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DNA Fingerprinting of Rice Lines for Salinity Tolerance at Reproductive Stage

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Abstract

Salinity is the most common abiotic stresses leading to the reduction of rice yield in many rice-growing areas of the world. This study was undertaken to assess the genetic diversity among saline treatment and susceptible rice lines using molecular marker (SSR). Salinity screening was performed at reproductive stage using sustained water bath following IRRI standard protocol. Twenty two rice lines were used for molecular analysis using three SSR markers: RM1287, RM342 and RM493 to determine salinity tolerance at reproductive stage. For DNA fingerprinting of rice Varieties, DNA was extracted from leaf samples using IRRI standard protocol. Amplified microsatellite loci were analyzed for polymorphism using Polyacrylamide Gel Electrophoresis (PAGE) and the result revealed that all the primers detected polymorphism among the rice lines analyzed. Using 3 SSR markers, a total of 25 alleles were detected among the 22 rice lines. The polymorphism information content (PIC) reflects the diversity allele frequency among the lines, which ranged from 0.59 to 0.88 with an average of 0.74. RM493 was the best marker for identification of genotypes as revealed by PIC values. The results of microsatellite marker based DNA fingerprinting analysis will be useful for the selection of parents for developing salt tolerant rice variety through molecular breeding.

Keywords: DNA fingerprinting; Salinity tolerance; Reproductive stage; *Oryza sativa*

Introduction

Rice production is affected by many biotic and abiotic stresses throughout the world. Among these abiotic stress alone contributes to about 50% of the total yield losses. Among the abiotic stresses soil/water salinity is considered as one of the major and prevalent stresses limiting rice production in the world [1]. Approximately 30% of the total irrigated land worldwide is salt-affected [2]. and that limits the total rice production in the world. Salinity is considered as one of important physical factors influencing rice production. At present, salinity is the second most widespread soil problem in rice growing countries after drought and is considered as a serious constraint to increase rice production worldwide [3].

In Bangladesh, rice occupies about 70% of the total cropped area of about 13.9 million hectares. Approximately 11% of the world's arable land is cultivated annually with rice [4], ranking next after wheat. Different cultures have preferences for different types of rice. Despite the annual production shortfall of 2 to 4 million metric tons, rice provides more than 80% of the food requirements for the common people of Bangladesh [5]. Furthermore, world population is increasing day by day. To fed with the increasing population it is becoming essential to utilize these saline soils either by reclamation of salinity or by growing salt tolerant plants [6]. Reclamation of salinity is difficult and expensive and not the permanent solution of the problem. Introduction of salt-tolerant variety is the realistic approach to obtain better yield under saline conditions [6,7]. Soil salinity is one of the major constraints to rice production in coastal areas of Bangladesh. To reduce salinity, major engineering structure and expensive soil amendments are needed. However, these amendments require large investments. Thus tailoring rice plants to adapt in salt stress prove condition to be practical and effective. In fact, a few varieties have been developed to cope with the varied soil and climatic conditions of the coastal areas. The urgent need therefore, for the development of saline tolerant varieties in the coastal areas of Bangladesh [8].

The conventional methods of plant selection for salt tolerance are not easy because of the large environment effects and the low narrow sense of heritability for salt tolerance [3]. The advanced biotechnological techniques provides adequate support to evaluate genetic variation in both phenotypic and genotypic levels. Molecular markers are powerful tools in the assessment of genetic variation, in the elucidation of genetic relationships within and among species and have demonstrated the potential to detect genetic diversity and to aid in the management of plant genetic resources [9-11]. SSR markers are ideal markers for constructing high-resolution genetic maps, assisting selection and studying the genetic diversity in germplasms. It has been extensively exploited for genome mapping and for wide range of population and evolutionary studies in Arabidopsis [12], rice [13] and other animal and plant species [14]. This marker has the advantage of reliability, reproducibility, discrimination, standardization and highly informative and cost efficiency over RFLPs. Microsatellite markers are proving valuable for tagging and mapping of salt tolerance genes [15]. The general objective of this study is DNA fingerprinting analysis and identification of the salt tolerant rice lines using microsatellite markers (SSR).

