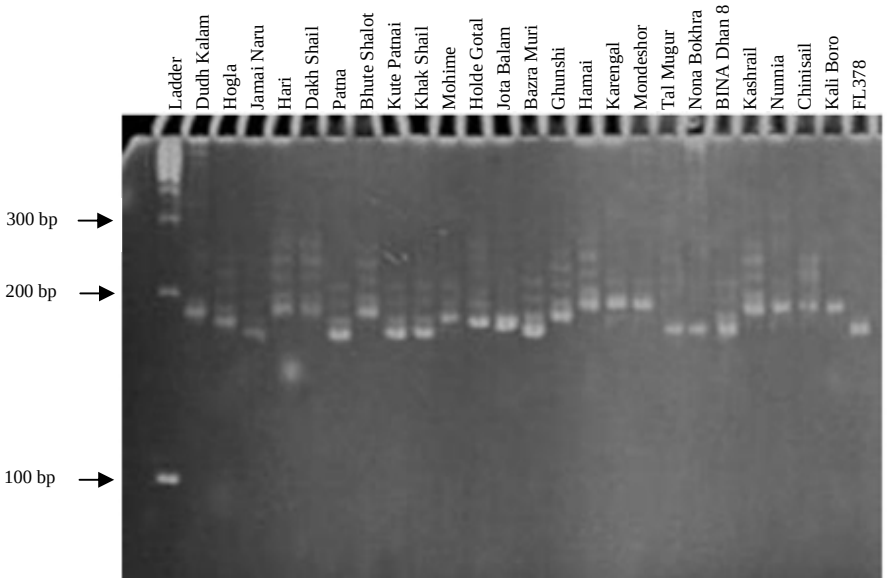

Fig. 1: Microsatellite profiles of 25 rice germplasms at loci RM585

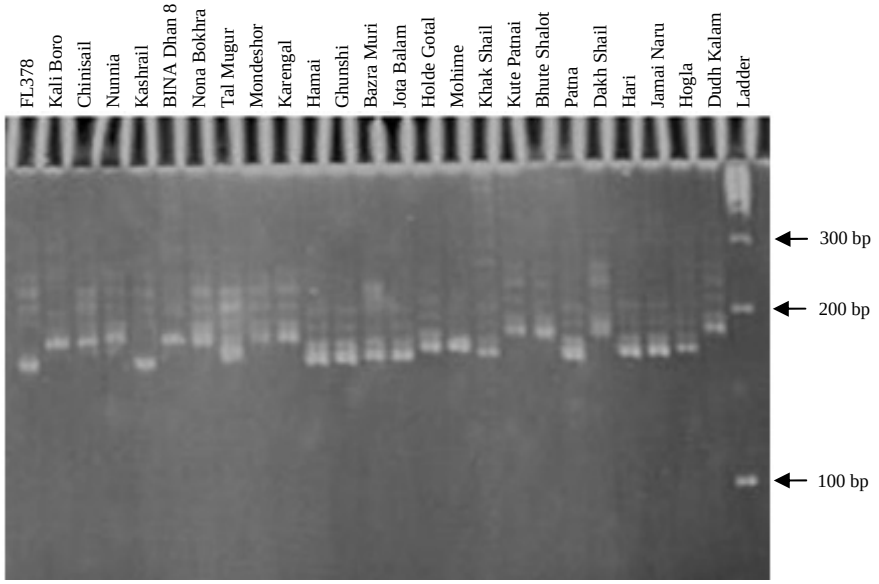

Fig. 2: Microsatellite profiles of 25 rice germplasms at loci RM510

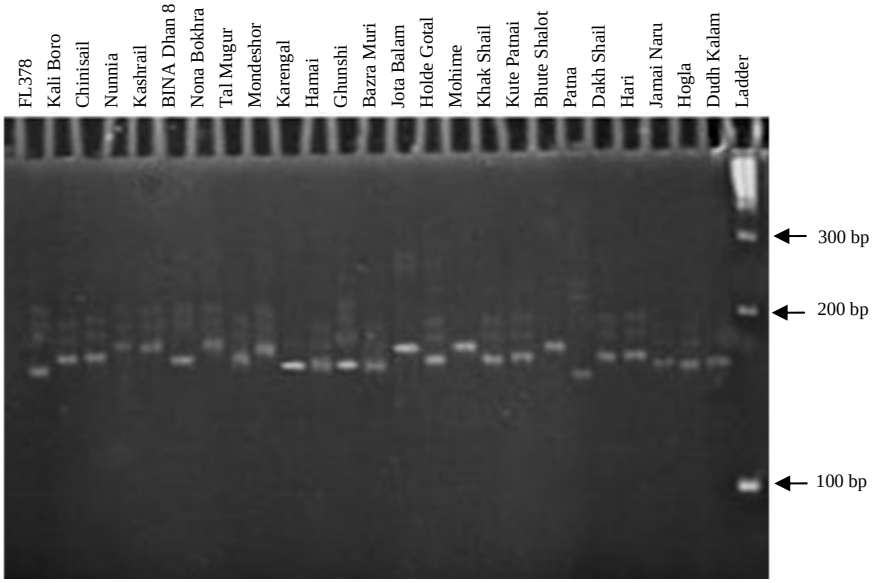

Fig. 3: Microsatellite profiles of 25 rice germplasms at loci RM336

Table 2: Genotyping data among 25 rice genotypes for 3 microsatellites (SSR)

Locus	Repeat motif*	Allele size ranges (bp)	Difference (bp)	Number of alleles	**Rare alleles	PIC	Gene diversity
RM510	(GA)15	165-199	34	12	4	0.8869	0.8960
RM336	(CTT)18	159-182	23	10	3	0.8533	0.8672
RM585	(TC)45	178-205	27	11	1	0.8940	0.9024
Mean	-	-	-	11	2.667	0.8781	0.8885

*Motif of the SSR and number of repeats as previously published. **Rare alleles are defined as alleles with a frequency less than 5%

germplasms by using 6 SSR markers[14]. As a measure of information of microsatellite, the average PIC value was 0.8781 with the range of 0.8869 (RM510), 0.8533 (RM336) and 0.8940 (RM585). The highest PIC value was observed in RM585 while the lowest in RM336. The higher PIC values to indicate that all these primers were capable of distinguishing between genotypes and highly informative. This result was in conformity with the findings of previous research[17,18]. The size of the alleles for RM510, RM336 and RM585 of 25 rice germplasms ranged from 165-199, 159-182 and 178-205 bp, respectively (Table 3). This result is not so similar to previous study that observed, the genetic diversity at each SSR locus was significantly correlated with the number of alleles detected, number of repeat motif and with the allele size range[19]. Marker RM510, RM336, RM585 detected 4, 3 and 1 rare alleles, respectively. Rare alleles are highly informative in fingerprinting of the varieties[20].

