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Full Length Research Paper

Analysis of genetic variability in rice cultivars of Arunachal Pradesh (India) using microsatellite marker

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The present investigation was carried out to analyze the genetic diversity of rice cultivars utilizing microsatellite or simple sequence repeat (SSR) markers. Twenty four (24) genotypes of rice were evaluated for polymorphisms after amplification with 30 SSR primer pairs. The results obtained depicted a wide range of variation among the varieties. The diversity parameters confirmed that Bordungsha F1, S5 and Baodhan Bordungsha (deep water rice) were most diverse and were distinctly different from the rests of the genotype and the dissimilarity coefficient ranged from 0.06 to 0.94. This study provides a clear picture of the genetic diversity of rice. Among the set of SSR primers tested, RM44 (0.942), RM495 (0.932), RM125 (0.998) and OSR13 (0.970) were more informative in comparison to other primers.

Key words: *Oryza sativa*, SSR markers, PCR, polymorphism, genetic variation.

INTRODUCTION

Rice is the world's most important cereal crop that provides food for more than half of the world's human population. It is a unique crop of great antiquity and akin to progress in human civilization (Chang, 2002). It is no longer a luxury food but has become the cereal that constitutes a major source of calories for the urban and the rural (Sasaki and Burr, 2000).

It is one of the few crop species endowed with the richest genetic diversity. Rice has one of the largest *ex situ* germplasm collections in the world (Jackson, 1994). This accessible collection of diverse cultivated and wild rice germplasm has made great contributions to rice breeding. Diversity of rice germplasm in Arunachal Pradesh is enormous.

Out of 7925 collections made by Assam Rice Collections series, ICAR and National Bureau of Plant Genetic Resources, 1866 collections are from Arunachal Pradesh, which is 24% of total germplasm.

Analysis of genetic diversity can be applied to studies to examine differences between members of the same species. On the other hand, comparison of the genetic composition of member of different species even over a wider taxonomic range can be done (Dale and Schantz, 2003).

Study about genetic variation is one of the most important methods for conservation of genetic resources. Recently, the use of microsatellites or simple sequence repeat (SSR) markers are being used for the molecular characterization and genetic diversity analysis of aromatic landraces (Sajib et al., 2012), fertility restorer genes for wild abortive (WA) cytoplasm (Shah et al., 2012) and salinity tolerance cultivars (Kanawapee et al., 2011) of rice.

Since the new marker system reveals difference at the DNA level, they represent an extremely powerful tool for assessment of genetic diversity in both cultivated and wild species of plants. Microsatellites or simply simple sequence repeats are DNA sequences with repeat

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lengths of a few base pairs. Compared with restriction fragment length polymorphisms (RFLPs), microsatellite markers detect a significantly higher degree of polymorphism in rice (Wu and Tanksley, 1993; Yang et al., 1994) and are especially suitable for evaluating genetic diversity among closely related rice cultivars.

About 2240 microsatellite markers are now available through the published high density linkage map (McCouch et al., 2002) or public database. SSR have become the molecular markers of choice for a wide range of applications in genetic mapping and genome analysis (Chen et al., 1997). For measuring genetic diversity, assigning lines to heterotic groups and genetic fingerprinting, microsatellite provide power of discrimination equal to or greater than that of RFLP in a more cost effective manner.

The present investigation has been made to analyze the genetic diversity of rice cultivars of Arunachal Pradesh (India) using microsatellite or SSR marker and to evaluate the accessions for polymorphism(s) after amplification with 30 SSR primer pairs.

MATERIALS AND METHODS

Plant materials

Twenty four (24) varieties of rice genotypes were used in the present study. The rice samples were obtained from the already stocked germplasm of ICAR, Division of Plant Breeding, and were grown in ICAR-RC, NEH Region, Umiam, and Meghalaya.

The accessions, Bordungsha F1 S, Bhatguti, Miatong, Khoujee 2, Khoujee 3, Sujanta 2, Bora type from Enow Village, Boradhan, Sujanta 5, Khogi Boga, Ranjit, Bihari, Bihari type (Enow Village), Khogi Lal, Dyong Collection, Barshagiri, Bordungsha F1 S4, Baodhan (Bordungsha), Bordungsha F1, Bordungsha F1 S2, Bordungsha F1 S5, Bordungsha F2 S2, Lampnah, *Oryza rufipogon* (cp) were identified by the name as given by the farmers and there were more than one accession with the same name, a field number and sample number was added after the name to allow them to be treated as different genotypes for analysis.

Polymerase chain reaction (PCR) amplification of DNA

The fresh leaves were used to extract DNA by the CTAB method (Ahmadikhah et al., 2009) and the qualitative analysis was done using Nanodrop N.D. 1000 software (ver 3.3.0). SSR primers of chromosome numbers 1 - 12 were used to estimate the genetic diversity of 24 Khamti rice varieties from Arunachal Pradesh (India) containing cultivated rice varieties and wild varieties. A total of 30 SSR primers were utilized for molecular profiling of the rice germplasm. The SSR primers (forward and reverse for each locus) were dissolved in Tris EDTA (TE) buffer and were tested across all the 24 samples. Out of 30 Primers, OSR13 was used for PCR amplification.

SSR banding score and SSR analysis

The amplified SSR DNA bands representing different alleles were scored as different genotypes because of the co- dominant nature of SSR markers and all the used varieties were compared with each other. Differences in molecular weight for PCR products amplified from SSR markers were manually measured using image analysis software ALPHAEASE (AlphaInotech HP, Japan).

The banding patterns from SSR analysis for each primer were scored individually by visual observation. The amplified profiles from all the primers used were scored and integrated together to form a data matrix (Rohlf et al., 1994). The band sizes were estimated using 50 bp molecular weight markers and 100 bp DNA ladder. The polymorphism information content (PIC) of each band was calculated and a dendogram was generated from the consensus tree file. Using the first three principal components, a principal component analysis (PCA) plot was also generated.

RESULTS

Banding pattern and SSR primers

In the present investigation, microsatellite or SSR markers from chromosome to 12 were used to characterize and to assess genetic diversity among 24 lines of rice belonging to *O. sativa* and *O. rufipogon*, utilizing a set of 30 SSR primers. The 24 genotypes were tested with all the 30 markers.

A total of 223 amplified alleles were obtained among 24 rice genotypes from 30 SSR primers. Most of the fragments amplified were found within 100 bp to 200 bp. Highest number of bands were recorded in primer RM447 (Figure 1e) followed by primer RM408 (Figure 1d). The overall molecular weight of the bands amplified using 30 SSR primer pair were in the range of 62 to 737 bp (Table 1). However, primer RM277 produced amplified fragments much below the predicted size. Genotype, Bihari showed highest band size of the primer RM484, which was above the expected band size indicating that this genotype was distinct from other genotype. The highest and the lowest molecular weight of a primer with the maximum frequency are shown in Table 2.

The rice genotypes amplified with the primer RM215 is shown in Figure 1a. The expected band size ranged from 126 to 161, while the observed band size was 136 to 154 (Table 1) with an average of 142 (Table 2). The arrows on the right that is, 150, 100, 50 bp indicate distance base pair of the marker (Figure 1a).

