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Molecular characterization and morphological clustering of exotic early maturing rice (*Oryza sativa* L.) lines

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Abstract

Characterization and variability analysis is important for the improvement of crop plant. This study aimed to evaluate the morphological and molecular variation of exotic early maturing rice (*Oryza sativa* L.) lines. A total of 32 exotic rice lines collected from different locations were genotyped and clustered using selected SSR markers. Based on morphological dendrogram, the lines were grouped into three clusters *viz.*I, II and III. Cluster I, cluster II and cluster III had 12, 11, 9 lines respectively. The results showed that the varieties were closely related belonging to the same cluster. DNA Markers namely Simple Sequence Repeats (SSR) is a useful tool for assessing genetic variations and resolving cultivar identities. Positive correlations were found between gene diversity, number of allele, the allele size range and the maximum number of repeats. Among the primers used RM147 identified more number of alleles and average PIC was 0.88. The UPGMA dendrogram based on Nei's (1972) genetic distance grouped the 32 rice lines into three major clusters. This result indicates that the line which formed grouped together, they are less diverse. A significant level of polymorphism based on morphological and molecular levels was observed. Being grouped into three clusters C1-4-11-7P-2P-1P and IR 79201-49-1-1-1 could be utilized as potential parents for the improvement of yield in early maturing rice lines.

Keywords: Early maturing rice, SSR markers, Cluster analysis, Genetic diversity

Introduction

Rice (*Oryza sativa* L.) is the staple food of more than 50% of the world's population (Aggarwal *et al*. 2002). The demand of rice increasing rapidly throughout the world. In Bangladesh, total crop production of 18.1 million tons and total demand is 35.3 million tons and cropping intensity is 183% (BBS, 2012). Therefore, to fill up the gap between production and demand, we need to increase cropping intensity nearly 300%. Short duration rice is an important feature to increase cropping intensity. Breeding for earliness is one of the basic objectives in breeding programs. The increased attention to the development of short duration plant species for prolonged food production under different conditions indicates the necessity of performing breeding experiments (Martin *et al.* 2008; Khodadadi *et al.* 2011). Genetic distance between parents and clustering are essential to get benefit of transgressed segregation. Growth and development of agricultural resources is mostly depend on genetic diversity among different crop plants and it is estimated that not even 15% of the potential diversity has utilized. This implies that thousands of valuable allelic variations of traits of economic significance remain unutilized (Hossain *et al.* 2007). Therefore, lines of distinct genetic structure are a good promise for the future rice crop improvement. Thus identification of genotypes and their inter-relationships is essential and it can be done by molecular markers. Molecular markers are powerful tools to detect genetic diversity and to aid in the management of plant genetic resources (Virk *et al.* 2000; Song *et al.* 2003; Teixeira da Silva, 2005). Microsatellite is faster and easier for exploiting genetic polymorphism among different lines and populations compared with other markers. These markers can detect a significantly higher degree of polymorphism in rice (Ni *et al*. 2002, Okoshi *et al.* 2004). The objectives present study was to evaluate the morphological and molecular variation among 32 exotic early maturing rice lines.

Materials and Methods

The current research was carried out at Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh-2202, during 2012 and 2013 seasons at Breeding field and Biotechnology laboratory.

Plant materials

Thirty two rice lines with diverse genetic background were used in this study. Of which thirty one were International Network for Genetic Evaluation of Rice (INGER) early maturing rice lines and one Bangladesh Institute of Nuclear Agriculture (BINA) developed short duration variety 'Binadhan-7' was used as check.