Genetic distance: The mean of genetic distances between germplasms was used to evaluate the genetic diversity of different germplasms. The values of pair-wise comparisons of Nei genetic distance (GD) between genotypes were computed from combined data for the 3 primers, ranged from 0.333-1.000 (Table 4). The lower genetic distance (0.333) was observed in Holde Gotal vs. Bazra Muri, Bazra Muri vs. Ghunshi, Karengalvs. Mondeshor, Nunnia vs. Kali Boro, Chinisail vs. Kali Boro, Jamai Naru vs. Hari and Jamai Naru vs. Kute Patnai. The means of genetic distances between germplasms were used to evaluate the genetic diversity of different germplasms. Most of the varieties of the lowest genetic distance were collected from Satkhira, only Nunnia, Chinisail and kali Boro were collected from Patuakhali. But it was observed that, varieties of Satkhira and Patuakhali showed a lower genetic distance with other varities from Satkhira and Patuakhali. FL378 showed a higher genetic distance from most of the varieties except Holde Gotal, Bazra Muri and Hamai. According to the relationship of lower genetic distance among the varieties of Satkhira, most of them were tolerant. A subset of 3 rice groups (including traditional and evolved Basmati and semi dwarf non-Basmati) was analyzed by using 19 SSR loci and 12 inter-SSR-PCR primers and they observed that the lowest genetic distance was among the traditional Basmati varieties, whereas

Table 3: Size and frequency of alleles at 3 SSR loci of 25 rice germplasms

Locus	Allele size (bp)	Allele frequency
RM 510	165	0.0400
	169	0.0800
	171	0.1600
	174	0.0400
	176	0.0800
	178	0.0800
	181	0.0800
	183	0.1600
	186	0.0400
	188	0.0800
	191	0.1200
	199	0.0400
RM 336	159	0.0400
	161	0.0400
	165	0.0400
	167	0.2000
	169	0.0800
	172	0.2000
	174	0.1200
	178	0.1200
	180	0.0800
	182	0.0800
RM 585	178	0.0800
	180	0.0400
	182	0.1200
	185	0.1200
	187	0.0800
	190	0.0800
	195	0.0800
	197	0.1200
	200	0.1200
	202	0.0800
	205	0.0800

the EB varieties showed the highest genetic distance by both the marker assays and they also reported that average genetic distance for the indica and japonica were 0.675 and 0.484, respectively[21].