The genotypes amplified with the primer RM 431 (Figure 1b) had expected band size ranged from 233 to 261, while the observed band size was 160 to 180, which was much below the expected minimum band size with an average of 177. Sujanta 5 (Lane 9) did not get amplified when primer RM433 was used (Figure 1c). The expected band size ranged from 216 to 248, while the observed band size was 210 to 228 (Table 1), with an average of 210 (Table 2).

The primer RM408 amplified all the 24 rice genotypes (Figure 1d). Bora type (lane 7), Boradhan (lane 8), Bihari type (lane 13) and Bordungsha F1 S5 (lane 21) produced double bands (Figure 1d). The expected band size

Table 1. List of SSR primers, annealing temperature, sequences and band sizes with chromosome number 1 to 12.

Chromosome	Primer Code	Anneal temp ºC	Forward primer	Reverse primer	Expected band size		Observed band size	
					Min.	Max.	Min.	Max.
1	RM495	55	aatccaaggtgcagagatgg	caacgatgacgaacacaacc	148	160	137	157
	RM431	55	tcctgcgaactgaagagttg	agagcaaaaccctggttcac	233	261	160	184
	RM237	55	caaatcccgactgctgtcc	tgggaagagagcactacagc	105	153	113	137
	RM283	61	gtctacatgtaccttgttggg	cggcatgagactctgtgatg	130	176	145	159
2	RM154	61	accctctccgcctcgcctcctc	ctcctcctcctgcgaccgctcc	148	230	145	187
	RM452	61	ctgatcgagagcgttaaggg	gggatcaaaccacgtttctg	192	213	169	198
3	RM338	55	cacaggagcaggagaagagc	ggcaaaccgatcactcagtc	178	184	172	189
	RM514	55	agattgatctcccattcccc	cacgagcatattactagtgg	229	278	224	260
	OSR13	53	catttgtgcgtcacggagta	agccacagcgcccatctctc	85	122	97	126
4	RM124	67	atcgtctgcgttgcggctgctg	catggatcaccgagctcccccc	257	289	227	242
5	RM507	55	ccttaagctccagccgaaatg	ctcaccctcatcatcgcc	234	257	236	248
	RM413	53	ggcgattcttggatgaagag	tccccaccatcttgtcttc	71	114	92	125
	RM161	61	tgcagatgagaagcggcgcctc	tgtgtcatcagacggcgctccg	154	187	142	180
6	RM162	61	gccagcaaaaccagggatccgg	caaggtcttgtgcggcttgcgg	191	244	207	244
	RM133	63	ttggattgttttgctggctcgc	ggaacacggggtcggaagcgac	226	237	202	211
7	RM125	63	atcagcagccatggcagcgacc	aggggatcatgtgccgaaggcc	105	147	122	128
	RM118	67	ccaatcggagccaccggagagc	cacatcctccagcgacgccgag	149	165	148	169
	RM455	57	aacaacccaccacctgtctc	agaaggaaaagggctcgatc	127	144	108	113
8	RM284	55	atctctgatactccaatccatcc	cctgtacgttgatccgaagc	139	159	136	148
	RM408	55	caacgagctaacttccgtcc	actgctacttgggtagctgacc	112	128	113	143
	RM447	55	cccttgtgctgtctcctctc	acgggcttcttctccttctc	95	146	101	131
	RM44	53	acgggcaatccgaacaacc	tcgggaaaacctaccctacc	82	132	103	125
	RM433	53	tgcgctgaactaaacacagc	agacaaacctggccattcac	216	248	210	228
	RM152	53	gaaaccaccacacctcaccg	ccgtagaccttcttgaagtag	133	157	135	153
9	RM316	55	ctagttgggcatacgatggc	acgcttatatgttacgtcaac	194	216	107	149
	RM215	55	caaaatggagcagcaagagc	tgagcacctccttctctgtag	126	161	136	154
10	RM271	5	tcagatctacaattccatcc	tcggtgagacctagagagcc	80	120	104	130
	RM484	55	tctccctcctcaccattgtc	tgctagccctctctctctctc	286	298	203	737
11	RM536	5	tctctcctcttagtttggctc	acacaccaacacgaccacac	223	247	209	219
12	RM277	55	cggtcaaatcatcacctgac	caaggcttgcaagggaag	104	121	62	68

ranged from 112 to 128, while the observed band size was 113 to 143 with an average of 126.

Out of 24 rice genotypes, Khoujee 3 (lane 5), Boardhan (lane 8), Barshagiri (lane 16) and Bordungsha F1 S5 (lane 21) did not get amplified with the primer RM447 (Figure 1e) whereas Baodhan Bordungsha (lane 18) and Lampnah (lane 5) produced double bands. The expected band size ranged from 95 to 146, while the observed band size was 101 to 131 (Table 1) with average of 109 (Table 2). The primer RM154 could not amplify the genotype Sujanta 5 (lane 9). Bora type (lane 7), Baodhan (lane 8) and Bordungsha F1 S5 (lane 21) produced double bands (Figure 1f). The expected band size ranged from 148 to 230, while the observed band size was 145

Table 2. List of the highest and lowest molecular weight with the maximum frequency of a primer.

Marker	Molecular weight		
	High	Low	Maximum frequency
RM316	149	107	134
RM133	211	202	202
RM215	154	136	142
RM162	244	207	214
RM277	68	62	67
RM283	159	145	147
RM284	148	136	140
RM338	189	172	172
RM431	184	160	177
RM433	228	210	210
RM452	198	169	189
RM455	113	109	111
RM495	157	137	148
RM507	248	236	248
RM514	260	224	239
RM536	219	209	212
RM118	169	148	162
RM125	128	122	128
RM237	137	113	130
RM154	187	145	156
RM408	143	113	126
OSR13	126	97	101
RM124	242	227	237
RM152	153	135	150
RM161	180	142	152
RM271	130	104	114
RM413	125	92	100
RM44	125	103	108
RM447	131	101	109
RM484	737	203	224

to 187 with an average of 156.

The genotype Khoujee 2 (Lane 4) did not get amplified with the primer RM507 (Figure 1g). The expected band size ranged from 234 to 257, while the observed band size was 236 to 248 (Table 1) with an average of 248 (Table 2).

The primer RM536 amplified all the rice genotypes (Figure 1h). The expected band size ranged from 223 to 247, while the observed band size was 209 to 219 (Table 1) which was below the expected minimum band size with an average of 212.

The expected band size with the primer RM514 ranged from 229 to 278, while the observed band size was 224 to 260 (Table 1) with an average of 239 (Table 2). The rice genotypes, Sujanta 2 (lane 6), Khogi lal (lane 14), Dyong (lane 15), Barshagiri (lane 16), Bordungsha F1 S4 (lane 17) and Bordungsha F2 S2 (lane 22) did not get amplified (Figure 1i) while Miatong (lane 3) and Bordungsha F1 S5 (lane 21) produced double bands.

The genotypes, Sujanta 2 (lane 6), Bora type (lane 7), Bihari (lane 12), (Khogi lal (lane 14), Baodhan Bordungsha (lane 18), Bordungsha F1 S2 (lane 20), (Bordungsha F1 S5 (lane 21), Bordungsha F2 S2 (lane 22), Lampnah (lane 23) did not get amplified with the primer RM162 (Figure 1j). Genotype Barshagiri (lane16) was found to produce double bands. The expected band size ranged from 191 to 244, while the observed band size was 207 to 244 (Table 1) with an average of 214 (Table 2).

