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Simple sequence repeat (SSR) markers for assessing genetic diversity among the parental lines of hybrid rice (*Oryza sativa* L.)

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The present scientific study was carried out at Tamil Nadu Rice Research Institute, Aduthurai with the objective of studying the genetic divergence among 51 restorer and five maintainer lines in terms of grain quality and fertility restorer genes (*Rf*$_3$, *Rf*$_4$ and *Rf*$_7$) using 55 primers of simple sequence repeat (SSR) markers. Among them, 37 SSR markers were found to be polymorphic and the number of amplified fragments ranged from one to five. The highest polymorphic information content (PIC) value (more than 0.60) was observed for eight primers *viz.,* AB 443, RM 3, RM 29, RM 226, RM 228, RM 304, RM 1812 and RM 3873 and average PIC value was 0.444. Cluster analysis using NTSYS generated dendrogram divided all the 56 parental lines into two distinct groups *viz.,* maintainer line (Group I) and restorer line (Group II) at 76% coefficient of similarity. Maintainer line group consisted of five genotypes (COMS 23B, COMS 24B, COMS 25B, CRMS 31B and CRMS 32B) and restorer line group had 51 genotypes. Further, at 80% similarity, all fifty one restorer lines were again grouped into nine clusters. With higher polymorphism revealed by SSR markers, parental lines having the similar genetic background from pedigree information were grouped into different clusters. The combination of pedigree analysis and SSR markers could be a more reliable method to study the diversity and grouping of parental lines of hybrid rice. Hybridization between diverse restorer and CMS groups identified from this study would be expected to yield hybrid combinations with premium grain quality and good fertility restoration.

Key words: Simple sequence repeat (SSR), maintainer and restorer genetic diversity, grain quality, fertility restoration.

INTRODUCTION

Rice (*Oryza sativa* L.) is the major staple cereal food crop fulfilling about 60% dietary requirement, 20% calorie and 14% protein requirement of the world's population. In the present decade, the rate of increase in rice production is lower (1.5% per year) than the increase in population (1.8% per year). The present world population of 6.3 billion is likely to reach 8.5 billion by 2030. Out of this, 5 billion people will be rice consumers and there is a need of 38% more rice by 2030. To meet this challenge there is a need to develop rice varieties with higher yield potential and greater stability (Khush, 2006). Hybrid rice technology is one of the strategy to meet this immense challenge imposed by ever growing populations. Hybrid rice varieties have clearly shown a yield advantage of 1.0

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to 1.5 tonnes per hectare (20 to 30%) over conventionally bred modern varieties (Virmani et al., 2003). India is the second country after China to develop and release the first rice hybrid during 1994 (Janaiah and Hussain, 2003). Breeding hybrid rice requires the development of elite parental lines in either a three-line or two-line system.

The heterotic potential of hybrids is associated with genetic divergence between their parental lines. Empirical evidence has indicated that establishment of divergent maintainer and restorer groups are the key for success of hybrid breeding programs using the CMS system. In order to increase hybrid rice breeding efficiency, the relationship between genetic diversity and heterosis can be investigated by using phenotypic traits and molecular markers (Julfiquar, 1985). Selection of diverse parents with desirable traits can be done based on the analysis using morphological, biochemical and or DNA markers. Though morphological characterization is an important methodology for studying diversity, it is cumbersome, whereas there are limitations in using biochemical markers as they are limited in number and are predisposed by environmental factors or the developmental stage of the plant (Winter and Kahl, 1995). DNA markers are the most widely used and are predominant due to their abundance and repeatability and they remain unaffected across different stages, seasons, locations and agronomic practices (McCouch et al., 1997). Of the several classes of DNA markers available, microsatellite or simple sequence repeat (SSR) markers are considered as most amenable for several applications including genetic diversity studies due to their multiallelic nature, high reproducibility, co-dominant inheritance, abundance and extensive genome coverage (McCouch et al., 2002). A carefully chosen set of SSR markers providing genome-wide coverage will facilitate an unbiased assay of genetic diversity, thus giving a robust, unambiguous molecular description of rice cultivars. SSR markers have been effectively used to study genetic diversity among closely related rice cultivars (Spada et al., 2004) and distantly related genotypes (Giarrocco et al., 2007). Hence the present investigation was taken up with the objective of studying the genetic divergence among the parental lines (Maintainer or 'B'lines and Restorer or `R` lines) of hybrid rice using Simple Sequence Repeat (SSR) markers which are distributed throughout the rice genome.

MATERIALS AND METHODS

Five maintainer or B lines (COMS 23A, COMS 24A, COMS 25A, CRMS 31A and CRMS 32A) and fifty one tester or Restorer lines (18 AICRIP parental lines, 13 recently stabilized breeding lines and 20 Advanced Cultures / lines under evaluation) formed the basic genetic materials for this study. The pure seeds of male sterile and maintainer lines were obtained from Paddy Breeding Station, Centre for Plant Breeding and Genetics, Coimbatore and the restorer lines were obtained from Tamil Nadu Rice Research Institute, Aduthurai. The parents were raised in a Randomized block design with three replications during September 2009 at Tamil Nadu Rice Research Institute, Aduthurai. The details of the chosen parental genotypes are given in Table 1.