Materials and Methods

Experimental site

The experiments were conducted at the experimental fields of Bangladesh Institute of Nuclear Agriculture (BINA), BAU, Mymensingh. 22 rice lines were grown during July-December, 2013.

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Molecular Characterization is done at the laboratory of BINA under Biotechnology division.

Experimental materials

22 rice lines were used with diverse genetic background. Rice lines with different genes were used in this study. A short description of the lines that were used in this study is listed in Table 1. To conduct the screening technique at the reproductive stage glass fiber tanks(any size with at least 22.0 cm height), Crude or non-refined salt, Electrical Conductivity meter, Well ground soil, collected from paddy field, Fertilizer (N, P and K).

Planting tray preparation:

Two experimental sustained water bath were prepared by ploughing followed by laddering weeds and stubbies were removed from the field. The land was mudded and leveled well before transplanting. At the final land preparation chemical fertilizers such as Urea, Triple Super Phosphate (TSP), Murate of Potash (MP) were applied.

Sowing of pre germinated seeds:

The seeds were soaked into water for 24 hours and incubated in petri dishes for 48 hours for quick germination in incubation. The pre germinated seeds were sown in trays in 3rd June, 2013. Soil and germination condition were as follows:

Tray-1: Dry seed+ Dry soil, Tray-2: Wet seed+ puddle

Data collection before treatment

To observed expression of Saltol gene measured the effect of salinity treatment on test entries compared with before treatment collected morphological markers information. Following of the morphological markers data is essential to measure the expression level of Saltol locus visibly at salinity condition. The number of total tiller per plant, total number of leaf per plant, leaf length and breath, plant height, plant type, and old and young leaves color etc. in this experiment, collected above morphological markers information of three or five plants of each entries.

Preparation of saline solution

To prepare the saline solution, I used 1 m height 6 pieces cylindrical shape water bath each of them contains 271 liter water. Prepared salinized water solution up to the desired EC level by dissolving crude salt (NaCl) in water while stirring. In this purpose 3 g/L and 4 g/L crude salt was used to make 14 dS/m saline solution [3]. Added 1897 g crude salt in 271 L water to make 14 dS/m saline solution. The plastic water bath container was filled up with salinized water solution. Following is the formula to make saline solution:

$$\text{Required amount of salt}(g) = \text{salt per liter for} 1\frac{dS}{m} \times \text{Desired Electric Cconductivity}$$

Where, 1dS electric conductivity per meter =135.5 g salt per liter.

Treatment setup

When the seedlings were at reproductive stage, water was siphoned out all from the trays and given a 24-h break. Then salinized water solution was prepared up EC 8 dSm^{-1} by dissolving crude salt. After then, normal setup watered with ordinary tap water and salinized setup watered with salinized setup watered with salinized water. The EC of the salinized water was monitored every week and adjust when necessary using crud salt and tap water. Salinity level was maintained until maturity.

Screening of rice lines at reproductive stage

The lines were evaluated for their tolerance to salinity in sustained water bath using IRRI standard protocol [3]. The experimental design was completely randomized design (CRD) with three replications. The evaluation was done at the experimental field of BINA, Mymensingh.

The modified standard evaluation score (SES) of IRRI was used to assess the visual symptoms of salt injury (Table 2). This scoring discriminates the tolerant, moderately tolerant and susceptible rice lines. Initial scoring was started at 15-day after salinization and final scoring was done at 21-day after salinization.

Harvesting

Harvesting was done upon the maturity of different lines. Different lines attain their maturity at different times. The date of harvesting was confined when 80% of the grain attained golden yellow color. Harvesting was completed by 30th October to 23th November 2013.

Data collection

Data were recorded on individual plant of the experimental tray. Among the studied characters, days to 50% flowering and plant height were recorded in the field and the remaining characters were recorded in the laboratory after harvesting. The characteristics were as follows……

- **Days to 50% flowering:** Recorded as days from sowing to 50% of the plants of each tray flowered.
- **Days to maturity:** recorded on the basis of the days from sowing to physiological maturity of the grains.
- **Plant height:** the length of main culms from the ground level to tip of its panicle was measured.
- **Tiller number per plant:** the total number of tiller as well as effective and non-effective tiller numbers was counted from each of the sample plant.