Genetic similarity analysis using UPGMA: A dendrogram was constructed based on the Nei's genetic distance calculated from the 33 SSR alleles (by 3 markers) generated from the 25 rice genotypes. All 25 rice germplasms could be easily distinguished. The UPGMA cluster tree analysis led to the grouping of the 25 germplasms in five major clusters (Fig.4).

In cluster 1, Dudh Kalam and Kashrail formed sub-cluster 1a while Jamai Naru, Kute Patnai and Hari formed sub-cluster 1b. Observed similarity value between Dudh Kalam and Kashrail was 0.333 and genetic distance value was 0.667. Jamai Naru and Kute Patnai are in the same position in dendrogram. Both of them are tolerant in SES scoring. Hari was on the other hand of sub cluster 1b. This variety was highly susceptible in SES scoring. Hari showed a similarity value of 0.333 with Dudh Kalam, Kashrail and Kute Patnai and 0.667 with Jamai Naru. All of these varieties of cluster 1 were collected from Satkhira except Kashrail.

Table 4: Summary of (Nei's, 1972) genetic distance values for 25 rice germplasms

Variety	Dudh kalam	Mohime	Holde Gotal	Jota Balam	Bazra Muri	Ghunshi	Hamai	Karengal	Mondeshor	Tal Mugur	Nona Bokhra	Hogla	BINA8	Kashrail	Nunnia	Chinisail	Kali Boro	FL378	Jamai Naru	Hari	Dakh Shail	Patnai	Bhute Shalot	Kute Patnai	Khak Shail
Dudh kalam	-																								
Mohime	1.0000	-																							
Holde Gotal	1.0000	0.6667	-																						
Jota Balam	1.0000	1.0000	1.0000	-																					
Bazra Muri	1.0000	1.0000	0.3333	0.6667	-																				
Ghunshi	1.0000	1.0000	0.6667	0.6667	0.3333	-																			
Hamai	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	-																		
Karengal	1.0000	1.0000	0.6667	1.0000	0.6667	0.6667	1.0000	-																	
Mondeshor	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.3333	-																
Tal Mugur	1.0000	1.0000	1.0000	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	-															
Nona Bokhra	1.0000	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	-														
Hogla	1.0000	0.6667	0.6667	1.0000	0.6667	0.6667	1.0000	0.6667	1.0000	1.0000	1.0000	-													
BINA8	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	0.6667	1.0000	-												
Kashrail	0.6667	1.0000	1.0000	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	-											
Nunnia	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	1.0000	0.6667	1.0000	1.0000	1.0000	-										
Chinisail	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	-									
Kali Boro	0.6667	1.0000	1.0000	1.0000	0.6667	1.0000	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.3333	0.3333	-								
FL378	1.0000	1.0000	0.6667	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	-							
Jamai Naru	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	1.0000	-						
Hari	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	1.0000	1.0000	1.0000	0.3333	-					
Dakh Shail	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	0.6667	0.6667	0.3333	1.0000	1.0000	1.0000	-				
Patnai	1.0000	1.0000	1.0000	0.6667	0.6667	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	1.0000	-			
Bhute Shalot	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	1.0000	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	1.0000	-		
Kute Patnai	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	1.0000	0.3333	0.6667	0.6667	1.0000	0.6667	-	
Khak Shail	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	0.6667	1.0000	1.0000	0.6667	0.6667	1.0000	1.0000	1.0000	0.6667	1.0000	1.0000	1.0000	-