Isolation of genomic DNA

Total genomic DNA was isolated from 56 parental lines at tillering stage (45 DAS) from fresh leaves of each genotype following the CTAB (cetyltrimethylammonium bromide) procedure as described by Dellapota et al. (1983). Fifty five SSR markers (Table 2) representing the entire rice genome associated with grain quality parameters (grain size, amylose content, alkali spreading value, cooking traits, protein content, plant height, panicle length, grains per panicle) and fertility restorer genes (*Rf*$_3$, *Rf*$_4$ and *Rf*$_7$) were selected from the published framework map (Akagi et al., 1996; Temnykh et al., 2000). Information regarding chromosomal localizations and repeat types of the SSRs can be found in the Web database (www.gramene.org). The isolated genomic DNA was quantified by NanoDrop^{TM} 1000 Spectrophotometer. Further, the isolated DNA was checked for intactness, homogeneity and purity by electrophoresis in 0.8% agarose gel.

PCR amplification

PCR reactions were carried out in Programmable Thermal Cycler (PTC) MJ research Inc. USA. The reaction volume was 15 µl containing 2 µl of genomic DNA, 1X assay buffer, 200 µM of dNTPs, 2 µM $MgCl_2$, 0.2 µM each primer (Forward and Reverse) and 1 unit of *Taq* polymerase (Banglore Genei). The temperature cycles were programmed as 95°C for 2 min, 94°C for 45 s, 55°C for 1 min, 72°C for 1.30 min for 35 cycles and additional temperature of 72°C for 10 min for extension and 4°C for cooling.

The amplified PCR products were separated in 3% agarose gel prepared in 1X TBE buffer stained with Ethidium bromide. The gel was run in 1X TBE buffer at a voltage of 90 V for a period of 45 min to 1 h. The gel was visualized in UV transilluminator and photographs taken using Alpha Digidoc gel documentation instrument. Clearly resolved, unambiguous bands were scored visually for their presence or absence with each primer. The scores were obtained in the form of matrix with '1' and '0', which indicate the presence and absence of bands in each variety respectively.

Data analysis

SSR markers scoring data were used to estimate the similarity on the basis of number of shared bands. Similarity was calculated with SIMQUAL function of NTSYSpc2.0 version that computes a variety of similarity and dissimilarity coefficients for qualitative data. The similarity matrix was used to generate dendrogram based on UPGMA. In order to estimate the congruence among dendrograms, cophenetic matrices for which marker and index type were computed and compared using the Mantel test. The PIC value of each locus was calculated as

$$PIC_j = 1 - \sum_{i-1 \text{ to } L} P_{ij}^2$$

Where, P_{ij} is the relative frequency of the ith allele for the locus j and is summed across all the alleles (L) over all lines. PIC provides an estimate of the discriminatory power of a locus by taking into account, not only the number of alleles that are expressed, but also the relative frequencies of those alleles. PIC values may range from 0 (monomorphic) to 1 (very highly discriminative), with many alleles in equal frequencies.

Table 1. Details of the parents used in the study.

S/N	Tester lines		Source
	Name of the entry	Parentage	
	AICRIP parental lines		
1	IET 19863	GR 11 / Pusa Basmati 1 / 6-12-1-4-1-3	AICRIP
2	IET 19922	IET 12356 / IR 64	
3	IET 20601	OR 1530 – 8 / IR 68181 – B- 49	
4	IET 20605	SYE – 1 / Tadukan	
5	IET 20881	Abhaya / SKL – 8	
6	IET 20885	Improved White Ponni / Tetep	
7	IET 20888	ADT 38 / CR 1014	
8	IET 20890	GR – 11 / Pusa Basmati 1 / 6- 12- 1- 4 – 1 – 4 -3 -2	
9	IET 20895	Erramallelu / RNR – M7	
10	IET 20896	Erramallelu / Kavya	
11	IET 20897	NLR – 145 / Kavya	
12	IET 20898	NLR – 145 / Kavya	
13	IET 20899	Tella Hamsa / Erramallelu	
14	IET 20904	Kavya / RNR – M7	
15	IET 20932	Keshava / NLR – 33655	
16	IET 20937	OR – 1530 – 8 / IR 68181 – B – 49	
17	IET 20944	Jaya / Mahsuri	
18	IET 20945	Kavya / BPT 5204 // Vijetha	
	Recently stabilized breeding lines		
19	AD 09522	Imp Rascadam / ASD 19 – 2-1-7-4-1	TRRI, Aduthurai
20	AD 09523	I.W.Ponni / Ratna – 1-1-2-3-1	
21	AD 09524	ASD 19 / WGL 32100 – 1-3-3-11-5	
22	AD 09525	ASD 19 / WGL 32100 – 13-2-2-1-1	
23	AD 09526	WGL 32100 / Swarna – 7-2-3-3-1	
24	AD 09527	BPT 5204 / Imp Rascadam – 4-7-1-2-1	
25	AD 09528	BPT 5204 / Imp Rascadam – 2-4-5-6-6	
26	AD 09529	I.W.Ponni / Kalajoha – 5 -1 – 5 - 4	
27	AD 09530	I.W.Ponni / Kalajoha – 21-3-3-3-1	
28	AD 09531	BPT 5204 / Azucena – 6-2-2-3-1	
29	AD 09532	ADT 43 / WGL 32100 – 7-3-3-2-2	
30	AD 09533	ADT 43 / WGL 32100 – 12-3-4-3-1	
31	AD 09534	ADT 43 / WGL 32100 – 3-3-7-10-1	
	Advanced Cultures / lines under evaluation		
32	AD 04072	Improved White Ponni / P 1110 – 5 – 1 - 2	TRRI, Aduthurai
33	AD 06084	ADT 42 / JGL 384	
34	AD 07073	ADT 43 / JGL 384	
35	AD 07076	ADT 43 / JGL 384	
36	AD 07083	ADT 43 / I. W. Ponni	
37	AD 07158	BPT 5204 / AD 02235	
38	AD 07309	Ajaya / IET 11613 – 22 – 5 – 2	
39	AD 08005	ADT 43 / ACK 03002	
40	AD 08009	ADT (R) 45 / AD 01236	
41	AD 08010	ADT (R) 45 / AD 01236	
42	AD 08013	ADT (R) 45 / ADT (R) 47	
43	AD 09193	ADT 43 / JGL 384	
44	AD 09194	ADT (R) 47 / Karnataka Ponni	

