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Molecular Characterization of Selected Landraces of Rice for Salt Tolerance Using SSR Markers

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ABSTRACT: Salinity stress is the major constraint in rice production. Selection for salinity tolerance genotypes of rice based on phenotypic performance alone is less reliable and will delay in progress in breeding. Recent advent of molecular markers, microsatellites or simple sequence repeats (SSRs) were used to find out salt tolerant rice genotypes. In this study three selected SSR markers viz. RM336, RM510 and RM3412 were used to screen the germplasm for salt tolerance. For genotyping rice germplasm, DNA was extracted from leaf samples using CTAB mini-prep method. The number of allele per locus was 10, with an average number of 10 per locus. The average gene diversity of overall SSR loci for the 25 genotypes was 0.8693, ranging from 0.8608 to 0.8768. The PIC values for 3 SSR markers varied from 0.8456 (RM336) to 0.8645 (RM510) with an average PIC of 0.8556. Unweighted Pair Group Method of Arithmetic Means (UPGMA) dendrogram, constructed from Nei's genetic distance produced three distinct clusters of 25 rice genotypes which is very much similar to Principal Component Analysis (PCA). It can be concluded that Jamai naru, Kajol shail, Hogla, Khak shail, Tal mugur, BINA dhan8 were salt tolerant compared to FL 478 because they showed a lower similarity value with FL 478. Marker RM510 showed the highest level of diversity due to high PIC value. This scientific information could be used for selection of suitable parents and development of salt tolerant rice varieties as well.

KEYWORDS: Saltol, Gene diversity, Genetic distance, microsatellite markers, Principal Component Analysis, UPGMA dendrogram.

1 INTRODUCTION

Rice is a highly polymorphic crop species with wide geographic distribution [1]. It is one of the most important world food crops, serving as the staple food for over one-third of the world's population [2]. It serves as the principal source of nourishment for over half of the global population and is the most important cereal crop. Rice breeders are increasingly challenged in the new century to meet the rapidly growing food demands of an increasing human population. Presently, growers in many regions have extended cultivation into marginal lands where salinity levels in soils are above thresholds affecting rice growth and yield. These instances, along with water conservation practices such as irrigating crops with marginal quality water have increased the need for genetic improvement of salt tolerance in rice. Unfortunately, there exists tremendous variation for salt tolerance within the species of rice [3]. Salinity ingress has lead to deterioration in the environment in the coastal areas with adverse implication on agriculture [4]. Progress in salinity tolerance breeding is slow due to the following aspects as limited knowledge in the genetics of tolerance, complexity of the several tolerance mechanisms involved, inadequate screening techniques, low selection efficiency and poor understanding of salinity and environmental interactions [5], [6]. Breakthrough in salinity tolerance breeding became feasible after the identification of major chromosomal regions (Qualitative trait loci, QTLs) underlying salinity (Saltol) stresses, the development and use of marker system for their speedy incorporation into modern high yielding and popular varieties through marker assisted

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backcrossing [7]. With the recent development in the field of molecular marker analysis, it is now feasible to analyze both the simple inherited traits and the quantitative traits and then identifying the individual genes controlling salinity tolerance which could facilitate selection in rice for this low heritable trait [8]. Looking into the above facts, the present investigation was carried out with the objective of assessing the presence and level of genetic diversity among O. sativa cultivars and for identifying microsatellite markers for salinity resistance genes.

2 MATERIALS AND METHODS

The experiment was conducted at the glasshouse, experimental field and Biotechnology Laboratory of Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh from May 2012 to March 2013. A total of 25 traditional and improved rice germplasm were collected to carry out the objectives of this research work. List of the rice genotypes are presented in Table 1.

Table1. List of rice genotypes used in the experiment

SL. No.	Genotypes	Origin and Identity	Source of Collection
1	Hogla	Satkhira	Bangladesh Institute of Nuclear Agriculture (BINA)
2	Jamai Naru		
3	Dakh Shail		
4	Patnai		
5	Kute Patnai		
6	Holde Gotal		
7	BazraMuri		
8	Ghunshi		
9	Tal Mugur		
10	Nona Bokhra		
11	Khak Shail		
12	Jota Balam		
13	Hamai		
14	Karengal		
15	Mondeshor		
16	Nona Kochi		
17	Kajol Shail		
18	Bhute Shalot		
19	Kashrail	Noakhali	
20	BINA dhan 8	IRRI	
21	FL 478		
22	BINA dhan 7		
23	Pokkali		
24	Jolkumari	Patuakhali	
25	Kalo Mota		