Table 1. List of rice lines with origin

Sl No	Lines Name	Origin	Sl No	Lines Name	Origin
1	IR 79246-105-2-2-4	IRRI	17	BP 1018F-BB8-13-BB4	INDONESIA
2	IR 73718-26-1-2-5	IRRI	18	IR 79525-20-2-2-2	IRRI
3	BP 10620F-BB4-13-BB8	INDONESIA	19	IR 80285-34-3-3-2	IRRI
4	IR 79538-1-1-1-1	IRRI	20	CT 18173-1-9-1-3-6-M	CIAT
5	IR 76494-28-1-2-2	IRRI	21	BP 10620F-BB4-2-BB4	INDONESIA
6	YN 2883-12-2-1	MYANMAR	22	PSB RC 64	INDIA
7	AD 02207	INDIA	23	IR 08N261	IRRI
8	BP 10620F-BB4-8-BB8	INDONESIA	24	RATNAGIRI 2	INDIA
9	C1-4-11-7P-2P-1P	CIAT	25	MTU-1113	INDIA
10	IR 79201-49-1-1-1	IRRI	26	KARJAT 5	INDIA
11	BP 10620F-BB4-12-BB8	INDONESIA	27	KHAZAR	IRAN
12	IR 82489-594-3-2-2	IRRI	28	IR 59552-21-3-2-2	IRRI
13	CT 18509-10-6-1VI-2	CIAT	29	C 2-9-9-2P-1P-3P	CIAT
14	IR 74052-153-5-3-1-3	IRRI	30	IR 39809-26-3-3	IRRI
15	PSD RC 2	IRRI	31	CT 18148-11-1-1-1-1-M	CIAT
16	IR 08N293	IRRI	32	BINA DHAN 7	BINA

Design and data collection

The experiment was laid out in a RCBD with three replications. The row to row and plant to plant distances were 20 cm and 15 cm, respectively. The following data Plant height (cm), Days to flowering, Days to maturity, Total tillers and effective tillers hill^{-1}, Filled and unfilled grains panicle^{-1}, 1000 seed weight (g), Yield plant^{-1} (g) were collected from field from randomly selected 5 plants of each unit plot.

Genotyping

Modified CTAB mini prep was used for DNA extraction for 21day-old seedling (IRRI, 1997). Parental polymorphism survey was done with eight SSR markers. Out of 5 markers, three polymorphic SSR markers viz., RM147, RM202 and RM215 were showed polymorphic and clear bands. Each PCR reaction carried out with 15.0µl reactions containing 1.5 µl 10 X buffer, 0.75 µl dNTPs, 1µl primer forward, 1µl primer reverse, 0.5 µl taq polymerase, 8.25 µl ddH_2O and 2.0 µl of each template DNA samples. PCR profile was maintained as initial denaturation at 94^oC for 5 min, followed by 34 cycles of denaturation at 94^oC for 1 min, annealing at 55^oC for 1 min and polymerization at 72^oC for 2 min; and final extension by 7 min at 72^oC. Then electrophoresis in 1.5% agarose gel was done after polymorphism in the PCR products and stained in ethidium bromide.

Primer test

Five primers of random sequence were screened for amplification of the DNA sequences. The details of the primers are given in Table 2. A final subset of three primers exhibiting good quality banding patterns and sufficient variability were selected for further analysis.

Table 2. Random primers used in the present study for polymorphism survey

Primer code	Forward Primer (Bases)	Reverse Primer (Bases)
RM47	ACTCCACTCCACTCCCCAC	GTCAGCAGGTCGGACGTC
RM147*	TACGGCTTCGGCGGCTGATTCC	CCCCCGAATCCCATCGAAACCC
RM167	GATCCAGCGTGAGGAACACGT	AGTCCGACCACAAGGTGCGTTGTC
RM202*	CAGATTGGAGATGAAGTCCTCC	CCAGCAAGCATGTCAATGTA
RM215*	CAAAATGGAGCAGCAAGAGC	TGAGCACCTCCTTCTCTGTAG

* Selected for SSR analysis in thirty two rice lines

Data Analysis

Morphological cluster were constructed by using Stat Graphics Plus for Windows 3.0 (Statistical Graphics Crop. Rockville, USA). Molecular weights for microsatellite products were estimated with AlphaEaseFC 4 software. The summary statistics including the number of alleles per locus, major allele frequency, genetic diversity and polymorphism information content (PIC) values were determined by using POWER MARKER (version 3.23). The unweighted pair-group method with arithmetic mean (UPGMA) dendrogram was drawn by using the software TREEVIEW.