Table 1. Contd.

45	AD 09203	ADT 43 / ADT 37	
46	AD 09206	ADT 43 / ADT 37	
47	AD 09216	ADT (R)45 / AD 01236	
48	AD 09222	ADT (R)45 / IET 18208	
49	AD 09223	ADT (R)45 / IET 18208	
50	AD 09231	PY 3 / ADT (R) 47	
51	AD 09241	AD 01236 / AD 01205	
Male sterile and Maintainer Lines			
52	COMS 23B		Coimbatore
53	COMS 24B		Coimbatore
54	COMS 25B		Coimbatore
55	CRMS 31B		Cuttack
56	CRMS 32B		Cuttack

RESULTS AND DISCUSSION

In the amplification of genomic DNA of 56 hybrid rice parental genotypes using 54 primers of SSR markers, 37 were found to be polymorphic. The number of amplified fragments ranged from one to five. Of the total amplified bands, the average polymorphic fragment per primer was 2.46. Among the 54 primers, RM 228, RM 304, RM 336 and RM 1812 produced the maximum number of alleles. Polymorphism revealed by RM 29 co segregating with width expansion ratio of cooked rice and RM3873 which is co segregating with fertility restoration gene (Rf_3) are presented in Figures 1 and 2. The PIC values representing allelic diversity and frequency among genotypes varied from one locus to another. The highest PIC value (more than 0.60) was observed for eight primers *viz.*, AB 443, RM 3, RM 29, RM 226, RM 228, RM 304, RM 1812 and RM 3873 while it was the lowest for RM 339 (0.035). The average PIC value was 0.444 (Table 3). The results of this study, that is, the number of alleles and PIC value are comparable to those reported by Cho et al. (2000), Yu et al. (2003), Thomson et al. (2007), Lapitan et al. (2007) and Seetharam et al. (2009). The above results provided an overview of the genetic diversity in all the genotypes.

The cluster analysis using NTSYS generated dendrogram (Figure 3) throws light on genetic similarity among the genotypes. It divided all the 56 parental lines into two distinct groups viz., maintainer group (Group I) and restorer group (Group II) at 76% coefficient of similarity (Table 4). Maintainer group consisted of five genotypes (COMS 23B, COMS 24B, COMS 25B, CRMS 31B and CRMS 32B) and restorer group had 51 genotypes. Further, at 80% similarity all fifty one restorer lines were again grouped into nine clusters. Fifteen (15) genotypes viz., AD 07073, AD 07076, AD 07083, AD 07309, AD 07158, AD 08005, AD 08009, AD 08010, AD 08013, AD 09193, AD 09194, AD 09203, AD 09206, AD 09216, and AD 09231 were grouped in cluster VI and all belonged to advanced cultures originated from TRRI, Aduthurai. Out of the 15 genotypes, seven and four had the common origin from ADT 43 and ADT (R) 45 as the female parents respectively. Out of the 13 recently stabilized breeding lines, most of them were grouped in cluster IV (AD 09526, AD 09531, AD 09532, AD 09533, AD 09534, AD 09528, AD 09530), followed by cluster III (AD 09522, AD 09524, AD 09525). The clusters II, V and VIII had one genotype each. The genotype AD 09529 (I.W.Ponni × Kalajoha) alone fell in a single cluster V. The genotypes from AICRIP were grouped exclusively in cluster I (6 genotypes) and VII (3 genotypes). Another set of seven genotypes viz., IET 20897, IET 20898, IET 20899, IET 20932, IET 20937, IET 20944 and IET 20945 were grouped in cluster VIII. One genotype each was grouped in cluster II (IET 20885) and III (IET 20904). In cluster III, the genotypes viz., AD 09522, AD 09524 and AD 09525 had ASD 19 as one of the parents. In cluster II, the entries IET 20885 and AD 09523 had I.W.Ponni as one of the parent. But another genotype AD 09523 also with I.W.Ponni as one of the parents fell into cluster VIII.