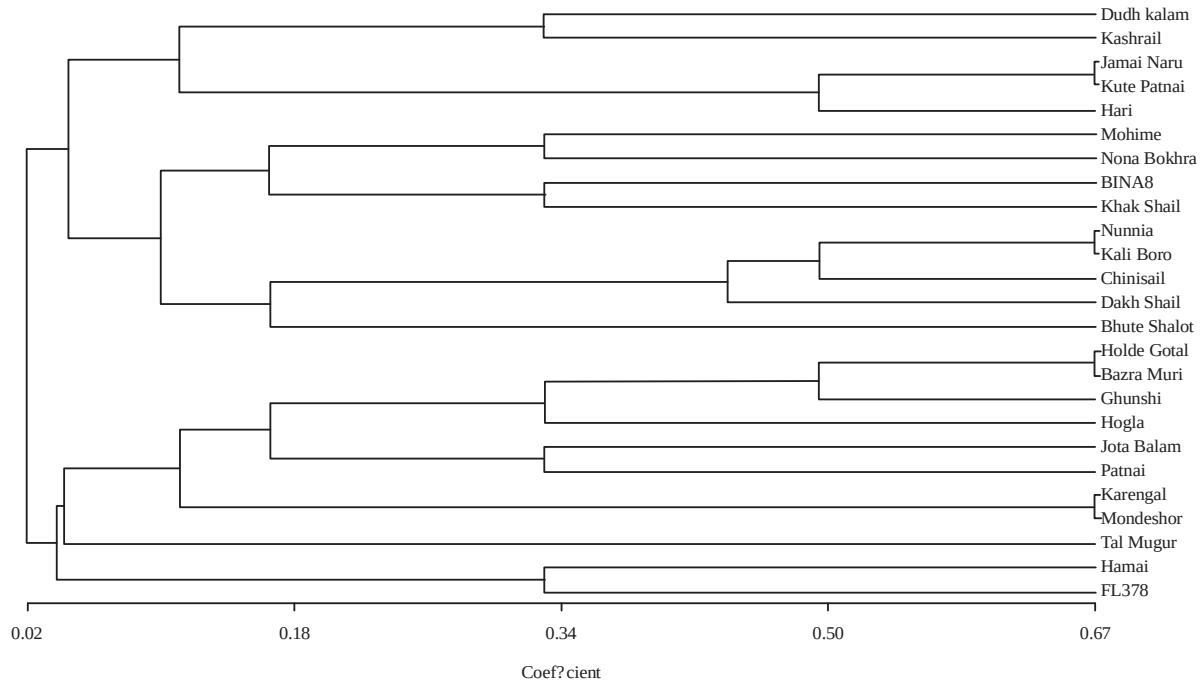

Fig. 4: Dendrogram for 25 rice germplasms derived from a UPGMA cluster analysis

Cluster 2 had two sub clusters (sub cluster 2a and sub cluster 2b). Sub cluster 2a had two sub-sub clusters. One sub-sub cluster was formed by Mohime and Nona Bokhra. Mohime was highly susceptible in SES scoring whereas Nona Bokhra showed tolerance. Both of these varieties were collected from Satkhira. Nona Bokhra showed a similarity value of 0.333 with Mohime. The other sub-sub cluster was formed by KhakShail and BINA Dhan 8. These varieties also showed a similarity value of 0.333. The main notable thing is that KhakShail was collected from Satkhira and BINA Dhan 8 from IRRI.

Sub cluster 2b had two sub-sub clusters. One sub-sub cluster was formed by Nunnia, Chinisail and Kali Boro. Nunnia showed a close similarity of 0.667 and 0.333 with Kali Boro and Chinisail, respectively. Kali Boro and Chinisail showed a similarity value of 0.667. It was observed that Nunnia and Kali Boro were in same position of cluster whereas Chinisail was at the other hand. All of these three varieties were highly susceptible by SES scoring. Dakh Shail was at the other hand of sub-sub cluster 2b. Dakh Shail also showed a close similarity of 0.667 with Kali Boro and 0.333 with Nunnia and Chinisail.

Dakh Shail was tolerant in SES scoring. It should be noted that Nunnia, Chinisail and Kali Boro were collected from Patuakhali but Dakh Shail was collected from Satkhira. The other hand of sub cluster 2b has one variety, which is Bhute Shalot. It is moderately tolerant by agronomic character. Bhute Shalot showed a similarity value of 0.333 with Nunnia and Dakh Shail.

