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Variety identification and genetic diversity analysis in rice (*Oryza sativa* L.) using STMS markers

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Abstract

In the present study, 14 rice specific sequence tagged microsatellite site markers (STMS) were used to develop fingerprints and to determine the genetic relatedness among 48 rice genotypes/cultivars. Ten of the 14 SSR primer pairs were found to be polymorphic, generating a total of 39 distinct reproducible alleles with an average of 2.79 alleles per primer pair and 22 (56.41%) of the 39 bands amplified emerged as polymorphic. The number of alleles per primer ranged from one to six. The polymorphic information content (PIC) value ranged from 0.03 to 0.7 with an average of 0.35. Cluster analysis based on Jaccard's similarity coefficient using unweighted pair group methods for arithmetic mean (UPGMA) grouped the 48 rice cultivars into five major clusters with an average similarity index of 0.87. A diagrammatic representation of DNA fingerprints of the rice cultivars based on 10 informative SSR markers was developed for ready reference, which are expected to be key in varietal identification as well as provide IPR to halt biopiracy.

Keywords: *Oryza sativa*; rice; DNA fingerprinting; genetic diversity; STMS

1. Introduction

Rice (*Oryza sativa* L.) constitutes the staple food for more than half of the world's population[1] including India and occupies largest area (43.95 million hectares) among all the crops grown in India as well as in all other rice growing countries worldwide with an average productivity of 2.42 tonnes /ha[2]. Although world rice production has been doubled in the past 50 years owing to introduction of new high yielding varieties and adoption of improved agricultural practices, nevertheless, still it will be inadequate to meet the global demands of the burgeoning population at the interface of dwindling natural resources, agricultural inputs and bioresources.

In a self pollinated crop like rice, one of the major challenges is the production and supply of adequate quantities of pure inbred and hybrid seeds to the farmers. Maintenance of high level of genetic purity of inbred and hybrid is inevitable to harness the benefit of heterosis as expected in this important life-line food crop for food security. Maintenance of genetic purity of seeds is essentially required to earn maximum benefit from varieties which even touches upto 40-45% more production under optimum management and 15-20% under sustenance mode.

Unambiguous identification of elite crop varieties is essential for their IPR protection, prevention of unauthorized commercial use and misuse of brand name by selling spurious seeds etc. A set of qualitative and quantitative characters known as "descriptors" are currently *in vogue* for varietal identification. Some of those characters, particularly those showing quantitative inheritance, interact with the environment in which the variety is grown and thus make the process of variety identification subjective and sometimes illusive due to masking effect of GxE interaction. In general, neither morphological nor biochemical markers alone could discriminate between the closely related indigenous varieties of a particular group.

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Unlike morphological and biochemical markers, an array of molecular markers are *in vogue* now a days, being based on DNA sequence variation, provide an unbiased means of identifying crop varieties, which is environment independent too. DNA markers are practically unlimited in number and are not affected by the environmental factors and/ or by the developmental stage of the plant[3]. Simple sequence repeat (SSR) or sequence tagged microsatellite sites (STMS) markers, in particular, because of their abundance, co-dominant inheritance, high polymorphism, reproducibility and amenability for automation[4-8] are preferred for precise and rapid identification and characterization of the varieties/genotypes with high reproducibility. Molecular markers, therefore, could be used as an alternative to conventional grow-out-test (GOT) by generating molecular identity profile (Molecular ID cards) of the varieties, by identifying specific markers for individual varieties and eventually the identified primers could be used in assessment of genetic purity of samples through PCR based techniques at a faster rate with ease and confidence from different seed lots collected from diverse sources.

The principal objective of the present study was to identify useful microsatellite (STMS) markers for studying genetic diversity among rice cultivars and to develop fingerprints precisely to facilitate unambiguous and quick variety identification of the rice genotypes/ cultivars under study.