In contrast to the morphological or biometrical traits, the molecular markers reveal polymorphism at the DNA level, suggesting a very powerful tool for characterization of genotype and estimation of genetic diversity. Among them, the microsatellite or SSR (simple sequence repeats) markers show a high potential for identification and estimation of genetic diversity (Hashimoto et al., 2004). In this study, newly stabilized breeding lines viz., AD 09527 and AD 09528 with the same genetic background (AD 09527: BPT 5204 × Imp Rascadam – 4-7-1-2-1, AD 09528: BPT 5204 × Imp Rascadam – 2-4-5-6-6) were grouped into separate clusters *viz.,* II and IV. Likewise, AD 09529 and AD 09530 had the same genetic background (AD 09529: I.W.Ponni × Kalajoha – 5 -1 – 5 – 4, AD 09530: I.W.Ponni × Kalajoha – 21-3-3-3-1) but were also grouped into separate clusters viz., IV and V.

Table 2. List of SSR primers used for diversity study.

Primer	Sequence	Annealing Temperature (°C)	Chromosomal location
RM431	F - TCCTGCGAACTGAAGAGTTG R - AGAGCAAAACCCTGGTTCAC	55.5	1
RM11	F - TCTCCTCTTCCCCCGATC R - ATAGCGGGCGAGGCTTAG	56.5	7
RM336	F - CTTACAGAGAAACGGCATCG R - GCTGGTTTGTTTCAGGTTCG	54.5	7
RM505	F - AGAGTTATGAGCCGGGTGTG R - GATTTGGCGATCTTAGCAGC	56.0	7
RM1812	F - CAGCTAGTGAGCTCCTAGTG R - GCTAACCCACCAACTTATTC	53	11
RM209	F - ATATGAGTTGCTGTCGTGCG R - CAACTTGCATCCTCCCCTCC	57	11
RM3	F - ACACTGTAGCGGCCACTG R - CCTCCACTGCTCCACATCTT	57.5	6
RM217	F -ATCGCAGCAATGCCTCGT R - GGGTGTGAACAAAGACAC	55	6
RM225	F - TGCCCATATGGTCTGGATG R - GAAAGTGGATCAGGAAGGC	54	6
RM247	F - TAGTGCCGATCGATGTAACG R - CATATGGTTTTGACAAAGCG	53	12
RM81	F - GAGTGCTTGTGCAAGATCCA R - CTTCTTCACTCATGCAGTTC	53.5	3
Wx (GBSS)	F - CTTTGTCTATCTCAAGACAC R - TTGCAGATGTTCTTCCTGAT	50.0	6
RM10	F - TTGTCAAGAGGAGGCATCG R - CAGAATGGGAAATGGGTCC	54	7
RM6	F - GTCCCCTCCACCCAATTC R - TCGTCTACTGTTGGCTGCAC	56.5	2
RM238	F - GATGGAAAGCACGTGCACTA R - ACAGGCAATCCGTAGACTCG	56.5	6
RM44	F - ACGGGCAATCCGAACAACC R - TCGGGAAAACCTACCCTACC	57.5	8
RM137	F - GACATCGCCACCAGCCCACCAC R - CGGGTGGTCCCCGAGGATCTTG	65.0	8
RM325A	F - GACGATGAATCAGGAGAACG R - GGCATGCATCTGAGTAATGG	53.5	8
RM331	F - GAACCAGAGGACAAAAATGC R - CATCATACATTTGCAGCCAG	52.0	8
RM339	F - GTAATCGATGCTGTGGGAAG R - GAGTCATGTGATAGCCGATATG	53.0	8
RM342	F - CCATCCTCCTACTTCAATGAAG R - ACTATGCAGTGGTGTCACCC	55.0	8
RM224	F - ATCGATCGATCTTCACGAGG R - TGCTATAAAAGGCATTCGGG	53.5	11
RM211	F - CCGATCTCATCAACCAACTG R - CTTCACGAGGATCTCAAAGG	53.5	2

Table 2. Contd.

Primer	Sequence	Annealing Temperature (°C)	Chromosomal location
RM29	F - CAGGGACCCACCTGTCATAC R - AACGTTGGTCATATCGGTGG	56.5	2
RM232	F - CCGGTATCCTTCGATATTGC R - CCGACTTTTCCTCCTGACG	54.5	3
RM215	F - CAAAATGGAGCAGCAAGAGC R - TGAGCACCTCCTTCTCTGTAG	55.5	9
RM190	F- CTTTGTCTATCTCAAGACAC R - TTGCAGATGTTCTTCCTGATG	50.5	6
RM204	F - GTGACTGACTTGGTCATAGGG R - GCTAGCCATGCTCTCGTACC	56.5	6
RM226	F- AGCTAAGGTCTGGGAGAAACC R - AAGTAGGATGGGGCACAAGCTC	58.0	1
RM140	F - TGCCTCTTCCCTGGCTCCCCTG R - GGCATGCCGAATGAAATGCATG	62.0	1
RM411	F - ACACCAACTCTTGCCTGCAT R- TGAAGCAAAAACATGGCTAGG	56.0	3
RM507	F - CTTAAGCTCCAGCCGAAATG R - CTCACCCTCATCATCGCC	55.0	5
RM1	F - GCGAAAACACAATGCAAAAA R - GCGTTGGTTGGACCTGAC	54.0	1
RM171	F - AACGCGAGGACACGTACTTAC R - ACGAGATACGTACGCCTTTG	56.0	10
RM228	F - CTGGCCATTAGTCCTTGG R - GCTTGCGGCTCTGCTTAC	55.0	10
RM6737	F - CATTGGGGGTGGATAAAGAG R - TATCCTCTACTCCCTCGGCC	55.5	10
AB443	F - CACTCGTATAGTAACTTGAGACGAT R - CCAAACAAGTTCCCACAATAGA	53.4	10
RM304	F - TCAAACCGGCACATATAAGAC R - GATAGGGAGCTGAAGGAGATG	53.5	10
RM3873	F - GCTAGCTAGGACCGACATGC R - CCTCCTCCTTATCCTCCCTG	56.0	1
RM3233	F - GTGGTGAGTAAACAGTGGTGG R - GAGAGCAGAGCAGAGGCAAC	57.0	1
RM6100	F - TCCTCTACCAGTACCGCACC R- GCTGGATCACAGATCATTGC	56.5	10
RM210	F - TCACATTCGGTGGCATTG R - CGAGGATGGTTGTTCACTTG	54.0	8
RM315	F - GAGGTACTTCCTCCGTTTCAC R - AGTCAGCTCACTGTGCAGTG	56.5	1
RM282	F - CTGTGTCGAAAGGCTGCAC R - CAGTCCTGTGTTGCAGCAAG	56.8	3
RM333	F - GTACGACTACGAGTGTCACCAA R- GTCTTCGCGATCACTCGC	56.5	6
RM234	F - ACAGTATCCAAGGCCCTGG R - CACGTGAGACAAAGACGGAG	56.5	7
RM250	F - GGTTCAAACCAAGCTGATCA R - GATGAAGGCCTTCCACGCAG	56.0	2