Sl no.	Lines	Source
G1	RC 191	IRRI*
G2	RC 192	IRRI*
G3	RC 193	IRRI*
G4	RC 217	IRRI*
G5	RC 221	IRRI*
G6	RC 222	IRRI*
G7	RC 225	IRRI*
G8	RC 227	IRRI*
G9	RC 229	IRRI*
G10	RC 251	IRRI*
G11	RC 249	IRRI*
G12	BRRI dhan11	BRRI
G13	BINAdhan7	BINA
G14	BINAdhan8	BINA
G15	BINAdhan 10	BINA
G16	BINAdhan 11	BINA
G17	Joli Aman	BINA
G18	BINAdhan 12	BINA
G19	BRRI dhan 29	BRRI
G20	Pajam	BRRI
G21	BRRI dhan 39	BRRI
G22	FL 478	BRRI

Table 1: The Sources of the lines that were used in this study.

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

Table 2: Modified standard evaluation score (SES) of visual salt injury at reproductive stage.

• **Panicle length:** panicle length was recorded as the distance cm from the last node of the rachis to tip of the main panicle which was randomly selected from each plant and the average was taken.

• **Filled grain number per plant:** the spikelet with kernel was considered as filled grain and counted from one selected panicle from each plant and the average was taken.

• **Unfilled grain number per plant:** the spikelet without kernel was considered as unfilled grain and counted from one selected panicle from each plant and the average was taken.

• **25 grain weight:** 25 clean and sun dried grains were counted from the sample plant after sun drying the samples and the average was taken at 14% seed moisture content.

• **Panicle weight**: whole panicle weight of each sample panicle was taken after cleaning and sun drying the samples and the average was taken at 14% seed moisture content.

• **Grain yield per plant:** Total grain weight (g) of each sample plant was taken after cleaning and sun drying the samples and the average was taken at 14% seed moisture content.

• **Genomic DNA isolation:** Juvenile, vigorously growing fresh leaf samples was collected from 21-30 days old seedlings for isolation of genomic DNA. Leaf samples were collected from selected Lines. Initially, healthy portion of the youngest leaves of the tiller was cut apart with sterilized scissors and washed in 70% ethanol and dH_2O and dried on fresh tissue paper to remove spore of microorganisms and any other source of foreign DNA. The collected leaf samples were then put into polythene bags and stored in a -20°C refrigerator. Leaf samples were taken from freezer prior to genomic DNA isolation and kept them in ice box until starting work. Leaf samples were held with sterilized forceps and cut into 1-3 cm piece with sterilized scissors. Genomic DNA samples of each genotype were extracted from vigorous, young growing leaf tissue using the mini preparation Modified CetylTrimethyl Ammonium Bromide (CTAB) method [16]. At first healthy portion of vigorous, young actively growing leaves were cut apart with sterile scissors and forceps. The leaf material was washed in sterile distilled water and ethanol as well as dried on tissue paper to remove excess water and spore of microorganisms and any other source of foreign DNA. Then the DNA from leaf tissues were extracted following step by step.

SSR markers and PCR amplification

Five SSR primer pairs were selected on the basis of the published rice microsatellite framework map for the genetic diversity analysis Finally 3 primers exhibiting good quality banding patterns and sufficient variability were selected for further analysis. Information regarding the original source, repeat motifs, primer sequences, expected length, chromosomal localizations and repeat types of the SSRs can be found in the Web database (http://www.gramene.org). Prior to DNA amplification, a PCR cocktail was prepared containing all required components. PCR cocktail was prepared into eppendorf tube, which was placed into ice. The PCR cocktail had total volume of 10.0 µl reaction mixture including 2 µl DNA based on salinity protocol, was placed in the PCR tubes and run in the DNA thermal cycler. 2.0 µl of each template DNA samples were pipette into the wells of the PCR tubes. The primers were pipetted first into PCR tubes compatible with the thermocyclear used (0.2 ml). For each DNA sample being tested, a pre-mix was then prepared including, in the following order: buffer, dNTPs, DNA template and sterile distilled water. *Taq* DNA polymerase enzyme was then added to the pre-mix. The pre-mix was then mixed well and aliquoted into tubes containing primers. The tubes were then sealed and placed in a thermocycler and the PCR reaction was started immediately. Amplified products were stored at -20°C until further use.