2. Materials and Methods

2.1 Plant materials

Mature, clean, dried seeds of 48 rice inbred lines were obtained from rice collections maintained at different research institutes in India like Central Agricultural Research Institute (CARI), Port Blair; Central Rice Research Institute (CRRI), Cuttack, Odisha; Narendra Dev University of Agriculture & Technology, Faizabad; Paddy Breeding Station, Tamilnadu Agricultural University, Coimbatore; Indian Agricultural Research Institute, New Delhi; Directorate of Rice Research, Hyderabad and Rice Research Station, Chinsurah, West Bengal (**Table I**). To undertake the present study, a random sample of 450 seeds of uniform shape, size and appearance, representing the pure seeds of each variety were used for conducting genetic diversity study and testing their genetic purity. Out of 450, 50 randomly drawn pure seeds of each variety were used for marker analysis and rests were used for Grow-out-test (GOT) following standard method as practiced for seed genetic purity test.

2.2 Molecular analysis

The genomic DNA was isolated from bulked leaf samples of 10 morphologically similar individual plants using Cetyl-Trimethyl Ammonium Bromide (CTAB) method[9]. Quantification of DNA was accomplished by analyzing the purified DNA on 0.8% agarose gel using diluted uncut lambda DNA (400 µg/mL, Bangalore Genei Pvt. Ltd.) as standard. DNA was diluted in $T_{10}E_1$ buffer to a concentration of about 12.5 ng/ µL for PCR analysis. The sequence information for the primer pairs was obtained from the publications[10-12] and were synthesized from GCC Biotech Pvt. Ltd., India.

2.3 PCR amplification

DNA amplification using 14 SSR primer pairs (**Table II**) selected for this study, was carried out in a 25µL reaction mixture containing 1X PCR assay buffer (50 mM KCl, 10 mM Tris-Cl, 1.5 mM $MgCl_2$), 200 µM each of dNTPs, 0.2 µM each of forward and reverse primers, 0.6 units of Taq DNA polymerase (Bangalore Genei Pvt Ltd., Bangalore, India) and 25 ng of genomic DNA template. The amplification reaction was carried out in a thermal cycler (Eppendorf AG22331, Hamburg, Germany). The first cycle consisted of denaturation of template DNA at 94°C for 5 min, primer annealing (55° C) for 1 min and primer extension (72°C) for 2 min. In the next 33 cycles, the period of denaturation was reduced to 1 min while the primer annealing and primer extension time remained as in the first cycle. The last cycle consisted only for primer extension (72°C) for 7 min.

The amplified products were separated by electrophoresis in 3 % Metaphor™ agarose (Lonzo, USA) gel containing 1mg/ml ethidium bromide The gel was run in 1X TBE buffer (pH 8.0). The size of the amplified fragments was determined by using size standards (Low range DNA ladder, MBI Fermentas, Lithuania). DNA fragments were visualized and documented under UV-light in a gel documentation system (Bio-Rad USA).

2.4 Scoring and data analysis

The amplified PCR products (amplicons) were scored only for distinct and reproducible bands as present (1) or absent (0) generating a binary data matrix for each primer genotype combination. The data entry was done into a binary data matrix as discrete variables. Jaccards's coefficient of similarity was calculated and a dendrogram (**Figure I**) based on similarity coefficient was generated using Unweighted Pair Group Method based on Arithmetic Mean (UPGMA) using the computer package NTSYS-PC[13]. The binary data matrix was subjected to bootstrapping using Win Boot programme to test the robustness of clustering pattern. Principal component analysis (PCA) was also carried out to depict the relationship among 48 rice genotypes in three dimensions. The polymorphic information content (PIC)

Table I: List of rice genotypes with their parentage deployed in the present study