Table 2. Contd.

S/N	Primer	Sequences	Annealing temperature (°C)	Chromosomal Location
48	RM208	F - TCTGCAAGCCTTGTCTGATG R - TAAGTCGATCATTGTGTGGACC	55.0	2
49	RM5	F - TGCAACTTCTAGCTGCTCGA R - GCATCCGATCTTGATGGG	55.0	1
50	RM490	F - ATCTGCACACTGCAAACACC R - AGCAAGCAGTGCTTTCAGAG	56.0	1
51	RM1108	F - GCTCGCGAATCAATCCAC R - CTGGATCCTGGACAGACGAG	55.5	10
52	RM5841	F - CCTCTCTCTCTCTCTCCCCC R - TGTTATTGGCACGTGGTGTG	57.0	10
53	RM6344	F - ACACGCCATGGATGATGAC R - TGGCATCATCACTTCCTCAC	55.5	7
54	RM443	F - GATGGTTTTCATCGGCTACG R - AGTCCCAGAATGTCGTTTCG	54.5	1
55	RM7003	F - GGCAGACATACAGCTTATAGGC R - TGCAAATGAACCCCTCTAGC	55.5	12

These results indicated that SSR analysis could be a better method to study the diversity of parental lines of hybrid rice.

Presently, molecular marker techniques have been widely used in studying genetic variation and diversity among populations, species and varieties (Hashimoto et al., 2004; Yu et al., 2005). The use of SSR markers for assessing genetic diversity in the present study indicated that tester lines had wider genetic bases than the CMS maintainer lines. In addition, there were clear and wider genetic differences between the CMS lines and the tester lines. The original two parental groups *viz.,* CMS lines and testers have become more complex, through the emergence of new patterns of heterotic combinations. Therefore, the partitioning of parental lines to new parental groups and subgroups (AICRIP cultures, stabilized breeding lines and advanced cultures) is more important than the recognition of two parental groups (that is, maintainer and tester/restorer group). Nghia et al. (1999) reported genetic diversity existing among thermo-sen-sitive genic male sterile (TGMS) lines was much lower than that among cytoplasmic genic male sterile (CMS) lines. The cluster analysis based on RAPD markers was able to reveal close genetic relationships between different rice genotypes used in the hybrid rice breeding program.

Also SSR markers are an indispensable complementation to pedigree analysis in the identification of parental groups. In general, the pedigree analysis is considered to have no effect on selection and mutation. Therefore, pedigree analysis cannot reveal the relationship between progeny and their parents exactly. On the contrary, SSR markers can detect genetic variation at DNA level. SSR analysis showed that CRMS 31B was more similar to CRMS 32B than to CMS lines from TNAU (Figure 3). A combination of pedigree analysis and SSR markers will be helpful in more reliable grouping.

Conclusion

In the present study, the maintainer lines and tester/ restorer lines of rice were clearly discriminated on the basis of SSR analysis. Furthermore, with higher polymorphism revealed by SSR markers, some of the parental lines having similar genetic background from pedigree information were also grouped into different clusters. The identi-