Results and Discussion

Cluster analysis of morphological traits

Ward's dendrogram: Dendrogram grouped of 32 lines of rice into three clusters (Fig 1). Binadhan-7 as control line is grouped in cluster II. with lowest (0.00) genetic distance, IR 79246-105-2-2-4, IR 73718-26-1-2-5, CT 18148-11-1-1-1-1-M, BP 1018F-BB8-13-BB4, AD 02207, BP 1018F-BB8-13-BB4,IR 80285-34-3-3-2, PSB RC 64, CT 18509-10-6-1VI-2, MTU-1113, KARJAT 5, KHAZAR, IR 39809-26-3-3were grouped in cluster I. IR 76494-28-1-2-2, IR 82489-594-3-2-2, IR 08N293, C 2-9-9-2P-1P-3P, IR 79525-20-2-2-2, IR 79201-49-1-1-1, BP 10620F-BB4-12-BB8, IR 08N261, RATNAGIRI 2 were grouped on cluster III.

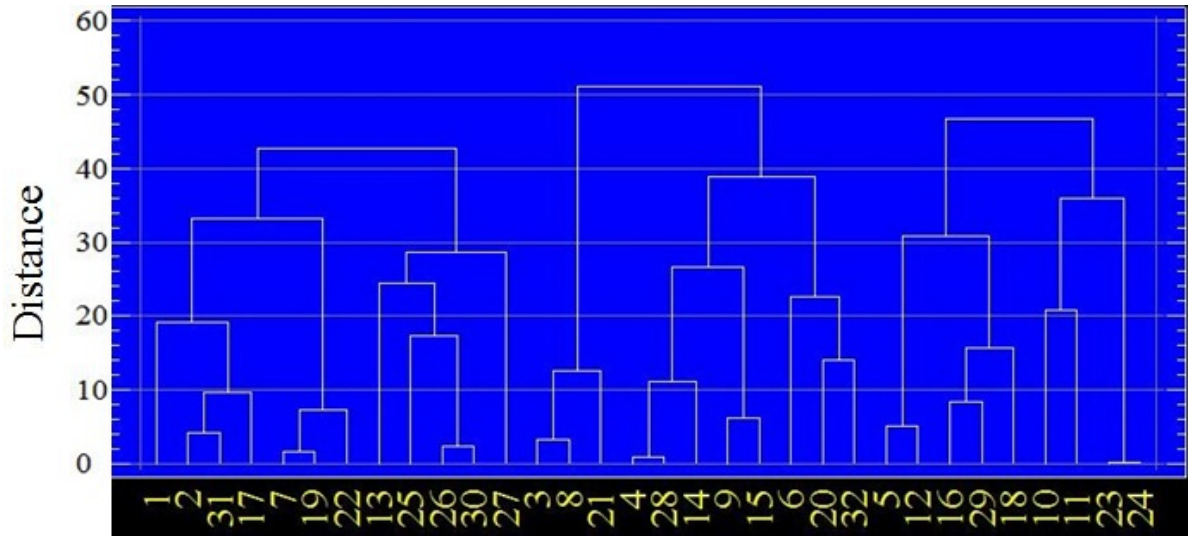

Fig.1. Dendrogram based on summarized data on differentiation among 32 lines of rice according to Ward's method

Genotyping through molecular markers: All the microsatellite loci (RM147, RM215 and RM202) amplified were found to be polymorphic. Using 3 primers across 32 varieties 45 allele were identified. The number of alleles ranged from eight to fourteen per locus. The locus RM147 had the highest number of alleles (14) and RM215 contain the number of alleles (12) while the locus RM202 had the lowest number of alleles (8) (Table 3). Yang *et al*. (1994) found up to 25 alleles for 10 microsatellite markers among 238 accessions of *Indica* and *Japonica* cultivars and landraces.