25 rice genotypes obtained from the Biotechnology Division of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh were used for molecular analysis. Total DNA was extracted from three weeks old seedlings by Cetyl Trimethy Ammonium Bromide (CTAB) method [9]. DNA concentration was measured using Spectrophotometer. For this study DNA quantification was done at central library of Bangladesh Agricultural University and the absorbance was taken at 260nm. 2000 µl dH_2O and 2µl DNA was used for this purpose. Each polymerase chain reaction was carried out in 13.0 µl reaction volume containing 1.5 µl 10x Buffer, 1.0 µl dNTPs, 1.0 µl Primer forward, 1.0 µl Primer reverse, 0.25 µl Taq polymerase, 7.25 µl ddH_2O and 1µl genomic DNA using Eppendorf and Applied biosystem thermal cyclers. Thermal cycler programme for PCR comprised 95ºC for 5 minutes for initial denaturation, followed by 36 cycles of 95ºC for 45 seconds, 55 to 65ºC for 45 seconds, 72ºC for 45 seconds and ending up with 7 minutes at 72ºC for the final extension. The annealing temperature was adjusted based on the specific requirements of different primer combinations. The PCR products were resolved by electrophoresis in 2 agarose gel containing 0.5 g/mL of Ethidium Bromide prepared in 1X TBE buffer at a constant voltage of 80v for period of 2h. The gel was visualized in UV transilluminator and documented using SYNGENE GENESNAP G-BOX gel

documentation system. Ethidium bromide staining of agarose gels generally revealed a multiple number of bands. The size of the most intensively amplified band for each microsatellite marker was determined based on its electrophoretic mobility relative to molecular weight marker (25 bp). Amplified products from microsatellite analyses were scored qualitatively for presence and absence of each marker allelegenotype combination. The SSR bands amplified by using given primers were further treated as a unit character. The data were entered into a binary matrix as discrete variables (e.g., 1 for presence and 0 for absence of character). The relevant primers were employed on the basis of polymorphism extent. The Polymorphic Information Content (PIC) value of the used marker was calculated by following a method developed by Anderson *et al.*, [10]. The pair-wise comparisons of the cultivars based on the proportion of unique and shared amplification products (alleles) were used to measure the genetic similarity by Dice coefficients. The Dice coefficients were employed by using Simqual sub-program in similarity routine of software NTSYS-pc version 2.2 (Exeter Software, Setauket, NY, U.S.A.) software package. The estimation of genetic similarity (F) were calculated by following the method described elsewhere [11]. The resultant similarity matrix data was employed to construct a dendrogram by using Sequential Agglomerative Hierarchical Nesting (SAHN) based on unweighted pair-group method with an arithmetic average (UPGMA) to infer genetic relationships and phylogeny among cultivars. The principal component analysis (PCA) was also done by using the subroutine EIGEN. All computations were done by using the NTSYS-pc, Version 2.2 package [12]. Further details are available elsewhere [13].

3 RESULTS AND DISCUSSION

3.1 DNA AMPLIFICATION AND CULTIVAR IDENTIFICATION

A total of ten microsatellite or SSR markers (i.e. RM 315, RM 7102, RM 336, RM 337, RM 3412, RM 510, RM 10890, RM 6737, RM 493 and RM 315) covering all 12 chromosomes were utilized to characterize and assess genetic diversity among thirty five rice varieties from different location of Bangladesh. Amplification profiles as revealed by RM337, RM3412, RM510 across a number of cultivars used is depicted in Figure 1,2,3. In this study, a considerable variability was found among different cultivars. In most of the cases, FL478 and other salt tolerant cultivars exhibited similar banding patterns. The used markers showed several bands, which were shared among the FL478 and other salt tolerant cultivars. Contrarily, the salt tolerant and non - salt tolerant cultivars of rice shared a few bands. The cultivar Jamai naru, Kajol shail, Hogla, Khak shail, Tal mugur, BINA dhan8 displayed unique bands in comparison with all other nonsalt tolerant genotypes. Many primers showed characteristic fragments in this cultivar, which were not produced in any of the other nonsalt tolerant cultivars used. Microsatellite markers have been used to investigate genetic diversity of a large number of cultivars in rice [14]. It has shown high levels of polymorphism in many crops including rice (*O. sativa* L.), wheat (*Triticum aestivum* L.), and maize (*Zea mays* L.). Kanawapee *et al.,* [15] evaluated genetic diversity of the 30 rice cultivars for salinity tolerance during the seedling stage using RAPD and simple sequence repeats (SSR) markers.

3.2 GENETIC DIVERSITY

According to Nei's [16], the highest level of gene diversity value (0.8768) was observed in loci RM510 and the lowest level of gene diversity value (0.8608) was observed in loci RM336 with a mean diversity of 0.8693 (Table 2). 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. The other primer showed a gene diversity value of 0.8704. The maximum number of repeats within the SSRs was also positively correlated with the genetic diversity. This result is consistent with previous work done by Heenan *et al.,* [17], 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.