Level of DNA polymorphism

The DNA polymorphism between the 24 genotypes was studied initially with 30 SSR markers. The number of alleles amplified by each primer ranged from 1 of RM277, RM507, RM124, RM125 and RM133 to 20 RM44 with a

Table 3. Primers showing polymorphisms with their PIC value.

Primer	No. of alleles amplified	PIC value
RM495	18	0.932
RM431	11	0.767
RM237	6	0.386
RM283	2	0.252
RM154	13	0.795
RM452	2	0.705
RM338	7	0.836
RM514	11	0.888
OSR13	16	0.970
RM124	1	0.082
RM507	1	0.082
RM413	9	0.846
RM161	8	0.694
RM162	3	0.656
RM133	1	0.498
RM125	1	0.998
RM118	4	0.493
RM455	7	0.816
RM284	12	0.727
RM408	17	0.859
RM447	10	0.608
RM44	20	0.942
RM433	5	0.395
RM152	8	0.842
RM316	5	0.081
RM215	7	0.885
RM271	13	0.894
RM484	2	0.533
RM536	2	0.087
RM277	1	0.373

total of 223 SSR alleles at average of 7.44 alleles (Table 3) per locus.

Among the set of SSR primers tested, RM44 (0.942), RM495 (0.932), RM125 (0.998) and OSR13 (0.970) came out to be more informative in comparison to other primers, as they could reveal more polymorphism (Table 3). PIC value of the primers RM316, RM237, RM277, RM283, RM536, RM507, RM124 and RM433 were low and, therefore, these were excluded from the analysis of genetic diversity. The PIC value ranged from a minimum of 0.081 in RM316 to maximum of 0.998 in RM125 (Table 3). The average PIC value considering each band generated by the 30 primers was 0.630. All the SSR primers revealed polymorphism of the rice genotypes that were used in the study. This indicates that sufficient polymorphism exists to allow distinction between any given two lines and these lines could be distinguished from all other varieties with a selection of these primers. Polymorphism information contents of the bands are presented in Table 3. Correlation between the consensus tree file and the individual similarity matrices were high (0.95 to 0.99) (Figure 2).

Genetic diversity of rice genotypes

A set of all 30 SSR markers was able to distinguish the 24 rice genotypes even though some accessions were closely related. Dendogram was generated based on the results of the unweighted pair group method with arithmetic mean (UPGMA) cluster analysis (Figure 3), calculated from 30 microsatellite marker alleles of 24 rice genotypes out of which one was *O. rufipogon*.

The dendogram constructed clearly distinguish the 24 rice genotypes into six different clusters (A to F) groups. Dissimilarity coefficient ranged from 0.06 to 0.94 (Figure 3). Bordungsha F1 S5 and Baodhan Bordungsha (deep water rice) were distinctly different from the rests of the

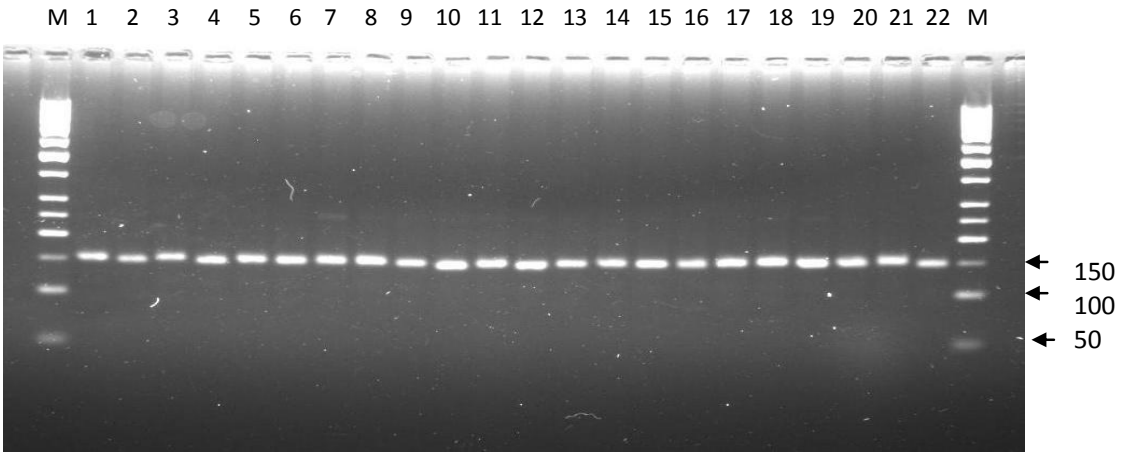

Figure 1a. The amplification profile of rice genotypes generated by the primer RM215 (Lanes 1 to 22). Lane 1, Bordungsha F1 S; lane 2, Bhatguti; lane 3, Miatong; lane 4, Khoujee 2; lane, Khoujee 3; lane 6, Sujanta 2; lane 7, Bora type; lane 8, Boradhan; lane 9, Sujanta 5; lane 10, Khogi boga; lane 11, Ranjit; lane 12, Bihari; lane 13, Bihari type; lane 14, Khogi lal; lane 15, Dyong; lane 16, Barshagiri; lane 17, Bordungsha F1, lane S4; 18, Baodhan Bordungsha; lane 19, Bordungsha F1; lane 20, Bordungsha F1 S2; lane 21, Bordungsha F1 S5; lane 22, Bordungsha F2 S2. Lane M, Marker.

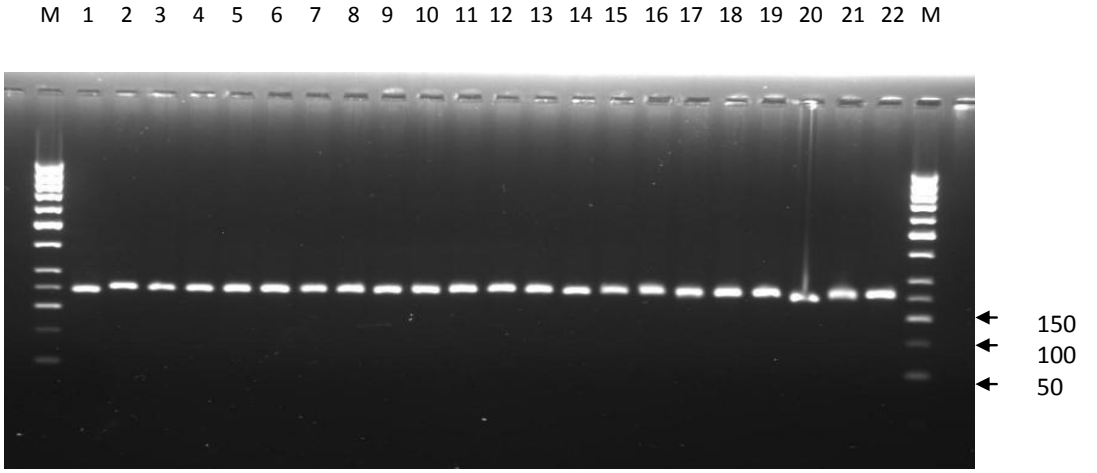

Figure 1b. The amplification profile of rice genotypes generated by the primer RM431 (Lanes 1 to 22). Lane 1, Bordungsha F1 S; lane 2, Bhatguti; lane 3, Miatong; lane 4, Khoujee 2; lane 5, Khoujee 3; lane 6, Sujanta 2; lane 7, Bora type; lane 8, Boradhan; lane 9, Sujanta 5; lane 10, Khogi boga; lane 11, Ranjit; lane 12, Bihari; lane 13, Bihari type; lane 14, Khogi lal; lane 15, Dyong; lane 16, Barshagiri; lane 17, Bordungsha F1, S4; lane 18, Baodhan Bordungsha; lane 19, Bordungsha F1; lane 20, Bordungsha F1 S2; lane 21, Bordungsha F1 S5; lane 22, Bordungsha F2 S2. Lane M, Marker.