Electrophoresis of the amplified product by page (0.8%) and visualization

The gel solution was prepared in a beaker with a magnetic stirring bar. Each PCR product was mixed with gel loading dye (bromophenol blue, xylene cyanol and sucrose) and electrophoresis was carried out in a mini vertical electrophoresis tank run on 8% polyacrylamide gels in TBE buffer. 2 µl of sample was added in each well. 25 bp DNA ladder was used for size determination. The cover was put on the tank. The electrodes were connected to the power supply and run for about 3-3.5 hr at 80 volts (running time may be variable depending on the size of the PCR fragments). The gel after electrophoresis was soaked in ethidium bromide (10 mg/ml) solution for 15- 20 min. The gel was viewed by the GEL Doc. The images that appeared on the computer were saved for analysis.

SSR data analysis

The size (in nucleotide base pairs) of the amplified band for each microsatellite marker was determined based on its migration relative to a molecular weight size marker (25 bp DNA Ladder) with the help of Alpha Viwer software (Alpha Innotech, USA). The summary statistics including the number of alleles per locus, major allele frequency, gene diversity and Polymorphism Information Content (PIC) values were determined using Power Marker version 3.23, NTSYS-pc was used to construct a UPGMA (unweighted pair group method with Arithmetic mean) dendogram showing the distance-based interrelationship among the genotypes. Genetic distance was calculated using the coefficient in PowerMarker.(version 3.25)

Results

In respect of primer RM 1287, allele size ranged from 159bp-172bp, whereas primer RM493, RM342, showed a range 203 bp-221 bp, 146 bp to 159 bp. Using 3 SSR markers, a total of 25 alleles were detected among the 22 rice lines. The average number of allele per locus was 8.33, with a range of 6 (RM1287) to 12 (RM493) (Table 2). Major allele is defined as the allele with the highest frequency and also known as most common allele at each locus. The size of the different major alleles at different loci ranges from 150bp (RM493) to 212 bp (RM342) (Table 3). On average, 38% of the 22 rice lines shared a common major

Locus	Repeat Motif*	Allele Size ranges(bp)	No. of alleles	*Rare alleles	Null allele	PIC
RM1287	(AG)17	159-172	6	-	2	0.75
RM493	(CTT)9	203-221	12	6	-	0.88
RM342	(CAT)12	142-159	7	4		0.59
Mean			**8.333**	**3.33**	**2**	**0.74**
Locus	**Repeat Motif***	**Allele Size ranges(bp)**	**No. of alleles**	***Rare alleles**	**Null allele**	**PIC**
RM1287	(AG)17	159-172	6	-	2	0.75
RM493	(CTT)9	203-221	12	6	-	0.88
RM342	(CAT)12	142-159	7	4		0.59
Mean			**8.333**	**3.33**	**2**	**0.74**

Table 3: List of 3 microsatellites (SSR) marker among 22 rice lines with their Repeat Motif, Allele size, No of alleles, Rare alleles, Null allele and PIC values.

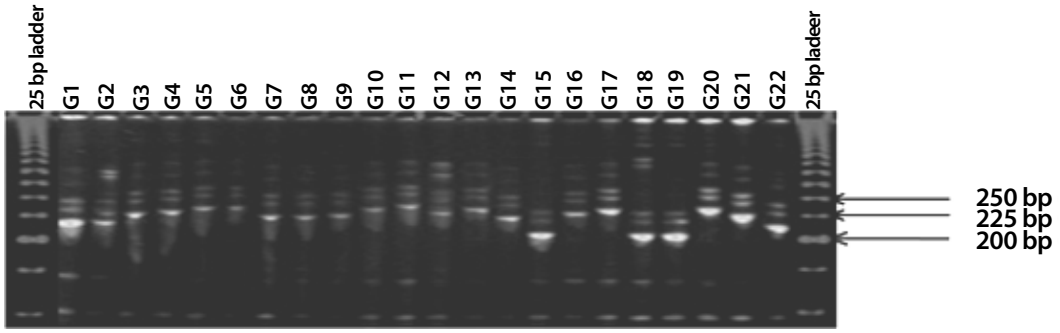


Figure 1: SSR profile of 22 rice lines using primer RM493.