3.3 PIC VALUES

Expected heterozygosity amongst 25 rice genotypes was observed in the range of 0.8456-0.8645, where in the marker RM 510 revealed the highest value of 0.8645 (Table 2). The average expected heterozygosity was 0.8556. The present result corroborates with the results of Davla *et al.*, [18], where molecular characterization of rice (*Oryza sativa* L) genotypes for salt tolerance using microsatellite markers including RM 336 was performed. Among the primers used in the present study, RM510 is highly informative since it recorded high PIC value (0.8645).

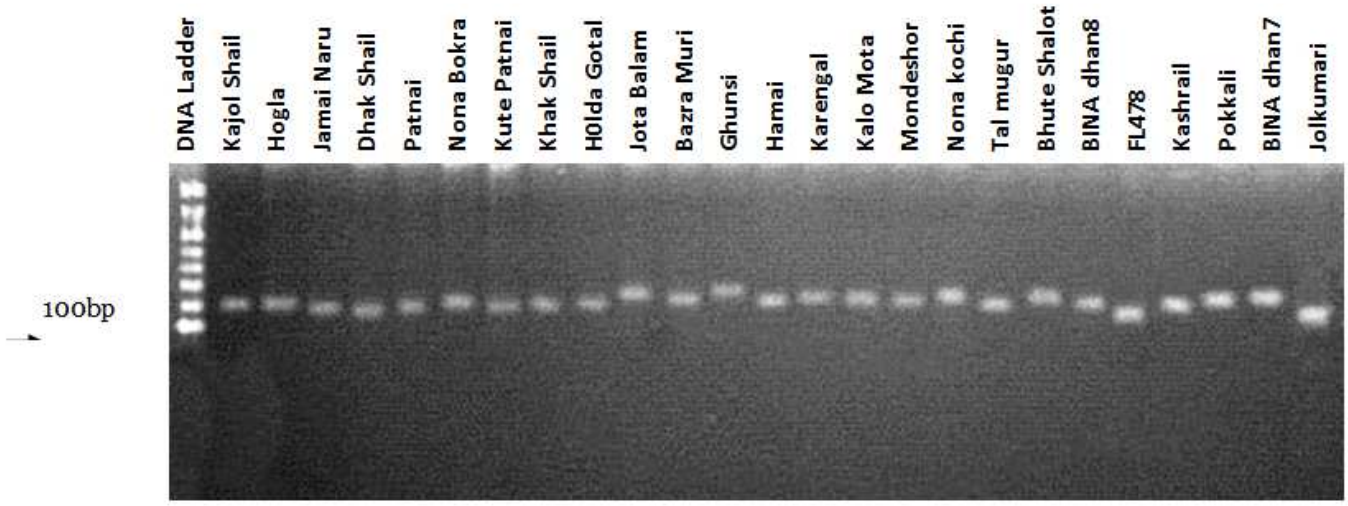

Fig.1. SSR Profiles of 25 Genotypes of Rice Using Primer RM336 (Ladder = 100bp)

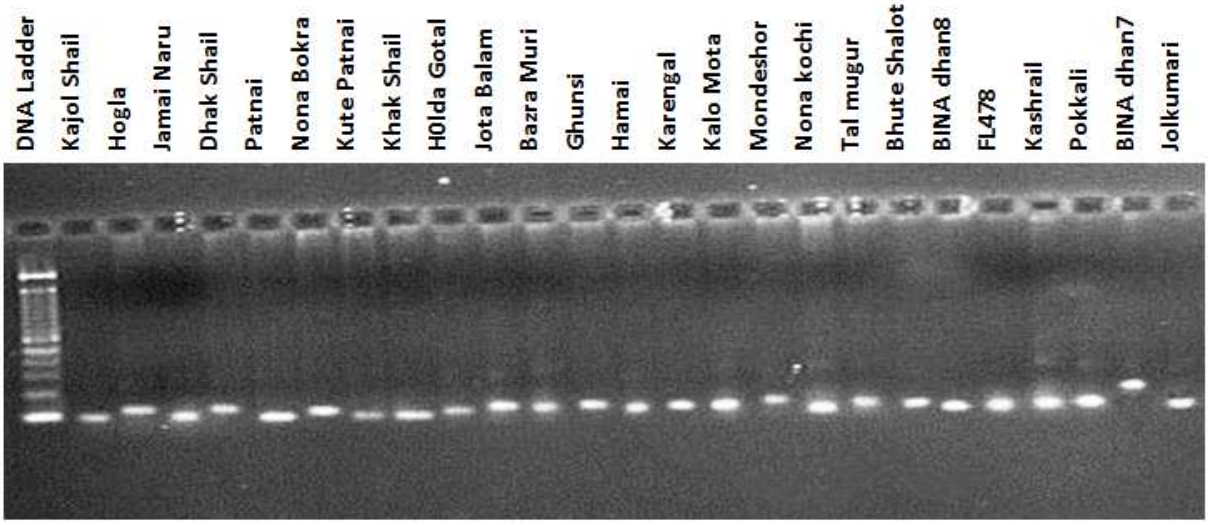

Fig. 2. SSR Profiles of 25 Genotypes of Rice Using Primer RM510 (Ladder = 100bp)