Cluster 3 showed two sub clusters (Sub cluster 3a and Sub cluster 3b). Sub cluster 3a had two sub-sub clusters. One sub-sub cluster of 3a had four varieties. One branch had Holde Gotal, Bazra Muri and Ghunshi whereas Holde Gotal and Bazra muri were in same position. Holde Gotal and Bazra Muri showed a highest similarity index value of 0.667. The remaining group had only one variety, which is Hogla. All of them were found tolerant by agronomic character. The other sub-sub cluster of 3a showed two varieties, which are Jota Balam and Patnai. Patnai showed a similarity value of 0.333 with Jota Balam, Ghunshi and Bazra Muri. It should be noted that Patnai was tolerant along with Holde Gotal, Bazra Muri and Ghunshi but Jota Balam was moderately tolerant by SES scoring.

Sub cluster 3b had two varieties which are Karengal and Mondeshor. These two varieties were moderately tolerant by agronomic trait. Karengal showed a similarity indexed value of 0.667 with Mondeshor. This is the highest similarity value obtained in this study.

Tal Mugur alone belonged to cluster 4 and this genotypes is separated from other 24 genotypes. Under salt stress, this line was tolerant. This variety showed a higher genetic distance (1.000) and a lower similarity (0.000) with FL 378. It showed similarity with BINA Dhan 8 and the value is 0.333. Higher genetic distance was previously found that is highly co-related to these findings[22].

Cluster 5 showed only two varieties which are Hamai and FL378. Hamai was found as moderately tolerant whereas FL378 was found as tolerant by SES scoring. They had a similarity value of 0.333. Based on above result, it may be concluded that, the maximum tolerant and moderately tolerant germplasms grouped in same cluster due to lower genetic distance. The dendrogram revealed that the genotypes that derivatives of genetically similar type clustered together.

The dendrogram related that the genotypes that derivatives of genetically similar type clustered together[23]. Rice genotypes are classified into 11 distinct groups that are similar of this finding[3].

CONCLUSION AND FUTURE RECOMMENDATIONS

The assessment of genetic diversity is an essential component in germplasm characterization and conservation. The results derived from analysis of genetic diversity at the DNA level could be used for designing effective breeding programs aiming to broaden the genetic bases of commercially grown varieties. From this study, it can be concluded from genotypic analysis that Holde Gotal, Bazra Muri and Hamai were salt tolerant compared to FL378. All of them were collected from Satkhira, Bangladesh. Holde Gotal and Bazra Muri also showed tolerance but Hamai showed moderately tolerance at the phenotypic analysis. Based on the diversity analysis, the salt tolerant high yielding genotypes from different clusters could be utilized for the recombination breeding program with high yielding genotypes to enhance the level of grain quality, tolerance and high yield.

This molecular characterization information could be helpful to the breeders for further planning of rice breeding program to improve grain quality, yield quality and specially tolerance to salinity. Similar result from different marker will help to optimize the marker analysis. It will also help to identify the gene related to salt tolerance and a detail analysis of genetic diversity, gene identification and transformation.

SIGNIFICANCE STATEMENTS

This study discovered some tolerant rice variety of Bangladesh along with genetic polymorphism and diversity. This information could be helpful for further planning of the rice breeding program to improve grain quality, yield quality and especially tolerance to salinity. The results may be utilized as the source of tolerant gene for elite molecular breeding and gene transformation and used as a baseline for improvement of rice varieties in Bangladesh.