genotype, Bordungsha F1 S and Miatong showed very little dissimilarity while their seed morphology were distinctly different. *O. rufipogon* (cp) genotype which was used as an out group, actually grouped with Bordungsha F1 S4 which was similar to *O. rufipogon* in seed morphology. Group A was the biggest and included 13 genotypes while group E and F included single genotype each. There was a doubt during collection of the genotypes that Khogi Boga was a selection from the variety 'Mashuri' made by the farmers while Bihari was actually the variety 'Pankaj'. Molecular analysis also indicated that the variety 'Ranjit' which was cross of 'Mashuri' and 'Pankaj' grouped with Khogi Boga and Bihari. Overall the genotypic analysis showed that although the collection was made from a very small area, there exists high degree of variability in the Khamti rice and there was a good scope of improvement by breeding and selection within the genotypes.

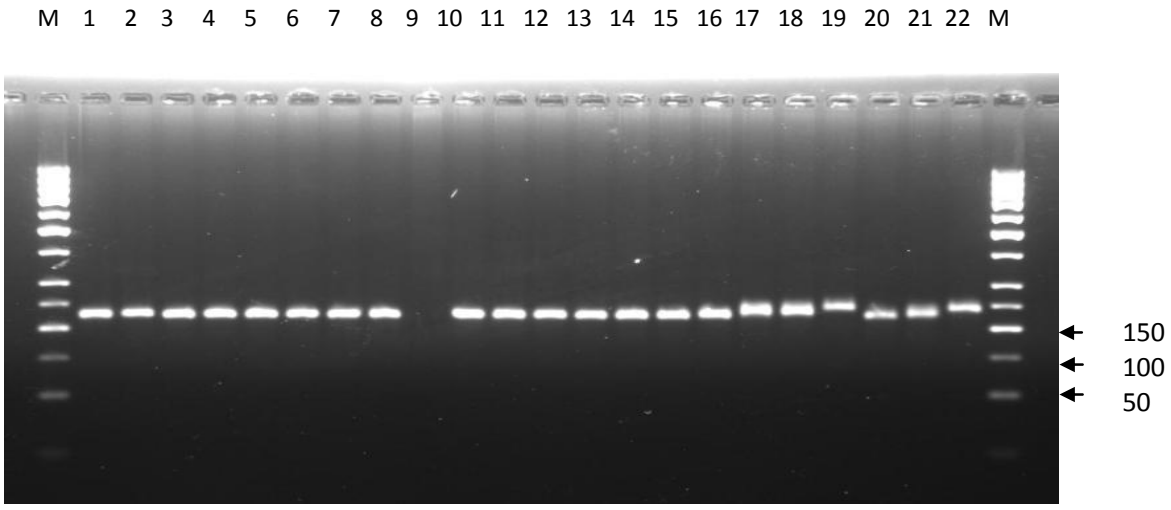

Figure 1c. The amplification profile of rice genotypes generated by the primer RM433 (Lanes 1 to 22). Lane 1, Bordungsha F1 S; lane 2, Bhatguti; lane 3, Miatong; lane 4, Khoujee 2; lane 5, Khoujee 3; lane 6, Sujanta 2; lane 7, Bora type; lane 8, Boradhan; lane 9, Sujanta 5; lane 10, Khogi boga; lane 11, Ranjit; lane 12, Bihari; lane 13, Bihari type; lane 14, Khogi lal; lane 15, Dyong; lane 16, Barshagiri; lane 17, Bordungsha F1, S4; lane 18, Baodhan Bordungsha; lane 19, Bordungsha F1; lane 20, Bordungsha F1 S2; lane 21, Bordungsha F1 S5; lane 22, Bordungsha F2 S2. Lane M, Marker.

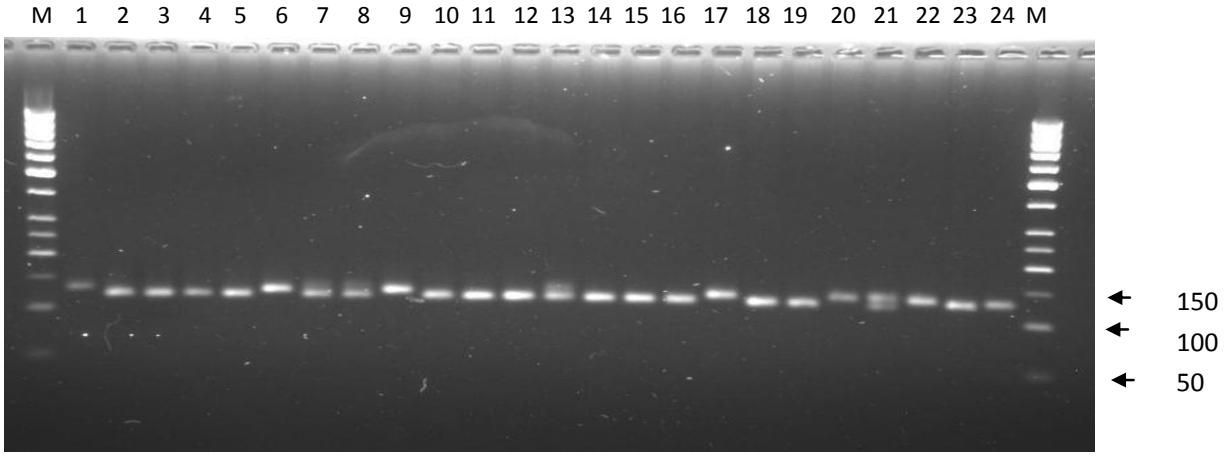

Figure 1d. The amplification profile of rice genotypes generated by the primer RM408 (Lanes 1 to 24). Lane 1, Bordungsha F1 S; lane 2, Bhatguti; lane 3, Miatong; lane 4, Khoujee 2; lane 5, Khoujee 3; lane 6, Sujanta 2; lane 7, Bora type; lane 8, Boradhan; lane 9, Sujanta 5; lane 10, Khogi boga; lane 11, Ranjit; lane 12, Bihari; 13, Bihari type; lane 14, Khogi lal; lane 15, Dyong; lane 16, Barshagiri; lane 17, Bordungsha F1, S4; lane 18, Baodhan Bordungsha; lane 19, Bordungsha F1; lane 20, Bordungsha F1 S2; lane 21, Bordungsha F1 S5; lane 22, Bordungsha F2 S2; lane 23, Lampnah; lane 24, *Oryza rufipogon*. Lane M, Marker.