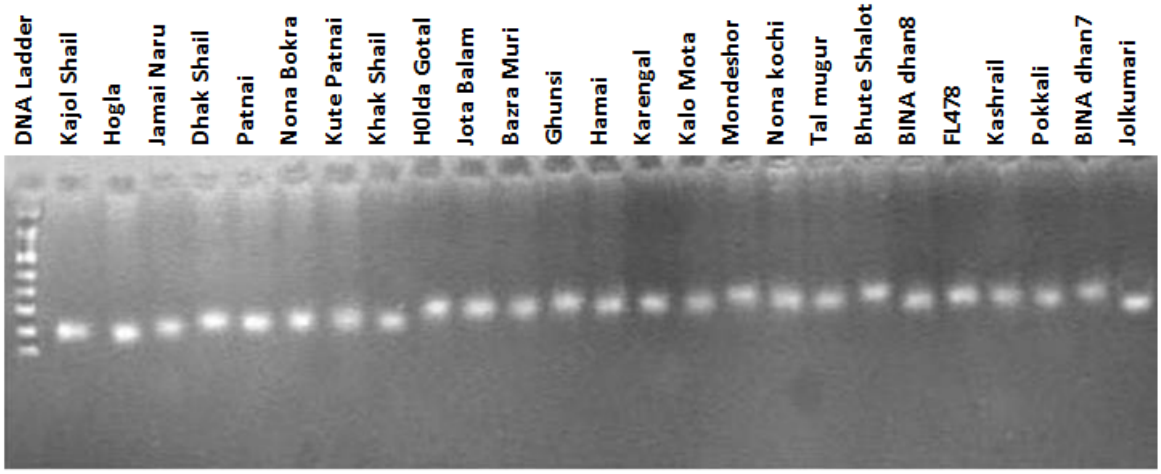

Fig. 3. SSR Profiles of 25 Genotypes of Rice Using Primer RM3412 (Ladder = 100bp)

The markers showed an average PIC value of 0.8556 which indicated that SSR markers used in this study were highly informative because only PIC values higher than 0.5 indicate high polymorphism. Markers with PIC values of 0.5 or higher are highly informative for genetic studies and are ex-tremely useful in distinguishing the polymorphism rate of a marker at a specific locus [19]. All loci are revealing a high degree of diversity among the accessions.

Table 2. Data on repeat motif, number of alleles, number of rare alleles, polymorphism information content (PIC) value and gene diversity) found among 25 rice genotypes for 3 microsatellites (SSR)

Locus	Repeat Motif*	Allele Size ranges(bp)	Difference (bp)	No. of alleles	PIC	Gene Diversity
RM336	(CTT)8	131-235	104	10	0.8456	0.8608
RM510	(GA)15	100-266	166	10	0.8645	0.8768
RM3412	(CT)17	164-338	74	10	0.8569	0.8704
Mean				10	0.8556	0.8693

** = Motif of the SSR and number of repeats as previously published on http://www.gramene.org*

3.4 NUMBER OF ALLELES AND ALLELE SIZE FREQUENCY OF ALLELES

The allele length for this 3 SSR markers varied from 100-338bp, whereas the highest allele length was recorded for RM 3412 338bp. In respect of primer RM336, allele size ranged from 131bp - 235bp, whereas primer RM510 showed a range 100bp - 266bp and primer RM3412 gave a range from 164bp - 338bp (Table 2). A total of 30 alleles were detected with an average number of alleles of 10 per locus (Table 3). Nearly similar observation was found by Thomson *et al.,* [20], where they got that the number of alleles per locus ranged from 4 alleles to 31 alleles with an average of 13 alleles per locus. Jain *et al.,* [21] got as like similar observation of number of allele per locus ranged from 3 to as high as 22 with average number of allele per locus 7.8. The allele frequency produced by different markers was20%. Similar results were also obtained by Singh *et al.*, [22].

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

Sl. No.	Locus	Allele size	Allele Frequency
1	RM337	131	0.0400
		140	0.0400
		149	0.0800
		159	0.0800
		170	0.2000
		181	0.1600
		193	0.0400
		206	0.2000
		220	0.0400
		235	0.1200
2	RM 510	100	0.0800
		105	0.1200
		125	0.0800
		133	0.1200
		141	0.0800
		149	0.2000
		158	0.1600
		168	0.0800
		188	0.0400
		266	0.0400
3	RM3412	164	0.0400
		174	0.0400
		209	0.1200
		222	0.0800
		235	0.0400
		318	0.1600
		338	0.1600
		359	0.0800
		381	0.2000
		405	0.0800