Sl. No.	Variety	Parentage
1	Nanjing 57161	BG 98-2/IR 661 (382012)
2	Zengua	Selection from INGER trial IRRI, Philippines
3	Swarna	Vasista/Mahsuri
4	Saraswathi	Pankaj x Patnai 23
5	S_1P_1 681032	S_1P_1 632063/Chianung Sen Yu7/Tainung Sen 12
6	ADT 41	Selection from dwarf mutant of Basmati-370
7	IR 18350-229-3	Exotic line from IRRI trial
8	Pusa 44	IARI5901-2 x IR 8
9	Taichung Sen Yu	Selection from INGER trial IRRI, Philippines
10	FR 43-B	Pure line selection of land race 'Betanasi'
11	Annada	MTU-15 xYakakaku Nantoku
12	Vikramarya	Vikram x PTB2 (Ponnayan)/RP-1125-1548- 1-4-3
13	Triguna	Swarna dhan x RP 1579- 38
14	IR66738-118-1-2 (Super-4)*	IR 66738 (Shen Nung 89-366 xSopon Jone)
15	Jaya	T(N)1 x T 141
16	Prabhat	IR 8 x MTU 3
17	Nalini	Pure line selection from Indian cultivar Sindurmukhi
18	Sasyasree	TKM6 x IR8
19	CSR 23	IR 64//IR4630-22-2-5-1-3/IR 964-45-2-2
20	Sarjoo 52	T(N)1 x Kashi
21	Vasumati	PR 109/Pakistani Basmati
22	Pusa Sugandh 3	IET 16313xPusa 2504-1-31
23	Anjali	PR19-2 x RR149-1129
24	IR8	Dee-gee-woogen x Peta
25	Sabita (IET-4786)	CR 10-114 / CR 10-115
26	Mahisugandha	BK79 x Basmati 370
27	IR67964-46-1-3-2 (Super-6)*	New Plant Type rice
28	IR 6763-63-1-3 (Super-9)*	New Plant Type rice
29	SR 26-B	Pure line selection from a land race of Odisha Kalambanka
30	Quing Levan No.1	Selection from INGER trial IRRI, Philippines
31	IR 64	IR 5857-33- 2-1 x IR 2061-465-1-5-5
32	Pusa Sugandh 2	IET 16310 x Pusa 2504-1-26
33	Salivahana	RP532 x Pankaj
34	MTL 113	IR 9782-111-2-1-2/IR 29
35	Samba Mahsuri	GEB24 x TN1 x Mahsuri
36	Shashi	IR50 x Patnai 23
37	Dinesh	Jaladhi 2 / Pankaj
38	IR 31851-6-3-3-2	Exotic line from IRRI trial
39	Heera	CR 404-48 x CR 289-1208
40	Dhanrasi	B32- Sel. 4/ *O.rufipogon*1/B 127
41	Mansarovar	RP31-49-2 x Leb Muey Nahng
42	Rasi	T(N)1 x CO 29
43	Dular	Traditional variety from India
44	PNR 519	Tainang 3 mutant x Basmati 370 x PNR- 417-3
45	Neeraja	IR 20 x IR 5
46	Hansaswari	Selection from a composite cross
47	BG 1639	BG 797/BG 300//85-1580/ Senerang
48	Millyang 55	Selection from INGER trial IRRI, Philippines

*Super rice denotes New Plant type rice evolved by IRRI, Philippines

value of each SSR marker was calculated from the formula:

PIC=1--ΣP^2_{ij} (P*ij* is the frequency of the jth allele (marker) for the ith SSR locus[14].

Primer resolving power (Rp) was calculated according to Prevost and Wilkinson formula[15] as mentioned below.

Rp= ΣIb_i (where Ibi=1- (2x|0.5-pi|); 'pi' is the proportion of accessions containing the ith band and Ibi is the informativeness of the ith band.