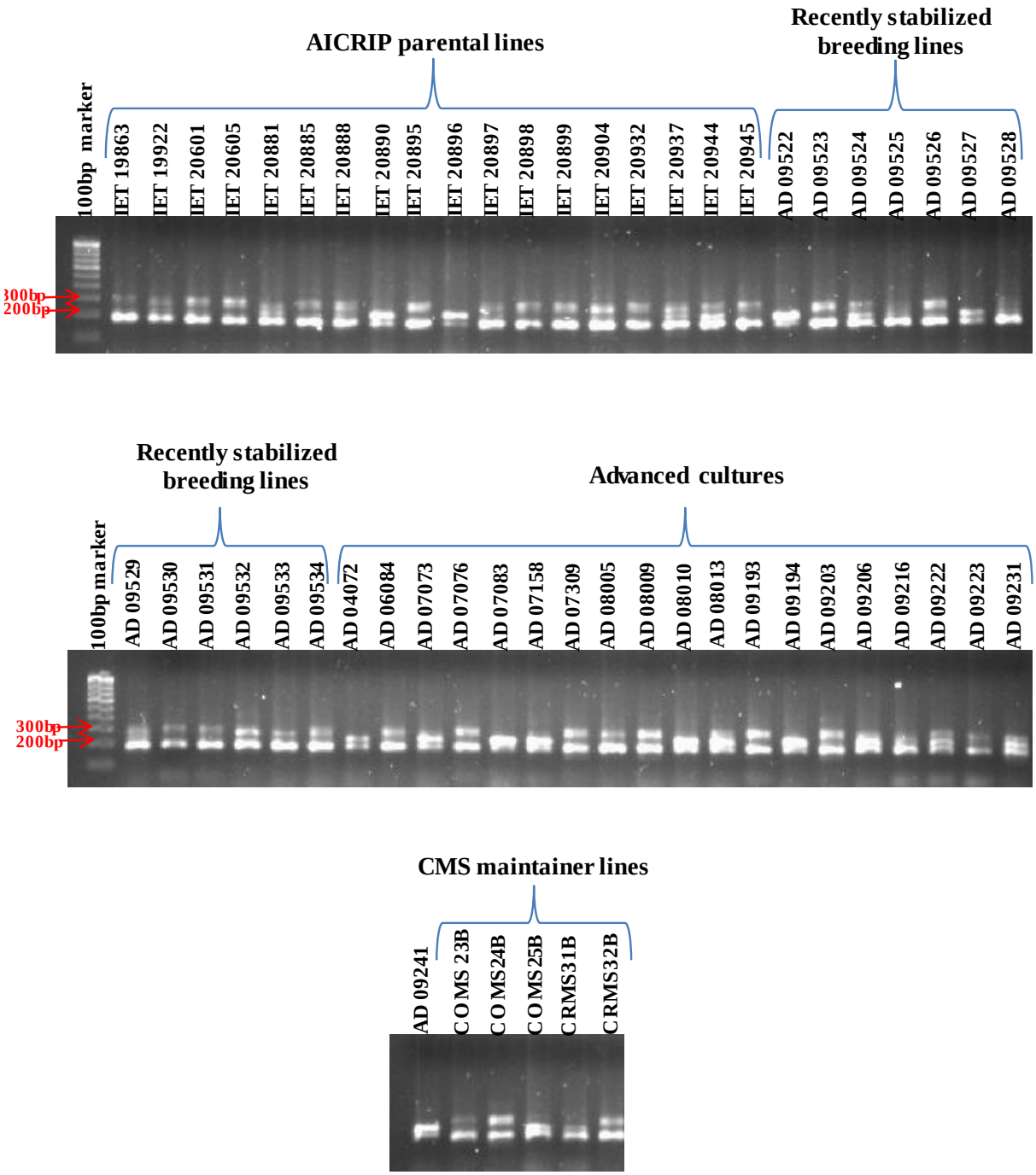

Figure 1. SSR polymorphism revealed by RM29 co segragating with width expansion of cooked rice.

fication of heterotic groups and patterns among breeding populations and lines provides fundamental information in order to help the plant breeders to gain more information on heterosis.

Systematic studies on classifying the breeding lines into heterotic groups are limited. Therefore, the grouping result based on SSR analysis might be helpful to identify heterotic groups for hybrid rice breeding.

Rice grain quality is mainly determined by the combination of many physical as well as chemical characters. Physical quality characters include kernel size, shape, hulling, milling percentage and head rice recovery. Chemical quality is mainly determined by amylose content, gelatinization temperature, gel consistency. High volume expansion and greater length wise expansion of kernel during cooking decides the consumer preference. Rice