DISCUSSION

Microsatellite markers have been used to investigate genetic diversity of a large number of cultivars in rice (Yang et al., 1994). 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. (2011) evaluated genetic diversity of the 30 rice cultivars for salinity tolerance during the seedling stage using RAPD and simple sequence repeats (SSR) markers. The cultivars were evaluated for polymorphisms after amplification with 20 random decamer primers and 20 SSR primer pairs. A total of 161 RAPD markers and 190 SSR alleles were produced

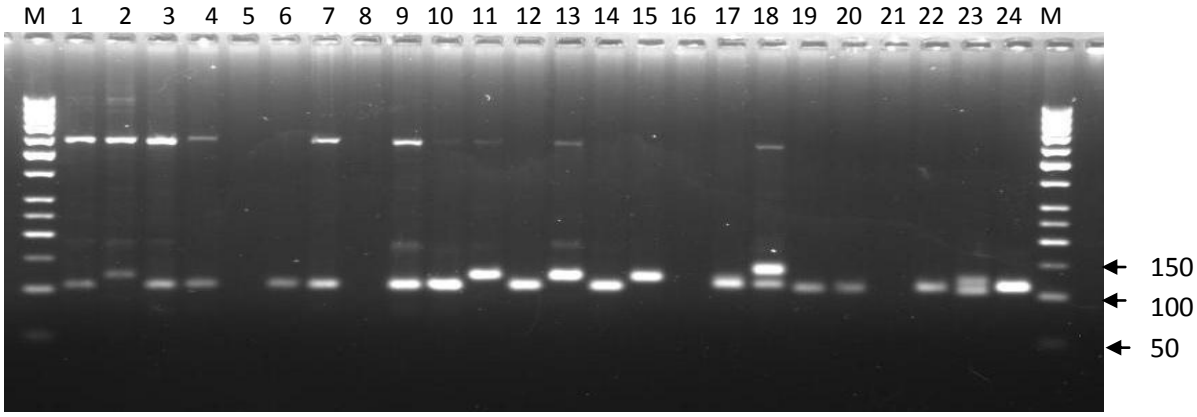

Figure 1e. The amplification profile of rice genotypes generated by the primer RM447 (Lanes 1 to 24). Lane 1, Bordungsha F1 S; lane 2, Bhatguti; lane, Miatong; lane 4, Khoujee 2; lane 5, Khoujee 3; lane 6, Sujanta 2; lane 7, Bora type; lane 8, Boradhan; lane 9, Sujanta 5; lane 10, Khogi boga; lane 11, Ranjit; lane 12, Bihari; lane 13, Bihari type; lane 14, Khogi lal; lane 15, Dyong; lane 16, Barshagiri; lane 17, Bordungsha F1, S4; 18, Baodhan Bordungsha; lane 19, Bordungsha F1; lane 20, Bordungsha F1 S2; lane 21, Bordungsha F1 S5; lane 22, Bordungsha F2 S2; lane 23, Lampnah; lane 24, *Oryza rufipogon*. Lane M, Marker.

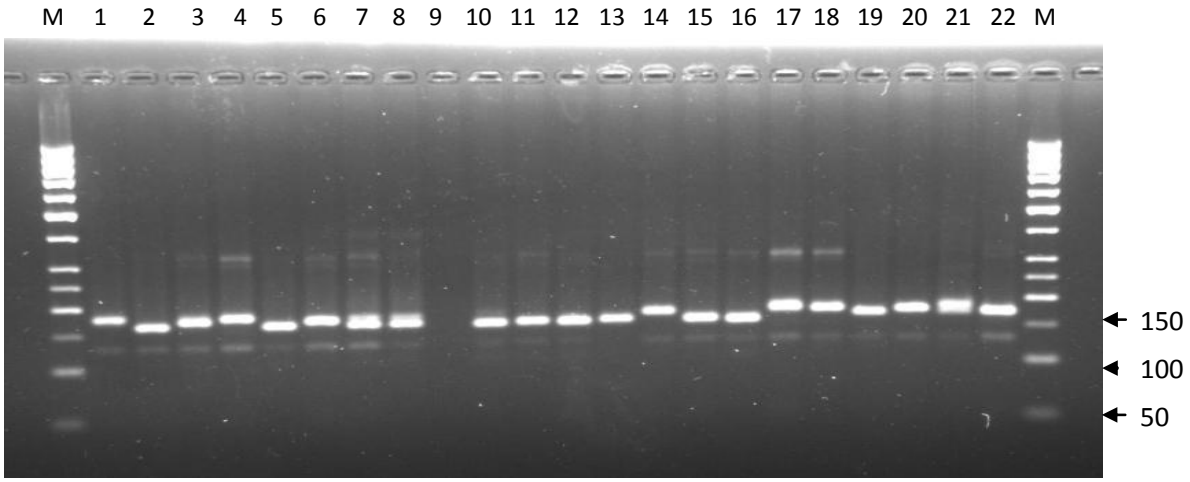

Figure 1f. The amplification profile of rice genotypes generated by the primer RM154 (Lanes 1 to 22). Lane 1, Bordungsha F1 S; lane 2, Bhatguti; lane 3, Miatong; lane 4, Khoujee 2; lane 5, Khoujee 3; lane 6, Sujanta 2; lane 7, Bora type; lane 8, Boradhan; lane 9, Sujanta 5; lane 10, Khogi boga; lane 11, Ranjit; lane 12, Bihari; lane 13, Bihari type; lane 14, Khogi lal; lane 15, Dyong; lane 16, Barshagiri; lane 17, Bordungsha F1, S4; lane 18, Baodhan Bordungsha; lane 19, Bordungsha F1; lane 20, Bordungsha F1 S2; lane 21, Bordungsha F1 S5; lane 22, Bordungsha F2 S2. Lane M, Marker.

which revealed 68.94 and 89.47% polymorphism, respectively. Mean genetic similarity coefficient was 0.82 for RAPD and 0.70 for SSR.

Thanh et al. (2012) evaluated the genetic diversity of 50 samples of *Afzelia xylocarpa* (Kurz) Craib based on the analysis of chloroplast 16S rRNA, non-coding regions between *trn*H-*trn*K, *trn*D-*trn*T and *ps*bC-*trn*S chloroplast genes using PCR-RFLP and RAPD markers and revealed that the all samples of *A. xylocarpa* (Kurz) Craib had low level of genetic diversity, as supported by the fact that the genetic similarity coefficients of the trees ranged from 49 to 100%.

In the present investigation a set of 30 SSR markers covering the rice genome were utilized for analysis to assess the genetic diversity of the rice of Arunachal Pradesh. Thirty (30) SSR markers were evaluated in 23 rice cultivars and *O. rufipogon*. All 30 markers were polymorphic across the 24 genotypes. A total of 223 alleles were detected with an average number of alleles of 7.44 per locus (Table 3). A similar study was