Table II: List of SSR primer pairs used in the study

Primer	CL	F/R Primer	TB	PB	PIC	Rp
RM 192	7	GCGGCGGATCATGAATTGCGAG CTTGTTCCCCGGCGTCGAGTCC	1	0	0.00	0.00
RM1150	6	ACAGTGGCCACAGTGTGTTG GGATTCGGGAGGTTGACG	2	1	0.48	0.08
RM 1356	4	GTAATCGCCAACCCTAGC AGATATGGGCTTCTAAAGTTTG	3	0	0.00	0.00
RM 3330	6	ATTATTCCCCTCTTCCGCTC AAGAAACCCTCGGATTCCTG	4	3	0.70	0.4
RM 3350	5	ACCACCACCACTACAGGCTC CCAGCTGCTCACTCACTCAC	1	0	0.00	0.00
RM 3530	1	GTAGATCCGGTCAGCTCCTC CAAGGAGATTCCCTTCCATG	2	1	0.39	0.4
RM 6818	6	GTAGATCCGGTCAGCTCCTC ACCATTTCCAGATGACTCGG	1	0	0.00	0.00
RM 7425	3	AGCCAGAGAGAGAGAGACGG ACATCAACACATTCCCCTCC	2	1	0.03	0.14
RM 334	5	GTTCAGTGTTCAGTGCCACC GACTTTGATCTTTGGTGGACG	2	1	0.33	0.04
RM 212	1	CCACTTTCAGCTACTACCAG CACCCATTTGTCTCTCATTATG	3	2	0.3	1.34
RM 264	8	GTTGCGTCCTACTGCTACTTC GATCCGTGTCGATGATTAGC	4	4	0.7	1.04
RM 154	2	ACCCTCTCCGCCTCGCCTCCTC CTCCTCCTCCTGCGACCGCTCC	6	6	0.36	1.64
RM 177	4	CCCTCTTAGACAGAGGCCAGAGGG GTAGCCGAAGATGAGGCCGCCG	5	1	0.13	0.7
RM 269	10	GAAAGCGATCGAACCAGC GCAAATGCGCCTCGTGTC	3	2	0.04	0.18
Total			**39**	**22**	**3.46**	**5.96**

CL: Chromosomal location; F/R Primer: Forward/Reverse primer; TB: Total number of bands; PB: Polymorphic bands; PIC: Polymorphic information content; Rp: Resolving power

The marker index (MI) for each SSR primer pair was calculated as MI=E (Hav)p, where E is the effective multiplex ratio [E = nβ where β is the fraction of polymorphic marker or loci] and (Hav)p represents the average heterozygosity for polymorphic marker[16].

3. Results and discussion

Fourteen STMS primer pairs were used to analyze 48 rice cultivars from diverse geographical locations. Out of 14 markers used, 10 (71.43%) revealed polymorphism and found to be informative. A total of 39 alleles were found to be amplified with an average of 2.85 alleles per primer pair. The number of bands per primer ranged from one (RM 192, RM 3350 and RM 6818) to six (RM 154) and the size of the amplified products varied from 0.1 kb to 1 kb. Of the 39 bands scored, 22 (56.41%) were found to be polymorphic. A diagrammatic representation (**Figure II**) of the DNA fingerprints of the rice genotypes under study based on these 10 markers has been developed.

Similarity indices estimated on the basis of all the 14 primer pairs ranged from 0.60 to 1.00. The UPGMA cluster constructed from STMS analysis grouped the 48 cultivars into five major clusters (I, II, III, IV & V). The first major cluster was further divided into two sub-clusters. The first sub-cluster (IA) consisted of five genotypes (all *indica* with Annada and Prabhat sharing a common parent MTU) and the second sub-cluster (IB) had two genotypes, both were selections from INGER trial, IRRI, Philippines thus showing their commonness. The second major cluster was further sub-clustered into three (IIA, IIB & IIC). The first sub-cluster (IIA) included 10 *indica* rice genotypes, wherein cent percent similarity was observed among genotypes 4, 12, 13, 44 and 46 (**Table I**). The second sub-cluster (IIB) comprised of five genotypes with hundred percent similarity between genotype 17 and 40. The third sub-cluster (IIC) had eight genotypes with highest similarity index (1.00) among genotypes 19, 24 and 27 and between genotypes 29 and 41. The third major cluster was sub-grouped into two. First sub-group, IIIA comprised of eight genotypes, wherein three genotypes viz., 14, 30 and 38 displayed maximum similarity (1.00). Second sub-group IIIB included only two genotypes, 10, a pure line selection from a landrace and 43, a traditional variety and the two showed hundred percent similarity.