Table 3. Frequency and no. of alleles found by 3 SSR loci across 32 lines of rice

Marker	Allele1	Covariance	No. of alleles	Frequency	2.5% l.b.	97.5% u.b.
RM147	157	0.00094	14	0.0313	0.5789	0.8947
RM215	431	0.00183	12	0.0625	0.5263	0.8947
RM202	427	0.00476	8	0.1875	0.4737	0.8421

Allelic and loci variation within the lines

Gene diversity: According to Nei's (1972), the highest level of gene diversity value (0.90) was observed in loci RM215 and the lowest level of gene diversity value (0.81) was observed in loci RM147 with a mean diversity of 0.86 (Table 4). It was observed that marker detecting the fewer alleles showed lower gene diversity than those detected higher number of alleles which revealed higher gene diversity. The maximum number of repeats within the SSRs was also positively correlated with the genetic diversity Herrera *et al*. (2008), also 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.

Allele size range: The size variation between the smallest and the largest allele at a given SSR locus was correlated with the number of alleles per locus. Thus, RM147 presented the smallest allele size range (129bp), while RM215 presented the largest allele size range (248bp) (Table 4).

PIC values: As a measure of the informativeness of microsatellites, the PIC values ranged from a low of 0.78 (RM202) to a high of 0.89 (RM215) and averaged 0.85 (Table 4).

Table 4. Data on repeat motif, PIC value and gene diversity (GD) found in 32 early maturing rice lines by 3 microsatellites (SSR)

Locus	Repeat motif	Allele Size ranges	Major Allele Frequency	Differences (bp)	PIC	Gene Diversity
RM147	(TTCC)5(GGT)5	157-286	0.22	129	0.88	0.89
RM215	(CT)16	431-679	0.16	248	0.89	0.90
RM202	(CT)30	427-531	0.31	104	0.78	0.81
Total					2.56	2.60
Mean			0.23		0.85	0.86