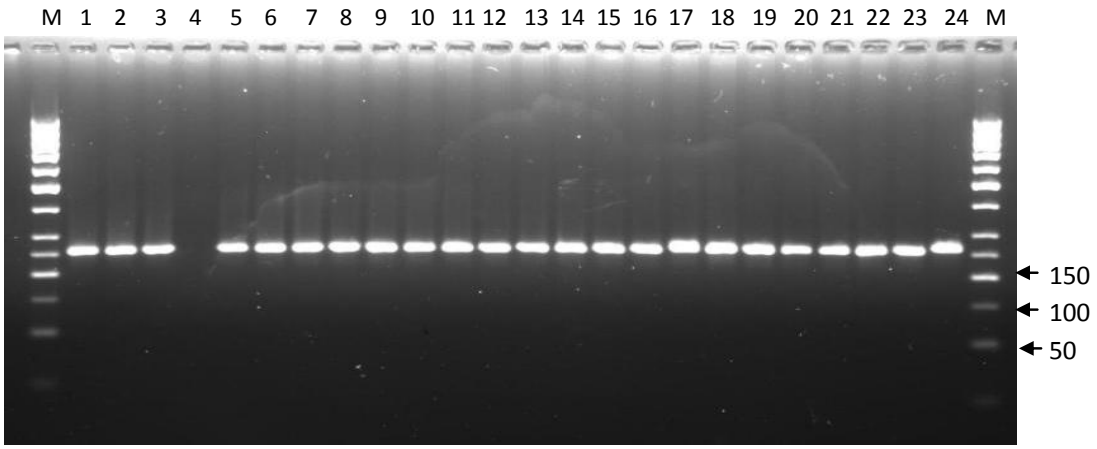

Figure 1g. The amplification profile of rice genotypes generated by the primer RM507 (Lanes 1 to 24). Lane 1, Bordungsha F1 S; lane 2, Bhatguti; lane 3, Miatong; lane 4, Khoujee 2; lane 5, Khoujee 3; lane 6, Sujanta 2; lane 7, Bora type; lane 8, Boradhan; lane 9, Sujanta 5; lane 10, Khogi boga; lane 11, Ranjit; lane 12, Bihari; lane 13, Bihari type; lane 14, Khogi lal; lane 15, Dyong; lane 16, Barshagiri; lane 17, Bordungsha F1, S4; lane 18, Baodhan Bordungsha; lane 19, Bordungsha F1; lane 20, Bordungsha F1 S2; lane 21, Bordungsha F1 S5; lane 22, Bordungsha F2 S2; lane 23, Lampnah; lane 24, *Oryza rufipogon*. Lane M, Marker.

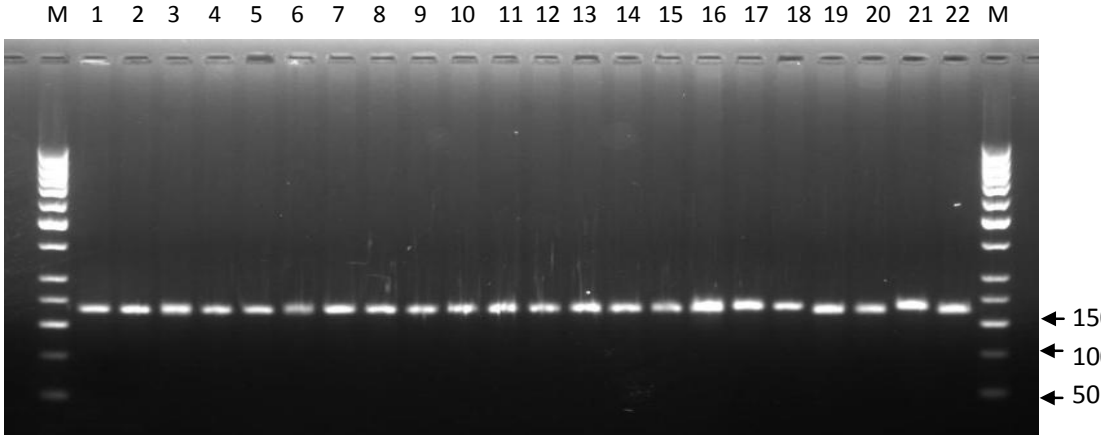

Figure 1h. The amplification profile of rice genotypes generated by the primer RM536 (Lanes 1 to 22). Lane 1, Bordungsha F1 S; lane 2, Bhatguti; lane 3, Miatong; lane 4, Khoujee 2; lane 5, Khoujee 3; lane 6, Sujanta 2; lane 7, Bora type; lane 8, Boradhan; lane 9, Sujanta 5; lane 10, Khogi boga; lane 11, Ranjit; lane 12, Bihari; lane 13, Bihari type; lane 14, Khogi lal; lane 15, Dyong; lane 16, Barshagiri; lane 17, Bordungsha F1, S4; lane 18, Baodhan Bordungsha; lane 19, Bordungsha F1; lane 20, Bordungsha F1 S2; lane 21, Bordungsha F1 S5; lane 22, Bordungsha F2 S2. Lane M, Marker.

conducted by Cao et al. (2006) where a total of 91 alleles with an average of 4.55 per locus (ranging from 75 to 257 bp) were generated by the 20 SSR primers, the highest number of alleles was 10. Another similar study conducted by Prasanna et al. (2006) generated 89 SSR alleles from 27 SSR markers at an average of 3.30 alleles per locus, length of amplified product ranged from 50 to 350 bp.

The PIC value ranged from a minimum of 0.081 in RM316 to maximum of 0.998 in RM125. PIC values and observed genetic distances showed the existence of large variability among the various accessions. The four SSR loci with PIC values equal to or greater than 0.932, RM44, RM495, RM125, and OSR13 showed a total allele number of 20, 18, 1 and 16, respectively. These indicated that markers with large number of alleles were informative for population studies. This study reveals the existence of considerable genetic variation in the Khamti rice genotypes of Arunachal Pradesh. Previous study conducted by Jayamani et al. (2007) analyzed a total of

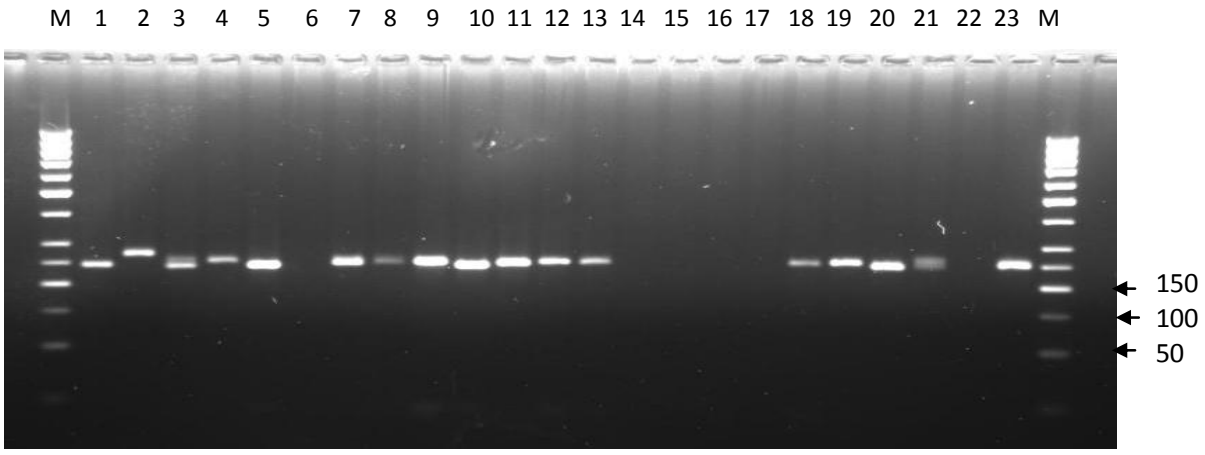

Figure 1i. The amplification profile of rice genotypes generated by the primer RM514 (Lanes 1 to 23). Lane 1, Bordungsha F1 S; lane 2, Bhatguti; lane 3, Miatong; lane 4, Khoujee 2; lane 5, Khoujee 3; lane 6, Sujanta 2; lane 7, Bora type; lane 8, Boradhan; lane 9, Sujanta 5; lane 10, Khogi boga; lane 11, Ranjit; lane 12, Bihari; lane 13, Bihari type; lane 14, Khogi lal; lane 15, Dyong; lane 16, Barshagiri; lane 17, Bordungsha F1, S4; lane 18, Baodhan Bordungsha; lane 19, Bordungsha F1; lane 20, Bordungsha F1 S2; lane 21, Bordungsha F1 S5; lane 22, Bordungsha F2 S2; lane 23, Lampnah. Lane M, Marker.