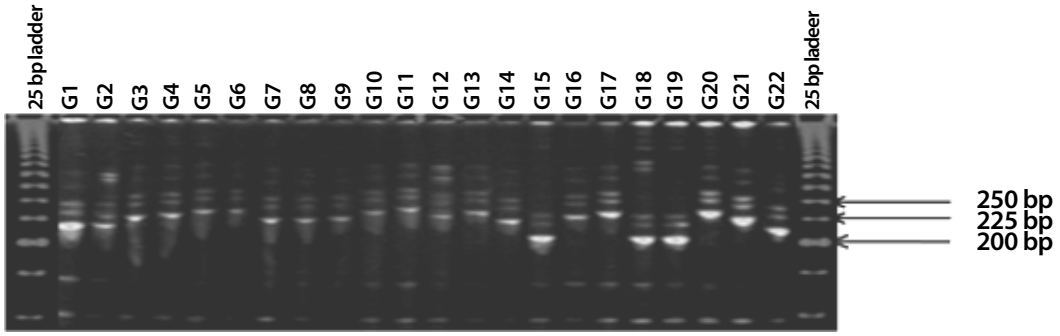


Figure 2: SSR profile of 22 rice lines using primer RM1287.

allele ranging from 18% (RM493) to 59% (RM342) common allele at each locus.

The highest gene diversity (0.89) was observed in loci RM493 and the lowest gene diversity (0.61) was observed in loci RM342 with a mean diversity of 0.76 (Table 3). It was observed that marker detecting the lower number of alleles showed lower gene diversity than those which detected higher number of alleles which revealed higher gene diversity.

Polymorphism information content (PIC) value is a reflection of allele diversity and frequency among the varieties. PIC value of each marker can be evaluated on the basis of its alleles. PIC varied significantly for all the studied SSR loci. In the present study, the level of polymorphism among the 22 rice lines was evaluated by calculating PIC values for each of the 3 SSR loci. The PIC values ranged from 0.59 (RM342) to 0.88 (RM493) with an average of 0.74 per locus (Table 2). The highest PIC value was 0.88 for RM493. The lowest PIC value observed 0.59 for RM 342 (Figures 1 and 2).

Genetic similarities were calculated from the data of coefficient (Table 4). The similarly matrix was used to determine the level of relatedness among the studied genotypes. Pair-wise estimates of similarity ranged from 0.33 to 1.00 and the average similarity among all 22 rice lines was 0.29. The lowest genetic distance (0.333) was observed in RC251 and BRRIdhan11, RC 251 and BINAdhan7, BRRIdhan11 and BINAdhan7, RC251 and BINAdhan11, BRRIdhan11 and BINAdhan11, BINAdhan7 and BINAdhan11, RC249 and BINAdhan12, RC249 and BRRIdhan29, RC249 and Pajam, RC251 and BRRIdhan39, BRRIdhan 39 and FL478, RC191 and RC193, RC221 and BINAdhan11, RC222 and BINAdhan11, RC252 and RC229, BRRI dhan 11 and RC229, BINAdhan 11 and RC229, BINAdhan 7 and RC229, BINAdhan 11 and RC229, RC225 and RC229, RC227 and RC229. The highest genetic distance of 0.100 was observed between a number of accession or variety pair. Some of them RC 217 and RC 227, RC 217 and RC 250, RC 217 and RC 252, RC217 and RC 221, RC 217 and RC 192, RC191 and RC251, RC249 RC249 and BINA dhan 10, RC191 and RC229, BINAdhan 8 and RC229, BINA dhan10 and RC229, RC225 and RC227, RC217 and RC227, RC217 and RC225, joliAman and FL478, JoliAman and BIRRI dhan 29, RC193 and JoliAman, RC217 and JoliAman etc (Figure 3 and Figure 4).