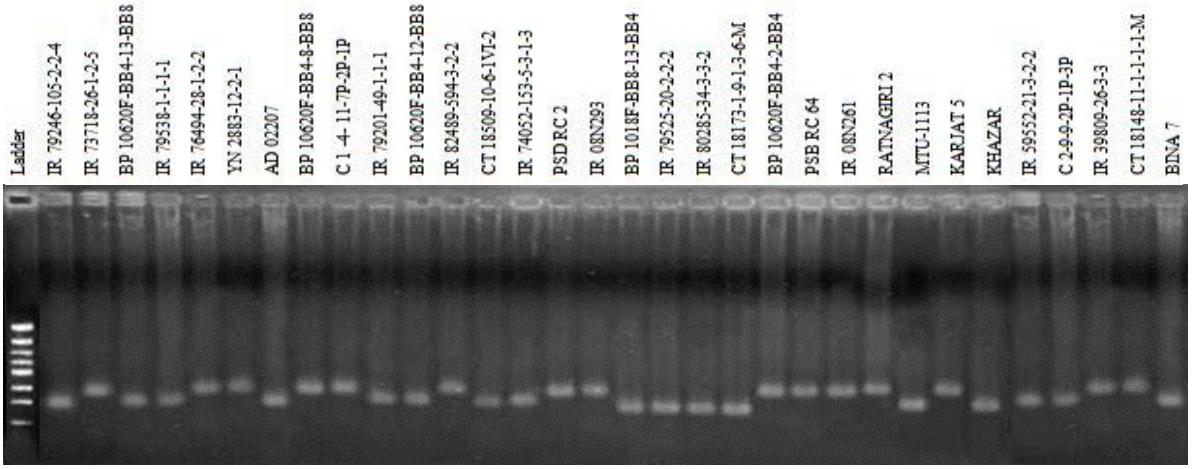

Fig. 2. Banding profiles of 32 rice genotype using primer RM147

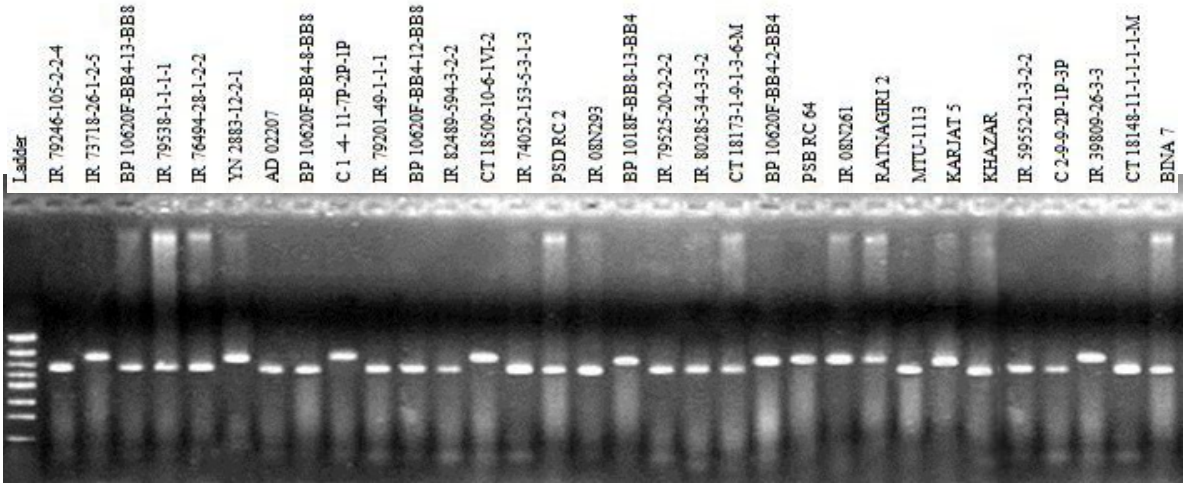

Fig. 3. Banding profiles of 32 rice genotype using primer RM202

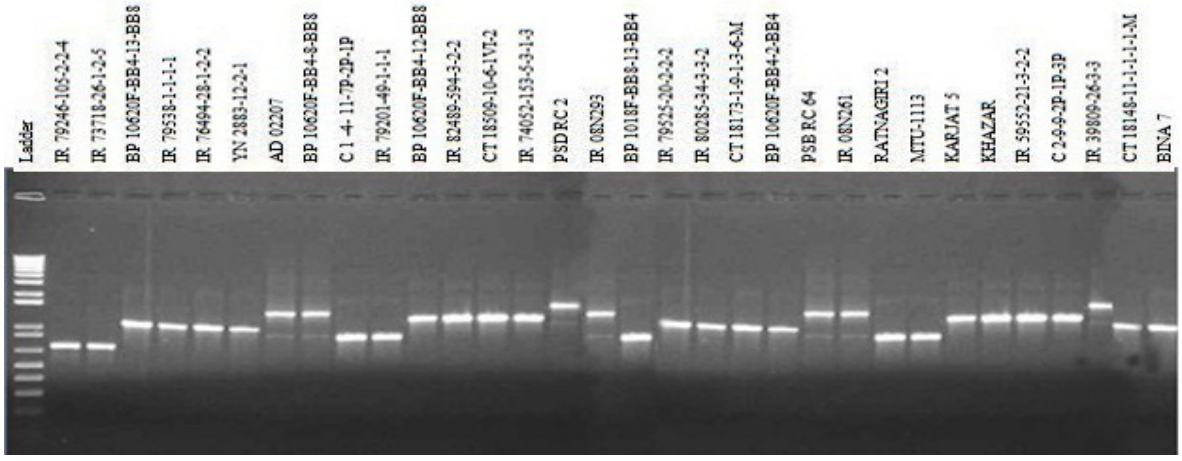

Fig. 4. Banding profiles of 32 rice genotype using primer RM215

Genetic distance

The values of pair-wise comparisons of Nei's (1972) genetic distance (D) between lines were computed from combined data for the 3 primers, ranged from 0.00 to 1.00 (Table 5). Higher genetic distance was observed between C1-4-11-7P-2P-1P vs IR 79201-49-1-1-1; IR 79246-105-2-2-4 vs IR 80285-34-3-3-2; AD 02207 vs BP 10620F-BB4-8-BB8; BP 10620F-BB4-2-BB4 vs Binadhan 7; KHAZAR vs IR 79525-20-2-2-2 lines pairs than the other lines combinations. The means of genetic distances between lines were used to evaluate the genetic diversity of different lines. Highly diversified lines could be useful in breeding programme to have potential genetic gains.