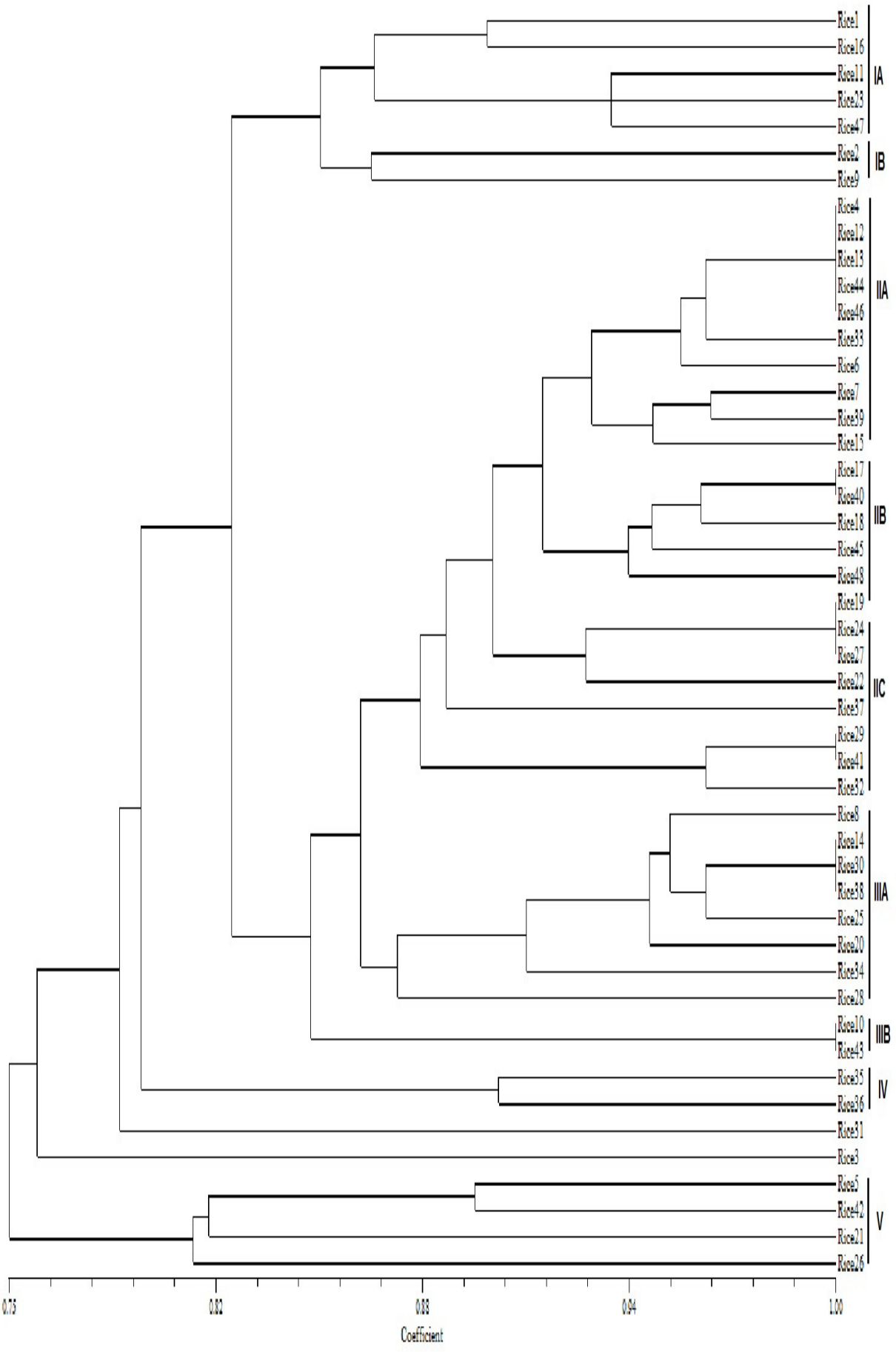

Figure I: Dendrogram constructed from UPGMA cluster analysis using Jaccard's similarity coefficient based on 10 SSR markers