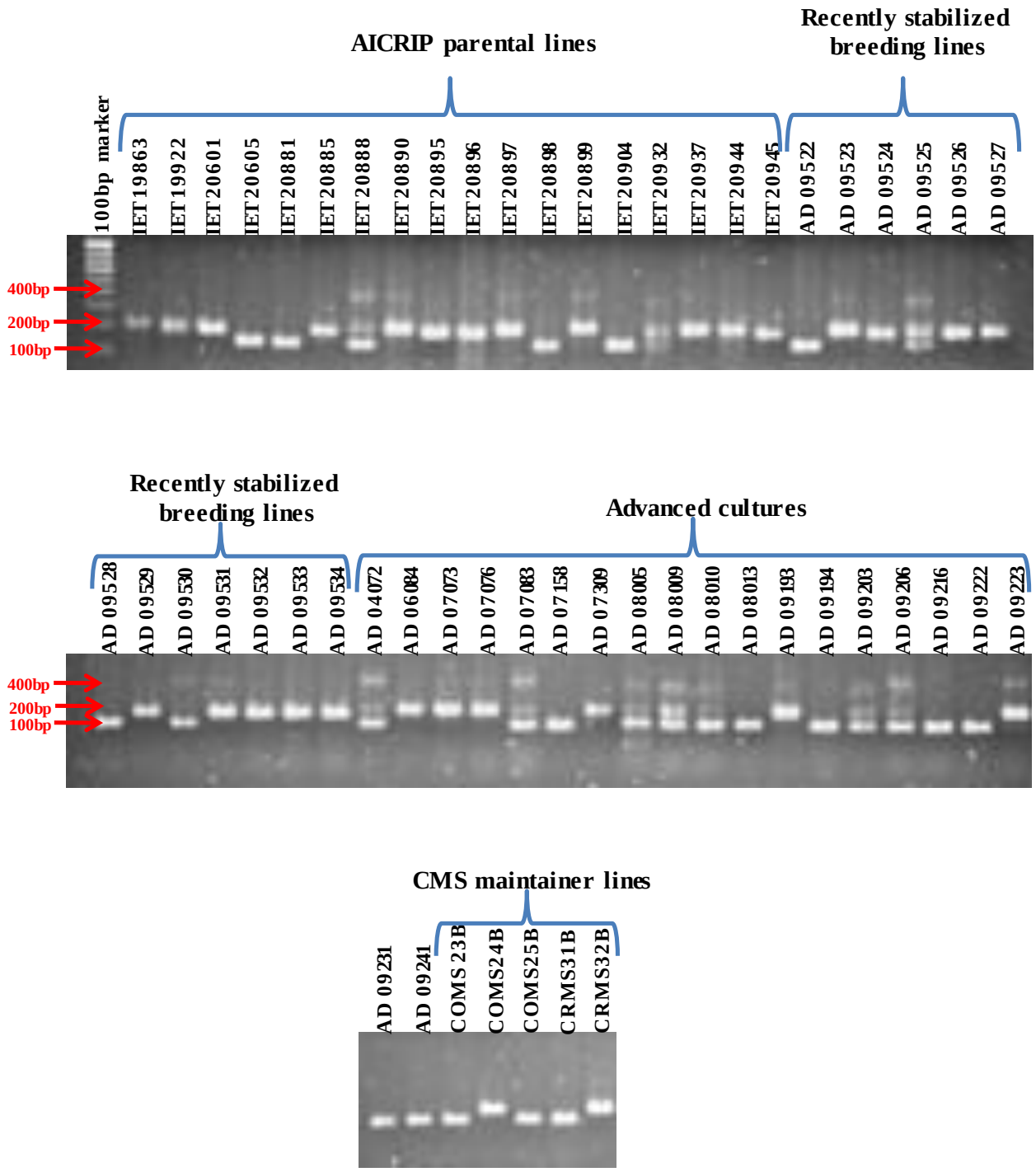

Figure 2. SSR polymorphism revealed by RM3873 co segragating with *Rf3* gene.

with soft to medium gel consistency, intermediate amylose content and gelatinization temperature is a preferred level for the consumers which determines the eating and cooking quality of rice grains (Bao et al., 2002). Amylose content is governed by the *waxy* (*Wx*) locus which is located on chromosome 6 (Septiningsih et al., 2003) and it is linked to the gene for alkali spreading score, which is also an indicator of temperature at which rice grain becomes gelatinous during cooking (Sano, 1984). SSRs used in this study were co-segregating with grain quality

Table 3. Number of alleles, percentage of polymorphism and PIC value of 37 polymorphic SSR markers.

S/N	Marker	Number of alleles	Number of polymorphic bands	Percentage of polymorphism	Polymorphic information content (PIC)
1	AB 443	2	2	100.00	0.716
2	RM 1	2	2	100.00	0.311
3	RM 3	3	2	66.67	0.602
4	RM 6	2	1	50.00	0.500
5	RM 29	3	3	100.00	0.641
6	RM 44	2	2	100.00	0.222
7	RM 140	2	1	50.00	0.337
8	RM 171	3	1	33.33	0.462
9	RM 204	3	2	66.67	0.528
10	RM 208	2	2	100.00	0.222
11	RM 209	2	1	50.00	0.494
12	RM 211	2	1	50.00	0.477
13	RM 224	2	1	50.00	0.469
14	RM 225	2	2	100.00	0.487
15	RM 226	3	3	100.00	0.603
16	RM 228	4	4	100.00	0.664
17	RM 232	2	1	50.00	0.469
18	RM 247	2	1	50.00	0.036
19	RM 304	**5**	4	80.00	0.708
20	RM 336	4	4	100.00	0.579
21	RM 339	2	1	50.00	0.035
22	RM 490	2	1	50.00	0.316
23	RM 505	3	2	66.67	0.525
24	RM 1108	2	1	50.00	0.500
25	RM1812	4	3	75.00	0.726
26	RM 3873	3	3	100.00	0.642
27	RM 6100	2	1	50.00	0.103
28	RM 137	2	1	50.00	0.494
29	RM 331	2	2	100.00	0.495
30	RM 190	2	1	50.00	0.320
31	RM 210	2	1	50.00	0.433
32	RM 250	2	1	50.00	0.397
33	RM 333	3	2	66.67	0.283
34	RM 411	2	1	50.00	0.274
35	Wx (GBSS)	2	2	100.00	0.380
36	RM 217	2	1	50.00	0.494
37	RM 234	2	2	100.00	0.497
	Mean	2.46	1.78	70.41	0.444