Table 5. Summary of Nei's (1972) genetic distance values among 32 rice lines

Sl.	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	26	27	28	29	30	31	32
1	0.0	0.7	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0
2	0.7	0.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0
3	1.0	1.0	0.0	0.7	1.0	1.0	0.7	1.0	1.0	0.7	0.7	1.0	0.7	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7	0.7	1.0	1.0	1.0
4	1.0	1.0	0.7	0.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7
5	0.7	1.0	1.0	0.7	0.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7	0.7	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0
6	1.0	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7
7	1.0	1.0	0.7	1.0	1.0	1.0	0.0	0.7	1.0	0.3	0.3	0.7	0.7	0.7	1.0	0.7	1.0	1.0	1.0	0.7	1.0	0.7	0.7	1.0	0.7	1.0	1.0	0.3	0.3	1.0	0.7	1.0
8	1.0	1.0	1.0	1.0	1.0	1.0	0.7	0.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7	0.3	1.0	0.7	1.0	1.0	1.0	0.7	0.7	1.0	1.0	0.7	0.7	1.0	1.0	0.7	1.0	0.7
9	1.0	0.7	1.0	1.0	0.7	1.0	1.0	1.0	0.0	0.7	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0
10	1.0	1.0	0.7	1.0	1.0	1.0	0.3	1.0	0.7	0.0	0.3	0.7	0.7	0.7	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	0.7	0.7	1.0	1.0	0.3	0.3	1.0	0.7	1.0
11	1.0	1.0	0.7	1.0	1.0	1.0	0.3	1.0	1.0	0.3	0.0	0.3	0.7	0.7	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	0.7	1.0	1.0	0.0	0.3	1.0	0.7	1.0
12	1.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	0.7	0.3	0.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	0.7	1.0	1.0	0.3	0.7	1.0	0.3	1.0
13	1.0	0.7	0.7	1.0	1.0	1.0	0.7	1.0	0.7	0.7	0.7	1.0	0.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0	0.7	0.7	0.7	0.7	1.0	1.0
14	1.0	1.0	1.0	0.7	1.0	1.0	0.7	1.0	1.0	0.7	0.7	0.7	0.7	0.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	0.7	0.7	0.7	0.7	0.7	1.0	0.7	0.7
15	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	0.0	0.7	1.0	0.7	1.0	1.0	0.7	0.7	0.7	1.0	1.0	1.0	0.7	1.0	1.0	0.7	1.0	0.7
16	1.0	1.0	1.0	1.0	1.0	1.0	0.7	0.3	1.0	1.0	1.0	1.0	1.0	1.0	0.7	0.0	1.0	0.7	1.0	1.0	1.0	0.7	0.7	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	0.7
17	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0
18	1.0	1.0	0.7	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	0.7	0.7	1.0	0.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	0.7
19	0.7	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7	0.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0
20	1.0	1.0	1.0	0.7	0.7	1.0	0.7	1.0	1.0	0.7	0.7	0.7	1.0	0.7	1.0	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	0.7	0.7	1.0	0.7	1.0
21	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	0.0	0.3	0.3	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	0.7
22	1.0	1.0	1.0	1.0	1.0	1.0	0.7	0.7	1.0	1.0	1.0	1.0	1.0	1.0	0.7	0.7	1.0	1.0	1.0	1.0	0.3	0.0	0.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0
23	1.0	1.0	1.0	1.0	1.0	1.0	0.7	0.7	1.0	1.0	1.0	1.0	1.0	1.0	0.7	0.7	1.0	1.0	1.0	1.0	0.3	0.0	0.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0
24	1.0	0.7	1.0	1.0	0.7	1.0	1.0	1.0	0.0	0.7	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0
25	0.7	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	0.7	0.7	0.7	1.0	0.7	1.0	1.0	0.7	1.0	1.0	0.7	1.0	1.0	1.0	1.0	0.0	1.0	1.0	0.7	0.7	1.0	0.7	1.0
26	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	0.7	0.7	1.0	1.0	1.0	1.0	1.0	1.0	0.7	0.7	0.7	1.0	1.0	0.0	0.7	1.0	1.0	0.7	1.0	1.0
27	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	0.7	0.7	0.7	0.7	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7	0.0	1.0	1.0	1.0	1.0	0.7
28	1.0	1.0	0.7	1.0	1.0	1.0	0.3	1.0	1.0	0.3	0.0	0.3	0.7	0.7	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	0.7	1.0	1.0	0.0	0.3	1.0	0.7	1.0
29	1.0	1.0	0.7	1.0	1.0	1.0	0.3	1.0	1.0	0.3	0.3	0.7	0.7	0.7	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	0.7	1.0	1.0	0.3	0.0	1.0	0.7	1.0
30	1.0	0.7	1.0	1.0	1.0	1.0	1.0	0.7	0.7	1.0	1.0	1.0	0.7	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0	0.7	1.0	1.0	1.0	0.0	1.0	1.0
31	1.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	0.7	0.7	0.3	1.0	0.7	1.0	1.0	1.0	1.0	0.7	0.7	1.0	1.0	1.0	1.0	0.7	1.0	1.0	0.7	0.7	1.0	0.0	1.0
32	1.0	1.0	1.0	0.7	1.0	0.7	1.0	0.7	1.0	1.0	1.0	1.0	1.0	0.7	0.7	0.7	1.0	0.7	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	0.0