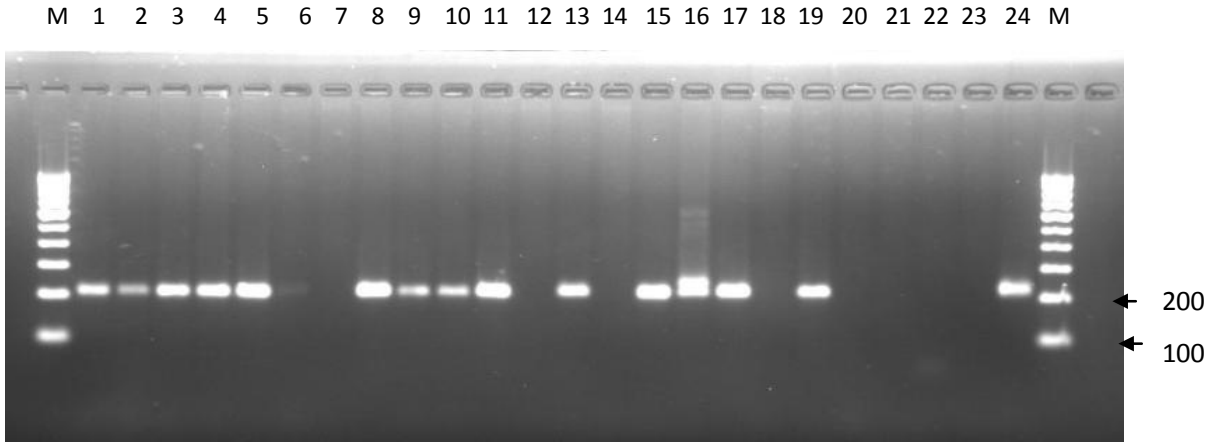

Figure 1j. The amplification profile of rice genotypes generated by the primer RM162 (Lanes 1 to 24). Lane 1, Bordungsha F1 S; lane 2, Bhatguti; lane 3, Miatong; lane 4, Khoujee 2; lane 5, Khoujee 3; lane 6, Sujanta 2; lane 7, Bora type; lane 8, Boradhan; lane 9, Sujanta 5; lane 10, Khogi boga; lane 11, Ranjit; lane 12, Bihari; lane 13, Bihari type; lane 14, Khogi lal; lane 15, Dyong; lane 16, Barshagiri; lane 17, Bordungsha F1, S4; lane 18, Baodhan Bordungsha; lane 19, Bordungsha F1; lane 20, Bordungsha F1 S2; lane 21, Bordungsha F1 S5; lane 22, Bordungsha F2 S2; lane 23, Lampnah; lane 24, *Oryza rufipogon*. Lane M, Marker.

176 rice accessions and two standard varieties using 24 SSR loci, 184 alleles were detected and the number of allele per locus ranges from three to 16 with an average of 7.7, PIC value ranging from a minimum of 0.179 to 0.894 with an average of 0.667. All loci were polymorphic among the rice accessions, revealing a high degree of diversity among the accessions.

Shah et al. (2012) assessed the genetic diversity among 22 rice genotypes representative of restorer, maintainer and male sterile lines using SSR markers. Among 30 SSR markers used, 25 SSR loci generated polymorphic patterns and a total of 231 alleles were amplified. The number of alleles per locus ranged from 5 to 17 with a mean of 9.4 alleles per locus. The PIC values for 25 SSR markers varied from 0.74 (RM195, RM10318, and RM258) to 0.92 (RM302).

Saini et al. (2006) evaluated the genetic diversity and patterns of relationships among the 18 rice genotypes representative of the traditional Basmati, cross-bred Basmati and non-Basmati (*indica* and *japonic*a) rice

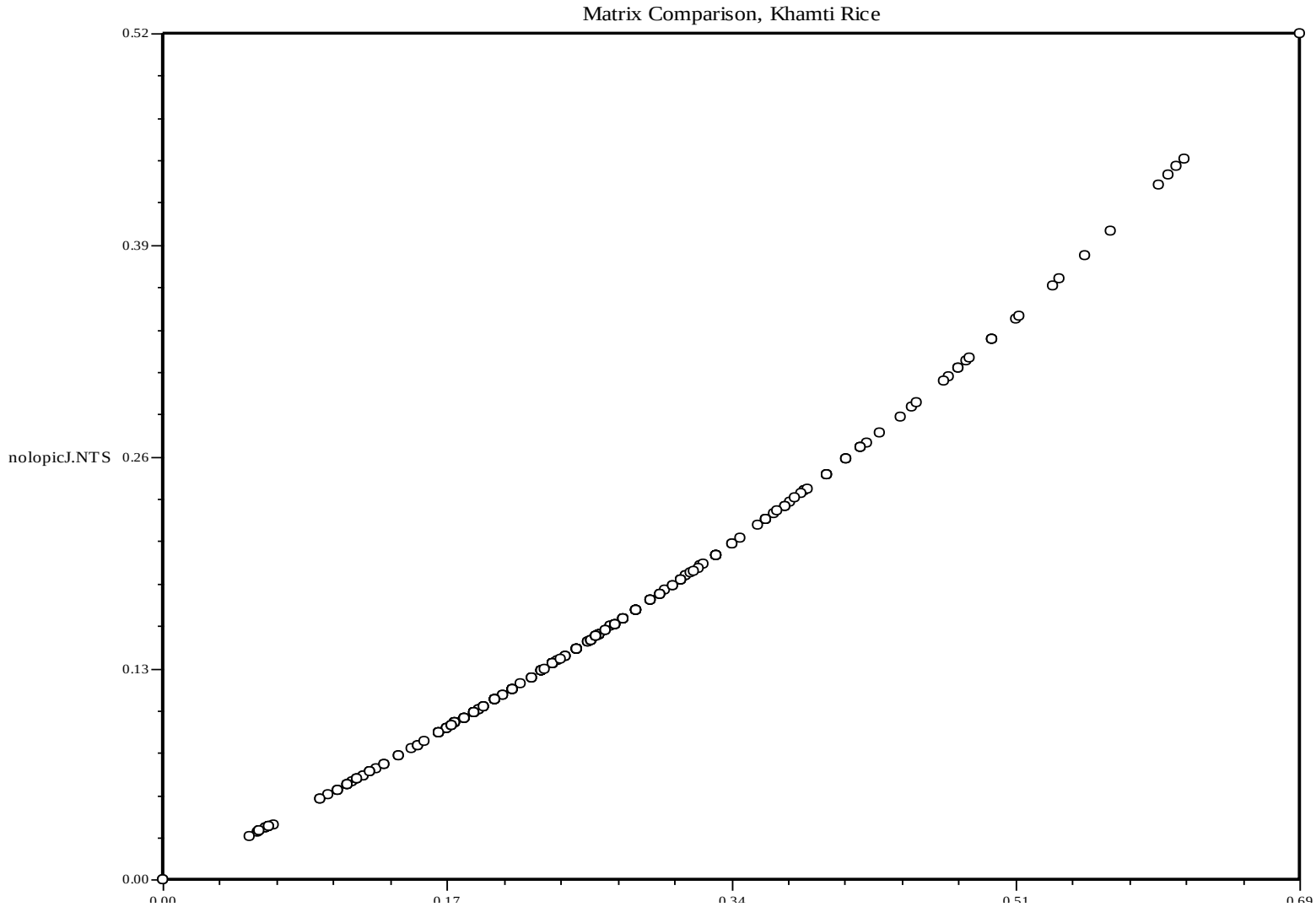

Figure 2. Comparing of the similarity and consensus tree matrices.