3.5 GENETIC DISTANCE-BASED ANALYSIS

Pair-wise comparison value of Nei [23] genetic distance (D) between varieties was computed from data of 3 primers and ranged from 0.333 to 1.000 (Table 4). 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 lower genetic distance (0.333) was observed in Holde Gotal vs. Bazra Muri, Bazra Muri vs. Ghunshi, Karengal vs. Mondeshor, Nunnia vs. Kali Boro, Chinisail vs. Kali Boro, Jamai Naru vs. Hari and Jamai Naru vs. Kute Patnai variety pair indicating that they are genetically much closer among the varieties. Most of the varieties of lowest genetic distance were collected from Satkhira, only Kalomota and Jolkumari 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. FL 478 showed a higher genetic distance with most of the varieties except Holde Gotal, Bazra Muri and Hamai. 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 by Nagaraju *et al.*, [24] and they observed that the lowest genetic distance was among the traditional Basmati varieties, whereas 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. The means of genetic distances between germplasms were used to evaluate the genetic diversity of different germplasms. Dhar *et al.*, [25] concluded that the values of pair-wise comparisons of Nei, [23] genetic distance (D) between varieties were computed from combined data for the 6 primers which ranged from 0.200 to 1.000.

3.6 SIMILARITY INDEX BASED ANALYSIS

A similarity index determines how closely the current plant community resembles either the potential natural community or some other reference community. The similarity index provides a distinct measurement in germplasm screening and diversity analysis after Nei genetic distance based analysis. In this study, the similarity value was ranged from 0.000 to 0.667 (Table 5). When the value is zero, it indicates that, there is no similarity between the variety pair. Highest similarity value was observed mostly between those varieties pairs which showed lowest genetic distance value in Nei genetic distance based analysis. Similarity Index value of 0.6667 was observed in Jota Balam vs. Bazra Muri, Jota Balam vs. Ghunsi, Bazra Muri vs. Holda Gotal, Talmugur vs. Pokkali, Talmugur vs. BINA dhan 7, Jamai Naru vs. Kuta Patnai and Dhak Shail vs. Bute Shalote. The lowest similarity value was observed in most of the varieties just as the highest genetic distance was observer in most of the varieties. Similar values of 0.77 to 0.98 were detected among 16 accessions of traditional, long-grain Iranian rice and 7 cultivars from other countries [26]. 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 [27]. 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 [28].

3.7 GENETIC SIMILARITY ANALYSIS USING UNWEIGHTED PAIR GROUP METHOD OF ARITHMETIC MEANS (UPGMA) AND PRINCIPAL COMPONENT ANALYSIS

The The multivariate nature of SSR markers has the unambiguous advantage of discriminating genotypes more precisely. The UPGMA analysis could reveal allelic richness of three clusters (Fig.4) for various sizes at a similarity coefficient level of 0.12. Among them Jamai naru, Kajol shail, Hogla, Khak shail, Tal mugur may be chosen as a parent for hybridization with any of the land races from other divergent cluster involving land races. SSR analysis resulted in a more definitive separation of cluster of genotypes indicating a higher level of efficiency of SSR markers for the accurate determination of relationships between accessions that are too close [29]. Grouping based on SSR markers, in general, agreed with the parental pedigree information provides indispensable information regarding the genetic diversity among the genotypes. Varieties and lines sharing the common ancestry were clustered in to the same group, indicating the efficiency of SSR markers in detecting the genetic diversity in rice [22].The PCA was also done to determine the genetic relationships among the rice varieties from different regions of Bangladesh (Fig. 5). The groupings identified by PCA were very similar to those identified by the UPGMA cluster analysis. Of 25 rice genotypes 7 cultivars formed a distinct cluster in the right side of the diagram, formed a distinct cluster in the right side of the diagram, separated from the main cluster. This cluster corresponded to the 8 varieties that appear as a major group in Fig.5. A second group of 18 genotypes corresponded well with the second and third group of varieties in the dendrogram.