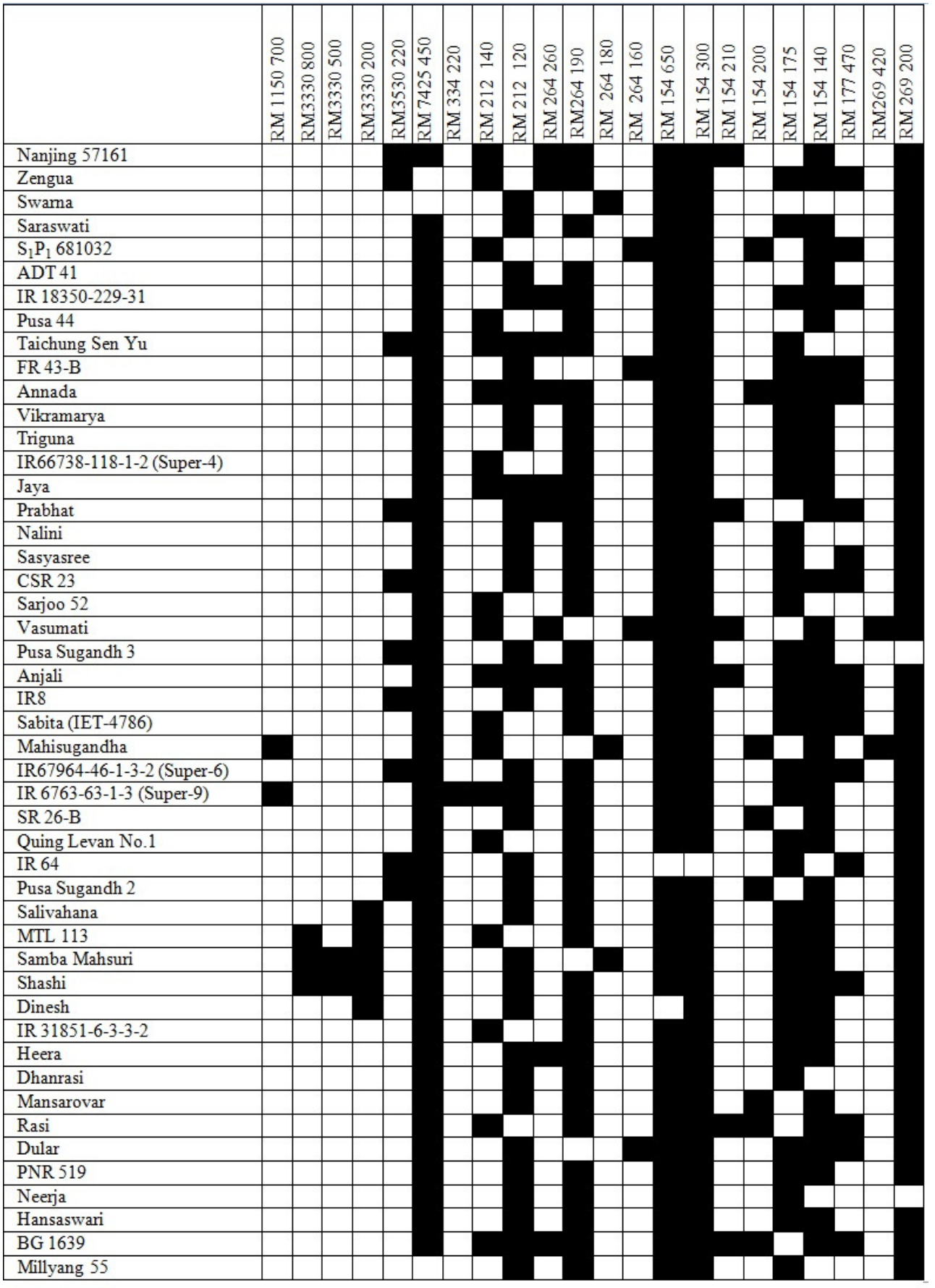

Figure II: Diagrammatic presentation of DNA fingerprints. Each row corresponds to a cultivar and each column represents the fragment pattern in respect of a marker across the cultivars. For instance, the first column (RM1150 700) denotes the pattern of a 700bp fragment, which was obtained with primer pair RM 1150 in two cultivars (Mahisugandha and IR 6763-63-1-3). The shaded regions represents the presence of bands.

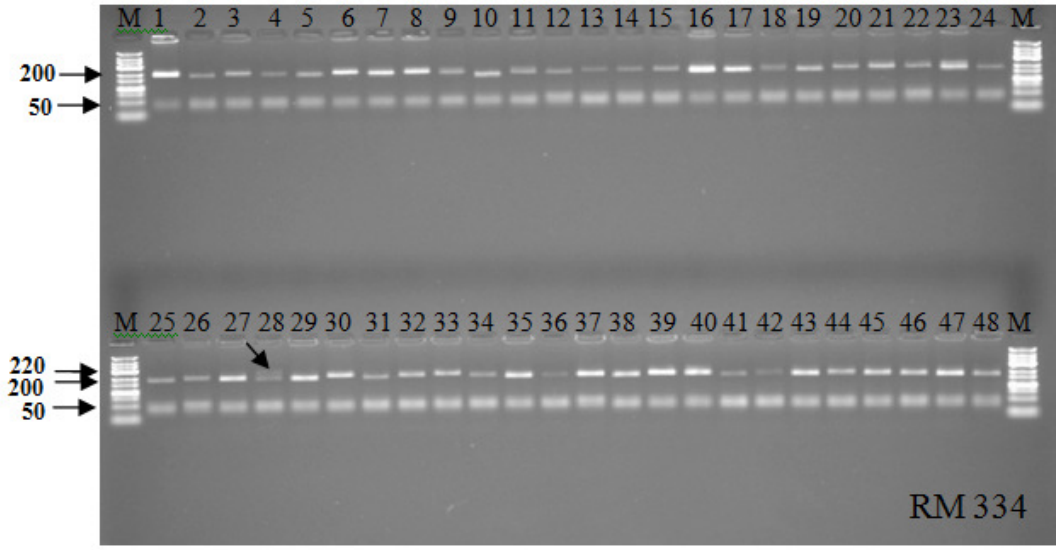

Figure III: DNA amplification pattern in using primer pair RM 334. Legends, M: Low range DNA Ladder. The numbers of lanes 1 to 48 correspond to the rice cultivars as listed in Table 1.

The fourth major cluster included two *indica* varieties, 35 and 36. The fifth major cluster consisted of four *indica* rice genotypes (5, 42, 21 and 26) that included two (21 & 26) highly scented, long and slender grained varieties. The two rice genotypes (31 & 3) were out-grouped in the cluster and genotypes 21 and 31 showed the lowest genetic similarity with a value of 0.60, since those two varieties are geographically reported to be very distant. The strength and robustness of the clustering pattern as indicated in the dendrogram and supported by Winboot, could bring genetically related rice cultivars together, thus formed closed clusters.

The polymorphism information content (PIC) values obtained in the present study ranged from 0.03 (RM 7425) to 0.7 (RM 3330 and RM 264) with an average of 0.35 (**Table II**). The estimates of Rp were found to be the highest for the primer RM 154 (1.64), followed by RM 212 (1.34) and was lowest for the primer RM 334 (0.04). The marker index (MI) calculated for the SSR marker system in the study was found to be 0.25.