varieties using amplified fragment length polymorphism (AFLP), inter-simple sequence repeat (ISSR) and SSR markers. All the

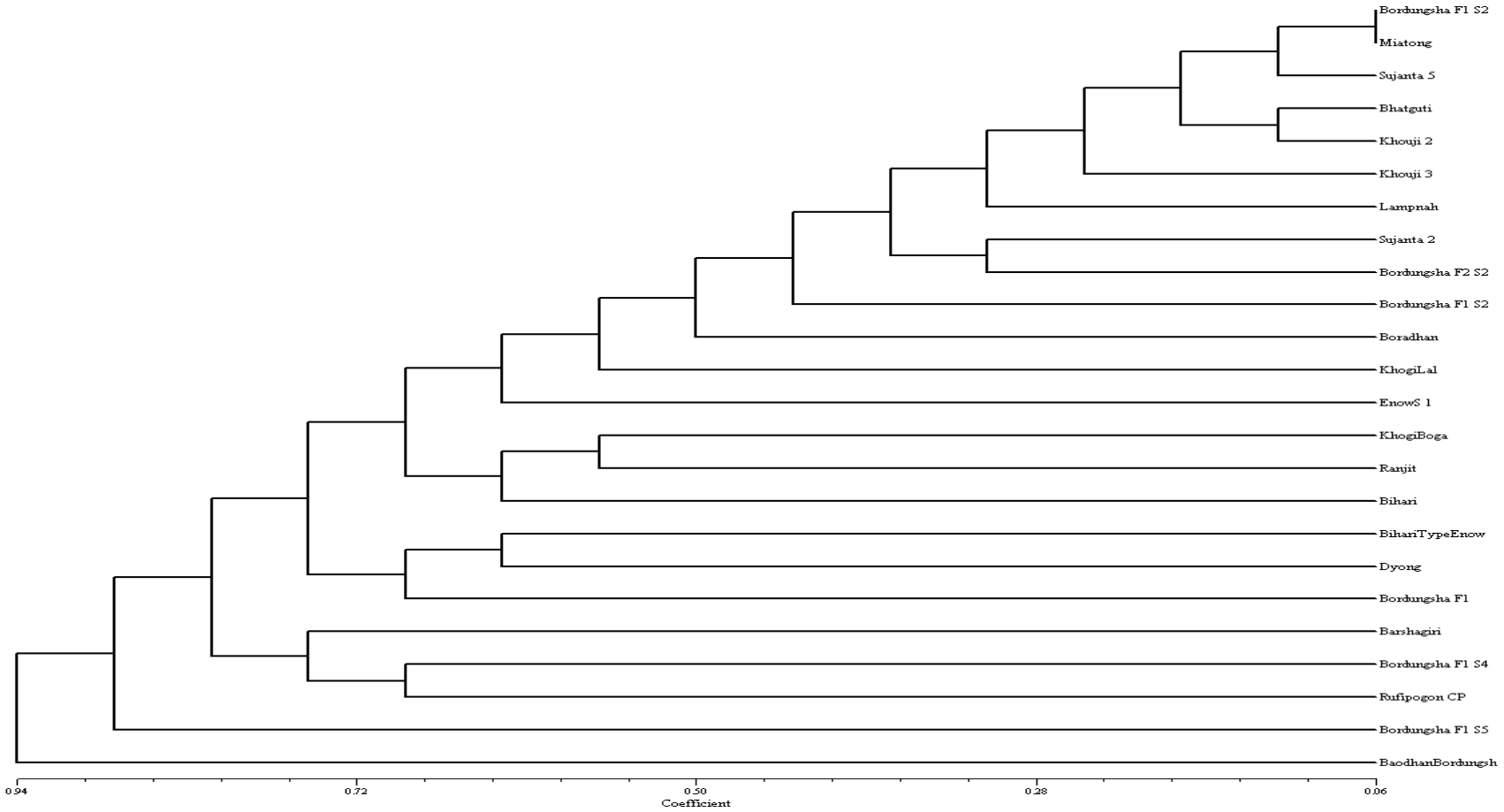

Figure 3. Dendrogram showing genetic diversity coefficient among Khamti rice genotypes. 1, Bordungsha F1 S; 2, Bhatguti; 3, Miatong; 4, Khoujee 2; 5, Khoujee 3; 6, Sujanta 2; 7, Bora type; 8, Boradhan; 9, Sujanta 5; 10, Khogi boga; 11, Ranjit; 12, Bihari; 13, Bihari type; 14, Khogi lal; 15, Dyong; 16, Barshagiri; 17, Bordungsha F1, S4; 18, Baodhan Bordungsha; 19, Bordungsha F1; 20, Bordungsha F1 S2; 21, Bordungsha F1 S5; 22, Bordungsha F2 S2; 23, Lampnah; 24, *Oryza rufipogon*.

three marker systems generated higher levels of polymorphism and could distinguish between all the 18 rice cultivars. A total of 171 (110 polymorphic), 240 (188 polymorphic) and 160 (159 polymorphic) bands were detected using five primer combinations of AFLP, 25 ISSR primers and 30 well distributed, mapped SSR markers, respectively.

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 (Ravi et al., 2003). The structure and length of SSRs are considered to be the major factors affecting microsatellite variability (Brinkman et al., 1998).

Conclusion

The correct determination of genetic diversity of varieties in the germplasm is important for rice breeding programs, allowing selection of the desired rice accessions for crossing. The present investigation was carried out to analyze the genetic diversity of Khamti rice utilizing microsatellite or SSR marker and evaluated for polymorphisms after amplification with 30 SSR primers pairs.

Twenty four (24) genotypes of rice from Arunachal Pradesh were studied for genetic diversity. The results obtained depict a wide range of variation among varieties. Through SSR analysis, diversity parameters confirmed that Bordungsha F1, S5 and Baodhan Bordungsha (deep water rice) were most diverse and were distinctly different from the rests of the genotype and the dissimilarity coefficient ranged from 0.06 to 0.94. This study provides a clear picture of the genetic diversity of rice.

Microsatellite or SSR markers of rice showed a level of polymorphism. Among the set of SSR primers tested, RM44 (0.942), RM495 (0.932), RM125 (0.998) and OSR13 (0.970) came out to be more informative in comparison to other primers, as they could reveal more polymorphism. The polymorphic SSR markers identified in this study not only reflected the conservation of SSR loci in *O. sativa*, but would also help in detailed molecular marker-based analysis of *O. sativa* ecotypes and *O. rufipogon* prevalent in Arunachal Pradesh.

Through SSR analysis, genotypes of rice showed that although the collection was made from a very small area, there exists high degree of variability in the Khamti rice and there is a good scope of improvement by breeding and selection within the genotypes.

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