Locus	Sample size	Major allele		Availabiliy	Gene diversity	Heterozygocity
		Size (bp)	Frequency (%)			
RM1287	22	160	36	1.0000	0.7810	0.0000
RM493	22	212	18	1.0000	0.8926	0.000
RM342	22	150	59	1.0000	0.6157	0.0000
Mean	**22**	**174**	**38**	**1.0000**	**0.7631**	**0.0000**

Table 4: List of 3 microsatellites (SSR) marker among 22 rice lines with their major allele, gene diversity.

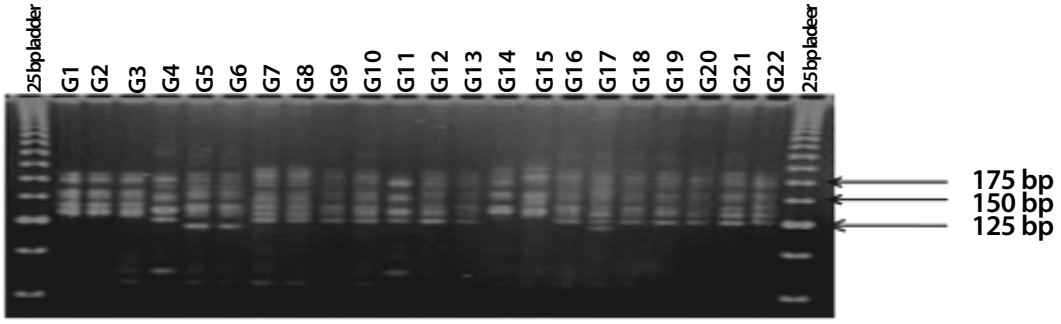


Figure 3: SSR profile of 22 rice lines using primer RM342.

UPGMA method was used for cluster analysis to differentiate the studied lines into groups based on similarity coefficient. Six clusters were made at genetic similarity level of 0.26-0.51 (Figure 4). The UPGMA cluster analysis led to the grouping of the 22 genotype in two major clusters. The two main clusters is cluster II and cluster III contains more lines. In cluster I RC191 and RC192 showed 100% similarity, and RC191, RC192 and RC193 showed 32% dissimilarity between them. In cluster II RC221, RC225, BINAdhan 8 and RC222 showed 32% dissimilarity among them. RC249 and RC251 showed 100% similarity, RC229 showed 32% dissimilarity with them, FL478 showed 68% dissimilarity with them in this cluster In cluster III BINAdhan10 and RC217 showed 100% similarity, BRRI dhan 11 showed 32% similarity with them, 56% dissimilarities showed among pajam and BIIRI dhan11, BINAdhan10, RC217. BINAdhan11 and BINAdhan12 showed 100% similarity. In cluster IV joliAman and RC227 showed 32% dissimilarities, BIRIdhan 29 showed 67% dissimilarities with JoliAmanAnd RC227. BINAdhan 7 And BIRRIdhan 39, each of tem from single cluster and they showed 100% dissimilarities.

Discussion

Using 3 SSR markers, a total of 25 alleles were detected among the 22 rice genotype. The average number of allele per locus was 8.33, with a range of 6 (RM1287 to 12 (RM493). These values were comparable to those reported earlier (8.42 alleles per locus; range 3-21) by that used 26 SSR loci to estimate genetic relationship among 69 Argentine rice accessions. An allele observed in less than 5% of the 22 accessions was considered to be rare. Rare alleles were observed at all of the SSR loci with an average of 3.33 rare alleles per locus and a total of 10 across all the loci. In general, markers detecting a greater number of alleles per locus detected more rare alleles. Marker RM 493 detected the highest number of alleles (12) and rare alleles (6). According to Jain et al. [17] rare alleles are highly informative in fingerprinting of the varieties.