Here, 1=IR 79246-105-2-2-4, 2=IR 73718-26-1-2-5, 3=BP 10620F-BB4-13-BB8, 4=IR 79538-1-1-1-1, 5=IR 76494-28-1-2-2, 6=YN 2883-12-2-1, 7=AD 02207, 8=BP 10620F-BB4-8-BB8, 9=C1-4-11-7P-2P-1P, 10=IR 79201-49-1-1-1, 11=BP 10620F-BB4-12-BB8,12=IR 82489-594-3-2-2, 13=CT 18509-10-6-1VI-2, 14=IR 4052-153-5-3-1-3, 15= PSD RC 2, 16=IR 08N293, 17=BP 1018F-BB8-13-BB4, 18=IR 79525-20-2-2-2, 19=IR 0285-34-3-3-2, 20=CT 18173-1-9-1-3-6,M, 21=BP 10620F-BB4-2-BB4, 22=PSB RC 64, 23=IR 08N261, 24= ATNAGIRI 2, 25=MTU-1113, 26=KARJAT 5, 27=KHAZAR, 28=IR 59552-21-3-2-2, 29=C2-9-9-2P-1P-3P, 30=IR 39809-26-3-3, 31=CT 18148-11-1-1-1-1-M and 32=Binadhan-7

UPGMA Dendrogram

Thirty two lines of the experiment were used to make dendrogram based on Nei's (1972) genetic distance using Unweighted Pair Group Method of Arithmetic Means (UPGMA). In this study 32 rice lines have been differentiated into three main clusters. The clusters were separated into several sub-cluster.

The dendrogram showed that the lines were closely related belonging to the same cluster while the lines KHAZAR, IR 59552-21-3-2-2 and C1-4- 11-7P-2P-1P belonging to different cluster suggesting that these varieties were genetically diverse in origin. The dendrogram revealed that the lines that are derivatives of genetically similar type clustered together.