Table 4. Summary of (Nei's, 1973) Genetic Distance Values for 25 Rice Germplasms

Genotypes	Kajol Shail	Jota Balam	Bazra Muri	Ghunsi	Hamai	Karengal	Kalo Mota	Mondeshor	Nona Kochi	Tal Mugur	Bhute Shalot	Hogla	BINA Dhan 8	FL 478	Kashrail	Pokkaly	BINA Dhan 7	Jolkumari	Jamai Naru	Dakh Sail	Patnai	Nona Bokra	Kute Patnai	Khak Sail	Holda Gotal
Kajol Shail	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.67	1.00	0.67	1.00	1.00	0.67	1.00	1.00	1.00	1.00	1.00	0.67	1.00	1.00	0.67	1.00
Jota Balam	1.00	0.00	0.33	0.33	1.00	0.67	0.67	1.00	1.00	1.00	0.67	1.00	1.00	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.67
Bazra Muri	1.00	0.33	0.00	0.67	0.67	0.67	0.67	1.00	1.00	1.00	1.00	1.00	1.00	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.67	1.00	1.00	0.33
Ghunsi	1.00	0.33	0.67	0.00	0.67	0.33	1.00	1.00	1.00	1.00	0.67	1.00	1.00	0.67	1.00	1.00	1.00	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Hamai	1.00	1.00	0.67	0.67	0.00	0.67	1.00	1.00	1.00	1.00	1.00	1.00	0.67	1.00	1.00	1.00	1.00	0.67	1.00	1.00	1.00	0.67	1.00	1.00	0.67
Karengal	1.00	0.67	0.67	0.33	0.67	0.00	0.67	0.67	1.00	1.00	1.00	1.00	1.00	0.67	1.00	0.67	0.67	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Kalo Mota	1.00	0.67	0.67	1.00	1.00	0.67	0.00	0.67	1.00	1.00	0.67	1.00	1.00	1.00	0.67	0.67	0.67	0.67	1.00	1.00	1.00	1.00	1.00	1.00	0.67
Mondeshor	1.00	1.00	1.00	1.00	1.00	0.67	0.67	0.00	0.67	1.00	1.00	1.00	1.00	1.00	1.00	0.67	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Nona Kochi	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.67	0.00	1.00	1.00	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.67
Tal Mugur	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	0.67	0.67	0.67	0.33	0.33	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.67	1.00
Bhute Shalot	1.00	0.67	1.00	0.67	1.00	1.00	0.67	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.67	1.00	0.67	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Hogla	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.67	0.67	1.00	0.00	1.00	1.00	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.67	0.67
BINA Dhan 8	1.00	1.00	1.00	1.00	0.67	1.00	1.00	1.00	1.00	0.67	1.00	1.00	0.00	0.67	0.67	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
FL 478	1.00	0.67	0.67	0.67	1.00	0.67	1.00	1.00	1.00	0.67	1.00	1.00	0.67	0.00	0.67	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Kashrail	0.67	1.00	1.00	1.00	1.00	1.00	0.67	1.00	1.00	0.33	0.67	0.67	0.67	0.67	0.00	0.67	1.00	0.67	1.00	1.00	1.00	1.00	1.00	0.67	1.00
Pokkaly	1.00	1.00	1.00	1.00	1.00	0.67	0.67	0.67	1.00	0.33	1.00	1.00	0.67	0.67	0.67	0.00	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
BINA Dhan 7	1.00	1.00	1.00	1.00	1.00	0.67	0.67	0.67	1.00	1.00	0.67	1.00	1.00	1.00	1.00	0.67	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Jolkumari	1.00	1.00	1.00	0.67	0.67	0.67	0.67	1.00	1.00	1.00	0.67	1.00	1.00	1.00	0.67	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Jamai Naru	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.67	1.00	0.67	0.33	0.67	1.00
Dakh Sail	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.67	0.00	0.67	0.33	1.00	1.00	1.00
Patnai	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.67	0.00	1.00	0.67	1.00	1.00
Nona Bokra	1.00	1.00	0.67	1.00	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.67	0.33	1.00	0.00	1.00	1.00	0.67
Kute Patnai	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.33	1.00	0.67	1.00	0.00	0.67	1.00
Khak Sail	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.67	1.00	0.67	1.00	1.00	0.67	1.00	1.00	1.00	0.67	1.00	1.00	1.00	0.67	0.00	1.00
Holda Gotal	1.00	0.67	0.33	1.00	0.67	1.00	0.67	1.00	0.67	1.00	1.00	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.67	1.00	1.00	0.00

Table 5. Similarity index of 25 rice germplasms

OTU	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	1.00																								
2	0.00	1.00																							
3	0.00	0.67	1.00																						
4	0.00	0.67	0.33	1.00																					
5	0.00	0.00	0.33	0.33	1.00																				
6	0.00	0.33	0.33	0.67	0.33	1.00																			
7	0.00	0.33	0.33	0.00	0.00	0.33	1.00																		
8	0.00	0.00	0.00	0.00	0.00	0.33	0.33	1.00																	
9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	1.00																
10	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00															
11	0.00	0.33	0.00	0.33	0.00	0.00	0.33	0.00	0.00	0.00	1.00														
12	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.33	0.00	1.00													
13	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.33	0.00	0.00	1.00												
14	0.00	0.33	0.33	0.33	0.00	0.33	0.00	0.00	0.00	0.33	0.00	0.00	0.33	1.00											
15	0.33	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.67	0.33	0.33	0.33	0.33	1.00										
16	0.00	0.00	0.00	0.00	0.00	0.33	0.33	0.33	0.00	0.67	0.00	0.00	0.33	0.33	0.33	1.00									
17	0.00	0.00	0.00	0.00	0.00	0.33	0.33	0.33	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.33	1.00								
18	0.00	0.00	0.00	0.33	0.33	0.33	0.33	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.33	0.00	0.00	1.00							
19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00						
20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	1.00					
21	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	1.00				
22	0.00	0.00	0.33	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.67	0.00	1.00			
23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.67	0.00	0.33	0.00	1.00		
24	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0..33	0.00	0.00	0.33	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.33	1.00	
25	0.00	0.33	0.67	0.00	0.33	0.00	0.33	0.00	0.33	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	1.00