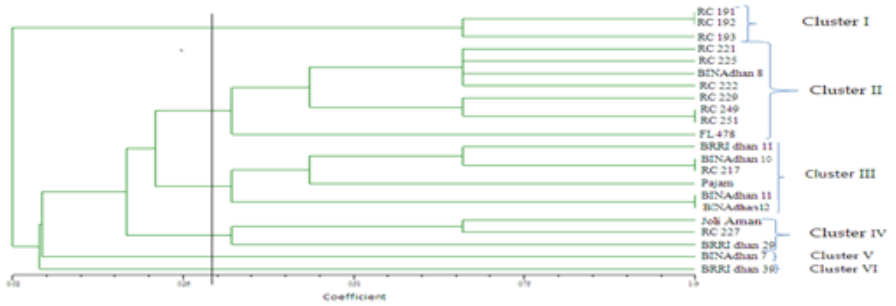
Major allele is defined as the allele with the highest frequency and also known as most common allele at each locus. The size of the different major alleles at different loci ranges from 150bp (RM 493) to 212bp (RM 342). On average, 37% of e 22 rice genotypes shared a common major allele ranging from 18% (RM493) to 59% (RM342) common allele at each locus [18]. observed that the frequency of the most common allele at each locus ranged from 15.38% (RM10701) to 37.51% (RM152).

The highest gene diversity (0.89) was observed in loci RM 493 and the lowest gene diversity (0.61) was observed in loci RM 342 with a mean diversity of 0.76 (Table 4). It was observed that marker detecting the lower number of alleles showed lower gene diversity than those which detected higher number of alleles which revealed higher gene diversity. This result is consistent with previous work done by Heenan et al. [19], who observed that the gene diversity at each SSR locus was significantly correlated with the number of alleles detected, number of repeat motif and with the allele size range. Dhar et al. [18] observed that the highest level of gene diversity value (0.8994) was observed in loci RM10701 and the lowest level of gene diversity value (0.7743) was observed in loci RM152 with a mean diversity of 0.8544.

In the present study, the level of polymorphism among the 22 genotypes was evaluated by calculating PIC values for each of the 3 SSR loci. The PIC values ranged from 0.59 (RM342) to 0.88 (RM493) with an average of 0.74 per locus (Table 4). These result revealed that markers RM493 would be best in screening 22 rice genotypes followed by RM1287 and RM342. Similar results were obtained where RM535 showed a PIC of 0.74 in several elite varieties of rice.

Similarity coefficients among various cultivars analyzed ranged from 0.54-0.98 (average 0.7). Similar values of 0.77-0.98 were detected among 16 accessions of traditional, long-grain, scented Iranian rice and 7 cultivars from other countries [20]. Similarity coefficients ranging from 0.36 to 0.96 were obtained among 45 accessions of AA-genome Oryza species from various locations suggesting a wider range of genetic variability [21]. As expected, similarity coefficients among 193 accessions of parental lines used at IRRI obtained from 26 countries were relatively low ranging from 0.22 to 0.68 [22].

UPGMA method was used for cluster analysis to differentiate the studied genotypes into groups based on similarity coefficient. Six clusters were made at genetic similarity level of 0.26-0.51. All of the 22 rice genotypes were grouped in four main clusters.



Here,G1- RC 191, G2 – RC 192, G3- RC 193, G4- RC 217, G5- RC 221, G6- RC 222, G7- RC 225, G8- RC 227, G9- RC 229, G10- RC 251, G11- RC 249, G12- BRRI dhan 11, G13- BINAdhan 7, G14- BINAdhan 8, G15- BINAdhan 10, G16- BINAdhan 11, G17- Joli Aman, G18- BINAdhan 12,G19- BRRI dhan 29, G20- Pajam, G21- BRRI dhan 39, G22- FL 478

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

Figure 4: UPGMA dendrogram based on Nei's genetic distance differentiation among 22 rice lines. Arrow line indicates the scale of genetic distance (0.00-1.00).

Finally, it can be said that the use of microsatellite markers divulged the larger range of similarity values for genotypes. Microsatellite markers offer a great opportunity for the assessments of genetic diversity and relationships, which can be helpful in future breeding programs. By using the data from clustering, intercrossing may be possible between distant rice varieties.