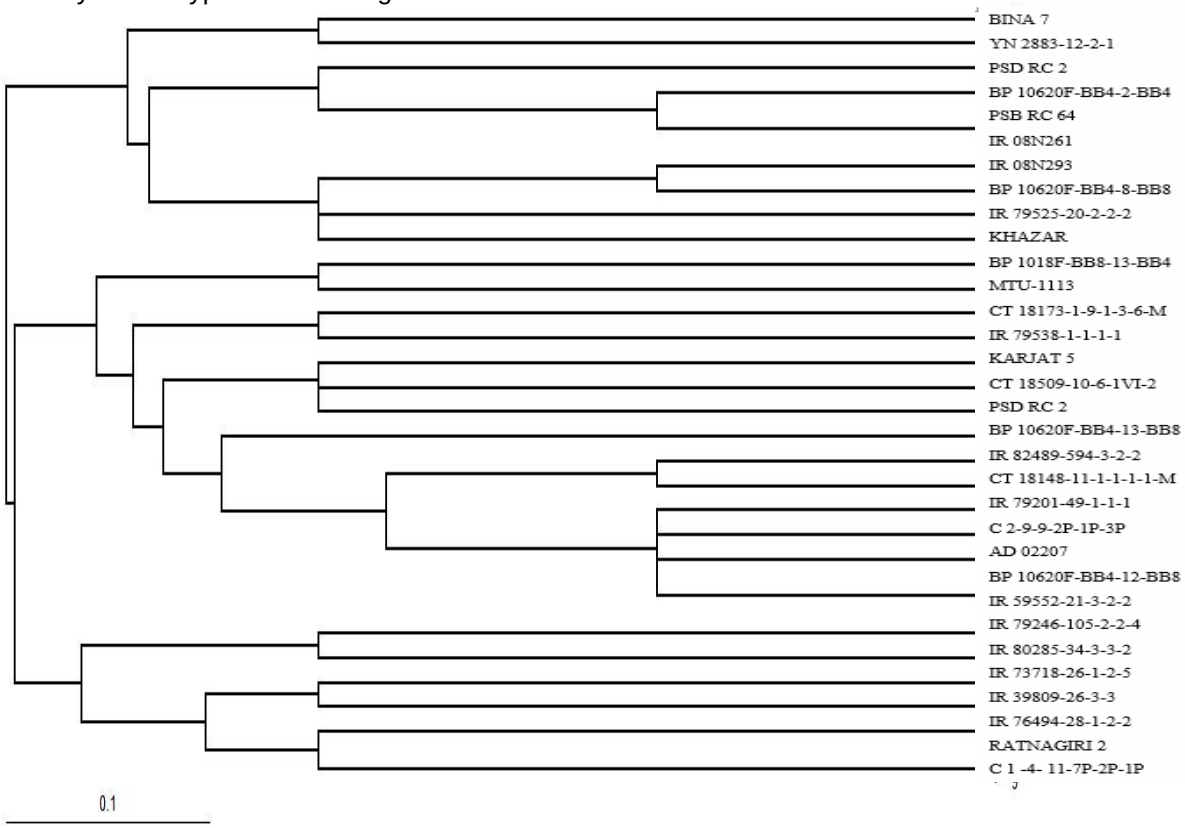

Fig. 5. UPGMA dendrogram based on Nei's (1972) genetic distance, summarizing the data on differentiation among 32 rice lines according to microsatellites marker analysis

Conclusion

The lines were grouped into three clusters *viz.*I, II and III for both morphological and molecular (UPGMA) dendrogram analysis. Hybridization among lines drawn from these widely divergent clusters with high yield potential would likely to produce heterotic combinations and wide variability in segregating generations. The results of molecular study showed that 14 alleles were identified by the each RM147, 12 and 8 alleles were identified by RM215 and RM202 respectively. As a measure of the informativeness of microsatellites, the PIC values ranged from a low of 0.78 (RM202) to a high of 0.89 (RM215) and averaged 0.85. According to Nei's (1972), the highest level of gene diversity value (0.90) was observed in loci RM215 and the lowest level of gene diversity value (0.81) was observed in loci RM202 with a mean diversity of 0.86. The identification and understanding of higher molecular diversity will be utilized in future breeding programme of developing more early maturing rice varieties. The use of more number of markers would be efficient to characterize the lines than used for the present study.

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