1=Kajol Shail, 2= Jota Balam, 3=Bazra Muri, 4=Ghunsi, 5= Hamai, 6=Karengal, 7=Kalo Mota, 8=Mondeshor, 9= Nona Kochi, 10=Tal Mugur, 11=Nona Bokra,12= Hogla,13= BINA dhan 8, 14= FL 478, 15= Kashrail,16= Pokkali, 17=BINA dhan 7, 18=Jolkumari, 19=Jamai Naru, 20=Dhak sail, 21=Patnai, 22=Bute Shalot , 23=Kute Patnai, 24=Khak Shail, 25=Holda Gotal

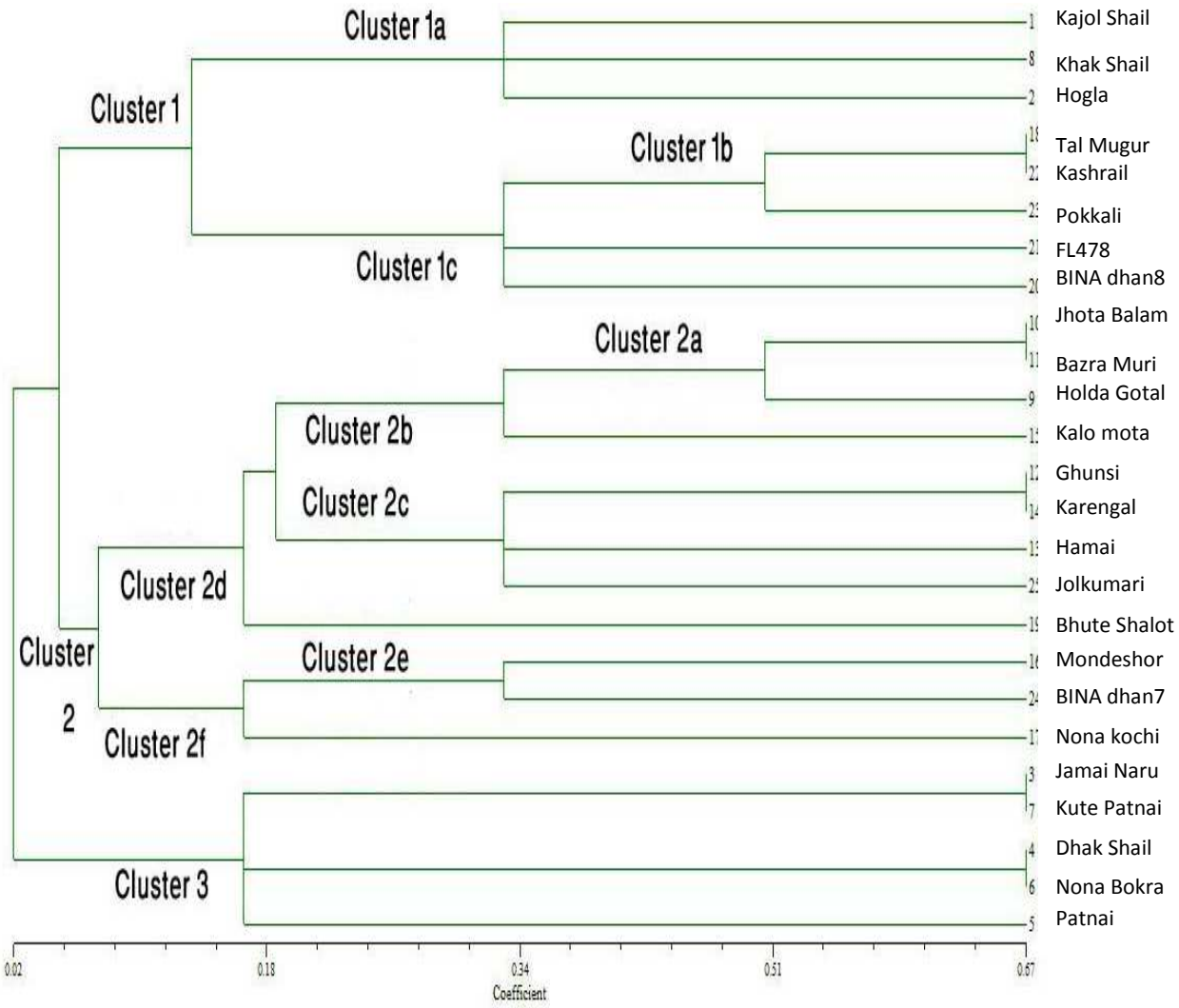

Fig. 4. Dendrogram for 25 rice germplasms derived from a UPGMA cluster analysis

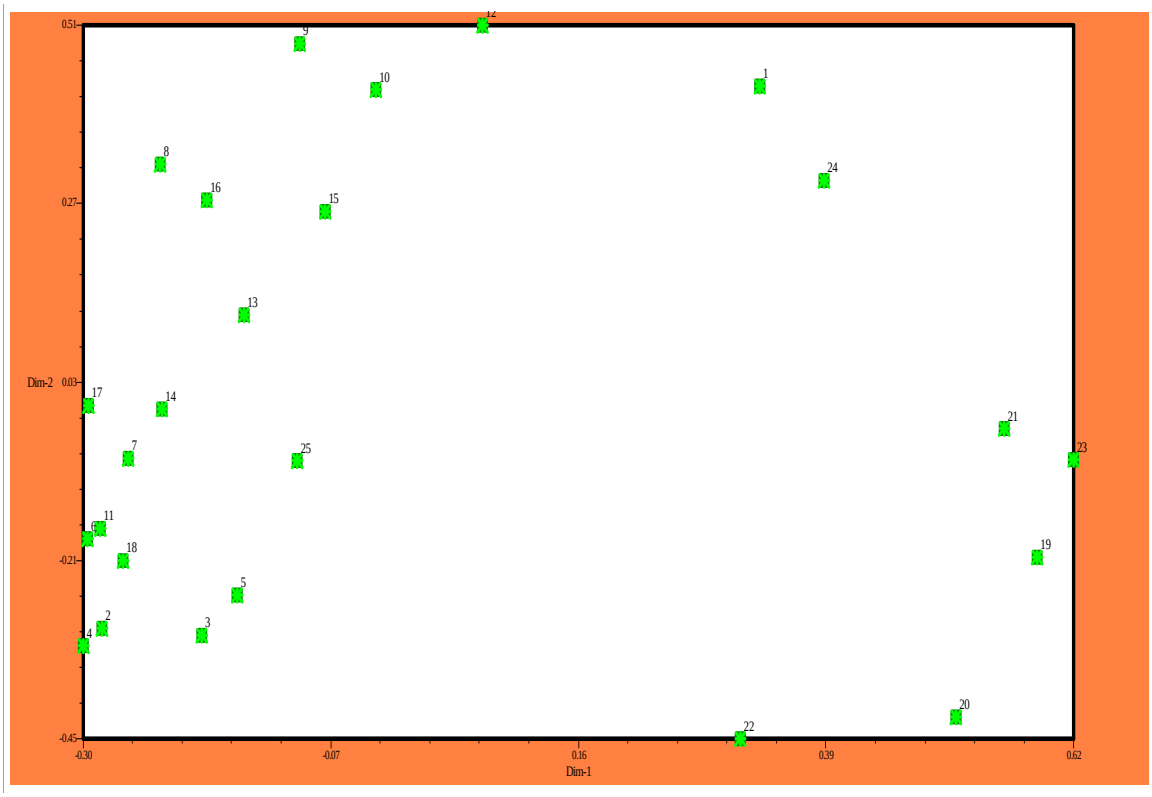

Fig.5. Principle Component Analysis grouping of 25 O. sativa species based on pooled SSR markers

1=Kajol Shail, 2= Jota Balam, 3=Bazra Muri, 4=Ghunsi, 5= Hamai, 6=Karengal, 7=Kalo Mota, 8=Mondeshor, 9= Nona Kochi, 10=Tal Mugur, 11=Nona Bokra,12= Hogla,13= BINA dhan 8, 14= FL 478, 15= Kashrail,16= Pokkali, 17=BINA dhan 7, 18=Jolkumari, 19=Jamai Naru, 20=Dhak shail, 21=Patnai, 22=Bute Shalot , 23=Kute Patnai, 24=Khak Shail, 25=Holda Gotal

4 CONCLUSION

The present study showed average number of alleles of all the rice genotypes were 10 over the three microsatellite loci. The Unweighted Pair Group Method of Arithmetic Mean (UPGMA) dendrogram based on Nei [21] genetic distance, resulted in three major clusters with several sub cluster. Through the present study, a total of 25 variety's specific alleles were identified with specific SSR primer. The result of the present study would be useful to know genetic variation, population structure, parentage assessment, genome mapping, Marker Assisted Selection (MAS), forensics, stock purity, etc. of different populations of the studied species before undertaking any breeding any breeding program, and also will be used as baseline information for further study. However, more extensive molecular data is needed in order to draw and conclusive remarks about the relationship between rice cultivars. Large number of samples would be necessary to determine if there are inherent differences in genetic distance between the rice cultivars. Moreover, using higher number of markers would give a clear idea about the genetic variation and genetic diversity which might be of greater interest for the plant breeders for the development of rice varieties. The results derived from analyze 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.

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