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REFERENCES

1. Singh, R.K. and B. Mishra, 1997. Stable genotypes of rice for sodic soils. Indian J. Genet. Plant Breed., 57: 431-438.
2. FAO., 2004. Mechanisms of salt tolerance: Sodium, chloride and potassium homeostasis in two rice lines with different tolerance to salinity stress. Food and Agriculture Organization, Rome.
3. Chakravarthi, B.K. and R. Naravaneni, 2006. SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa* L.). Afr. J. Biotechnol., 5: 684-688.
4. Gnanesh, A.U., V. Krishna, R.S. Kumar, Venkatesh, S.R.S. Kumar and H.E. Shashidhar, 2012. Regeneration of plantlets from mature embryo calli of Western Ghats land race cultivar of rice, *Oryza sativa* L. Indian J. Exp. Biol., 50: 164-170.
5. Amirjani, M.R., 2010. Effect of NaCl on some physiological parameters of rice. Eur. J. Biol. Sci., 3: 6-16.
6. Haque, S.A., 2006. Salinity problems and crop production in coastal regions of Bangladesh. Pak. J. Bot., 38: 1359-1365.
7. Islam, M.M., M.N.H. Mondol, R.M. Emon, S.N. Begum, S.K. Bhowmik and A.K. Hasan, 2007. Screening of salt tolerant rice genotypes using SSR markers at seedling stage. Bangladesh J. Prog. Sci. Tech., 5: 45-48.
8. Haq, T.U., J. Gorham, J. Akhtar, N. Akhtar and K.A. Steele, 2010. Dynamic quantitative trait loci for salt stress components on chromosome 1 of rice. Funct. Plant Biol., 37: 634-645.
9. Haq, T.U., J. Akhtar, S. Nawaz and R. Ahmad, 2009. Morpho-physiological response of rice (*Oryza sativa* L.) varieties to salinity stress. Pak. J. Bot., 41: 2943-2956.
10. Bhowmik, S.K., S. Titov, M.M. Islam, A. Siddika, S. Sultana and M.H. Haque, 2009. Phenotypic and genotypic screening of rice genotypes at seed-ling stage for salt tolerance. Global J. Biotechnol. Bio-Chem., 4: 126-131.
11. Gregorio, G.B., 1997. Tagging salinity tolerant genes in rice using Amplified Fragment Length Polymorphism (AFLP). Ph.D. Thesis, University of the Philippines, Los Banos College, Laguna, Philippines.

12. Islam, M.M., 2004. Mapping salinity tolerance genes in rice (*Oryza sativa* L.) at reproductive stage. Ph.D. Thesis, University of the Philippines Los Banos, College, Laguna, Philippines.
13. Bhuiyan, M.A.R., 2005. Efficiency in evaluating salt tolerance in rice using phenotypic and marker assisted selection. M.Sc. Thesis. Bangladesh Agricultural University, Mymensingh, Bangladesh.
14. Dhar, P., M. Ashrafuzzaman, S.N. Begum, M.M. Islam and M.M.H. Chowdhury, 2012. Identification of salt tolerant rice genotypes and their genetic diversity analysis using SSR markers. Int. J. Biol. Sci., 2: 45-50.
15. Anderson, J.A., G.A. Churchill, J.E. Autrique, S.D. Tanksley and M.E. Sorrells, 1993. Optimizing parental selection for genetic linkage maps. Genome, 36: 181-186.
16. Nei, M., 1972. Genetic distance between populations. Am. Naturalist, 106: 283-292.
17. Mohammad-Nejad, G., A. Arzani, A.M. Rezai, R.K. Singh and G.B. Gregorio, 2008. Assessment of rice genotypes for salt tolerance using microsatellite markers associated with the saltol QTL. Afr. J. Biotechnol., 7: 730-736.
18. Aliyu, R.E., A.K. Adamu, S.O. Alonge, S. Muazu and G. Gregorio, 2011. Tagging and validation of SSR markers to salinity tolerance QTLs in rice (*Oryza* sp). Proceedings of the International Conference on Biology, Environment and Chemistry, Volume 1, (IPCBEE'10), Singapore, pp: 328-332.
19. Herrera, T.G., D.P. Duque, I.P. Almeida, G.T. Nunez, A.J. Pieters, C.P. Martinez and J.M. Tohme, 2008. Assessment of genetic diversity in Venezuelan rice cultivars using simple sequence repeats markers. Electron. J. Biotechnol., Vol. 11. 10.2225/vol11-issue5-fulltext-6.
20. Jayamani, P., S. Negrao, M. Martins, B. Macas and M.M. Oliveira, 2007. Genetic relatedness of Portuguese rice accessions from diverse origins as assessed by microsatellite markers. Crop Sci., 47: 879-886.
21. Nagaraju, J., M. Kathirvel, R.R. Kumar, E.A. Siddiq and S.E. Hasnain, 2002. Genetic analysis of traditional and evolved Basmati and non-basmati rice varieties by using fluorescence-based ISSR-PCR and SSR markers. Proc. Natl. Acad. Sci., 99: 5836-5841.
22. Bhowmik, S.K., M.M. Islam, R.M. Emon, S.N. Begum, A. Siddika and S. Sultana, 2007. Identification of salt tolerant rice cultivars via phenotypic and marker-assisted procedures. Pak. J. Biol. Sci., 10: 4449-4454.
23. Ashraf, M., H.R. Athar, P.J.C. Harris and T.R. Kwon, 2008. Some prospective strategies for improving crop salt tolerance. Adv. Agron., 97: 45-110.