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References

1. Ren ZZ, Zheng V, Chinnusami J, Zhu X, Cui K (2010) RAS1, a quantitative traitlocus for salt tolerance and ABA sensitivity in Arabidopsis. PNAS 107: 5669-5674.
2. Rengasamy P (2006) World salinization with emphasis on Australia. J Exp Bot 57: 1017-1023.
3. Gregorio GB, Senadhira D, Mendoza RD (1997) Screening rice for salinity tolerance. IRRI Discussion Paper Series no. 22. Manila (Philippines). International Rice Research Institute 1-30.
4. Cantrell RP, Hettel GP (2004) New challenges and technological opportunities for rice based production systems for food security and poverty alleviation in Asia and the Pacific. In: FAO (Food and Agriculture Organization) Rice Conference, FAO, Rome, Italy, 12-13.
5. Jalaluddin M, Nakai H, Yamamoto T (2007) Genetic diversity and DNA fingerprinting of some modern Indica and Japonica rice. Sabrao Journal of Breeding and Genetics 39: 43-52.
6. Saeed M, Abdel Hafiz Adam D, Guo W, Zhang T (2012) A cascade of recently discovered molecular mechanisms involved in abiotic stress tolerance of plants. JIB 16: 188-199.
7. Ashraf M, Athart HR, Harris PJC, Kwong TR (2008) Some prospective strategies for improving crop salt tolerance. Adv Agron 97: 45-110.
8. ABSPII (Agricultural Biotechnology Support Project II) 2006: Drought tolerant rice and salinity tolerant rice. Agricultural Biotechnology Support Project II-South Asia, 26.
9. Virk PS, Newbury JH, Bryan GJ, Jackson MT, Ford-Lloyd BV (2000) Are mapped or anonymous markers more useful for assessing genetic diversity. Theor Appl Genet 100: 607-613.
10. Song ZP, Xu X, Wang B, Chen JK, Lu BR (2003) Genetic diversity in the northernmost Oryza rufipogon populations estimated by SSR markers. Theor Appl Genet 107: 1492-1499.
11. Teixeira da Silva JA (2005) Molecular markers for phylogeny, breeding and ecology in agriculture. In: Thangadurai D, Pullaiah T, Tripathy L (Eds) Genetic Resources and Biotechnology (Vol. III), Regency Publications, New Delhi, India, 221-256.
12. Innan H, Terauchi R, Miyashita NT (1997) Microsatellite polymorphism in

natural populations of the wild plant Arabidopsis thaliana. Genetics 146: 1441-1452.

13. Yang XH, Peng XJ, Yang GH, Feng LL, Wang K, et al. (2008) Preliminary function analysis of rice OsRab7 in salt tolerance and construction of its genetic transformation vector. Journal of Wuhan Botanical Research 26: 1-6.
14. Powell W, Machary GC, Provan J (1996) Polymorphism revealed by simple sequence repeats. Trends Plant Sci 1: 215-222.
15. Nguyen TL, Zhikang L, Buu BC (2001) Microsatellite markers linked to salt tolerance in rice. Omonrice 9: 9-21.
16. IRRI 1997: International Rice Research Institute. Annual Report for 1997. Los Banos, Laguna, Philippines. 308.
17. Jain S, Jain RK, McCouch SR (2004) Genetic analysis of Indian aromatic and quality rice (*Oryza sativa* L.) germplasm using panels of fluorescently-labeled microsatellite markers. Theoretical and Applied Genetics 109: 965-977.
18. Dhar P, Ashrafuzzaman M, Begum SN, Islam MM, Chowdhury MMH (2012) Identification of salt tolerant rice genotypes and their genetic diversity analysis using SSR markers. International Journal of Biological Science 2: 45-50.
19. Heenan DP, Lewin LG, McCaffery DW (2000) Salinity tolerance in rice varieties at different growth stages. Australian Journal of Experimental Agriculture 28: 343-349.
20. Moumeni A, Samadi BY, Leung H (2003) Genetic diversity and relatedness of selected Iranian rice cultivars and disease resistance donors assayed by simple sequence repeats and candidate defense gene markers. Euphytica 131: 275-284.
21. Ren F, Lu BR, Li S, Huang J, Zhu Y (2003) A comparative study of genetic relationships among the AA-genome Oryza species using RAPD and SSR markers. Theoretical and Applied Genetics 108: 113-120.
22. Yu SB, Xu WJ, VijaYakumr CHM, Ali J, Fu BY, et al. (2003): Molecular diversity and multilocus organization of the parental lines used in the International Rice Molecular Breeding Program. Theoretical and Applied *Genetics* 108**:** 131-140.

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