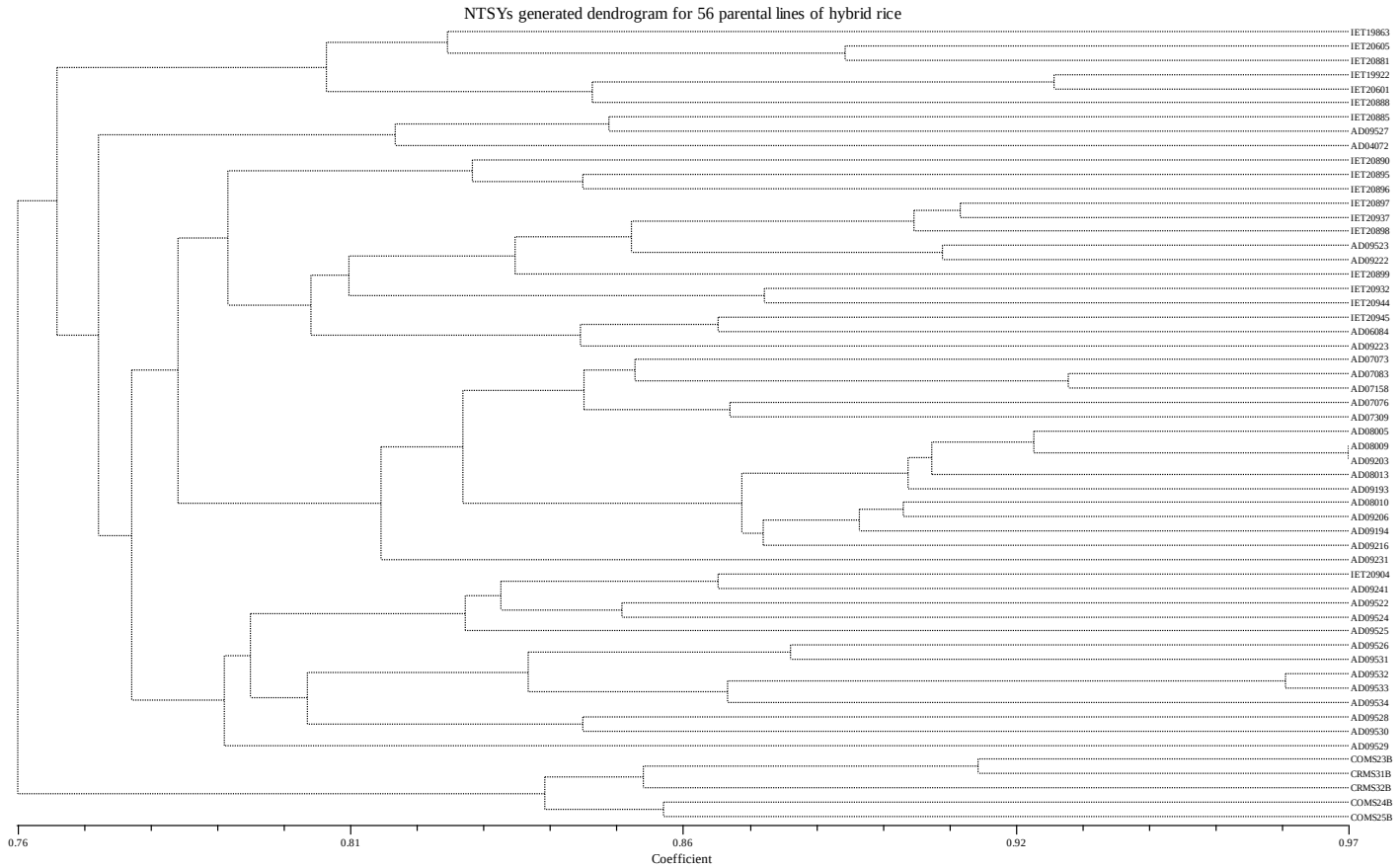

Figure 3. NTSYs generated dendrogram for hybrid rice parental lines from TNRRI.

Table 4. Clustering pattern of 56 rice genotypes based on SSR markers information.

Cluster	Genotype	Number of genotype
I	IET 19922, IET 19863, IET 20601, IET 20605, IET 20881, IET 20888	6
II	IET 20885, AD 09527, AD 04072	3
III	IET 20904, AD 09522, AD 09524, AD 09525, AD 09241	5
IV	AD 09526, AD 09531, AD 09532, AD 09533, AD 09534, AD 09528, AD 09530	7
V	AD 09529	1
VI	AD 07073, AD 07076, AD 07083, AD 07309, AD 07158, AD 08005, AD 08009, AD 08010, AD 08013, AD 09193, AD 09194, AD 09203, AD 09206, AD 09216, AD 09231	15
VII	IET 20890, IET 20895, IET 20896	3
VIII	IET 20897, IET 20898, IET 20899, IET 20932, IET 20937, IET 20944, IET 20945, AD 09523, AD 06084, AD 09222, AD 09223	11
IX	COMS 23B, COMS 24B, COMS 25B, CRMS 31B, CRMS 32B	5
	Total	56

and fertility restorer genes (*Rf*$_3$, *Rf*$_4$ and *Rf*$_7$). The diverse restorer lines identified from this SSR based diversity study will be hybridized with diverse group of CMS lines (COMS23A, COMS24A, COMS25A, COMS31A, and COMS32A) to develop hybrid combinations with superior grain quality and greater restoration ability. It could maximize opportunities to obtain superior hybrid combinations because unrelated parents would be expected to contribute unique desirable alleles at different loci. It is generally accepted that molecular markers represent genetic variation at DNA level, providing more accurate measures of relationships between individuals without the influence

of environmental variation.

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