The PIC values are dependent on the genetic diversity of the cultivars chosen. Lower PIC value may be the result of closely related genetic architecture. The number of alleles amplified by a primer and its PIC values also depend upon the repeat number and the repeat sequence of the microsatellite sequence[17-20]. Ni *et al.*[20] showed that larger repeats and GA-repeats yield higher number of alleles and higher PIC values. Contrary to this, Temnykh *et al.*,[17] reported that (CTT), and AT-rich trinucleotide repeats amplified with higher efficiency and revealed greater polymorphism overall.

In the present study, RM 154 and RM 264 having (GA) repeat produced 6 and 4 alleles and had PIC value of 0.36 and 0.7, respectively. For RM 177 $[(AG)_8]$ and RM 3330 $[(CT)_{15}]$, 5 and 4 alleles with PIC value of 0.13 and 0.7 were observed, respectively.

A genotype specific allele with respect to a particular STMS marker was observed uniquely. RM 334$[(CTT)_{20}]$ yielded 3 alleles with one rare allele/ signature band specific for a rice genotype (IR 6763-63-1-3) (**Figure III**) which can be used as a marker of choice for correct identification and genetic purity test.

Extensive research has led to the development of a large number of varieties in rice. Hence, identification and molecular characterization of genotypes and varieties is of utmost importance for genetic improvement, in protecting plant breeders' rights (PBR)[21,22] and halting abuse in commercial use. Molecular characterization of the genotypes provides precise information about the extent of genetic diversity and selection of appropriate or potential parents for breeding programme for higher productivity and value addition. With the aid of microsatellite markers and clustering pattern, different distantly related rice genotypes may be combined by intercrossing to get superior varieties/hybrids with highest heterosis owing to superior genetics.

Microsatellite markers have proved to be a reliable tool in assessing parental polymorphism and genetic purity in rice[23,24]. There were several reports on rice cultivar classification using molecular markers. Zhu *et al* [25] used 32 primers in 48 *indica* and *japonica* rice cultivars. Twelve aromatic rice landrace were characterized using 24 SSR primers[26] while Kanawapee *et al* [27] used two marker system viz., RAPD and SSR for genetic diversity assessment involving 30 rice cultivars differing in respect of salinity tolerance.

In the present study, a total of 14 microsatellite markers were used involving 48 rice genotypes for their

characterization and identification and to assess the level of genetic variation among the genotypes. The average similarity coefficient of 0.87 suggested that there was a low level of genetic diversity among 48 rice genotypes under reference. Further, the STMS markers that showed hundred percent similarity should be avoided for molecular characterization of those genotypes under study, which displayed cent percent similarity. This could be attributed to their similar genetic background or close pedigree relationship. However, to overcome this, other STMS markers developed across the rice genotypes are recommended for molecular characterization studies.

Conclusion

In nutshell, it is concluded that microsatellite markers is proved to be potential marker in genetic purity test and may easily be used to supplement conventional GOT and isozyme profile to make correct identification of a variety as well as may be used with ease and confidence to unzip genetic relatedness based on which genetically distant parents may be chosen for hybridization programme to capitalize the benefits of heterosis breeding, which finally leads into more productivity as well as production of a self pollinated crop on which the global life line depends heavily for its sustenance, perpetuation and prosperity.

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Conflict of interest

The author's declares none.

References

1. Ohtsubo K & Nakamura S, Cultivar identification of rice (*Oryza sativa* L) by polymerase chain reaction method and its application to processed rice products. *J Agr Food Chem*, **55** (2007) 1501-1509.
2. Anon. All India Area, Production and Yield of Rice along with Coverage under Irrigation, Agriculture Statistics at a Glance 2014, Department of Agriculture and Cooperation, Directorate of Economics and Statistics, Govt. Of India, New Delhi, pp:452.
3. Winter P & Kahl G, Molecular marker technologies for plant improvement. *World Journal of Microbiology & Biotechnology,* **11** (14) (1995) 438-448.
4. McCouch SR, Chen X, Panaud O, Temnykh S, Xu Y, Cho YG, Huang N, Ishii T & Blair M, Microsatellite marker development, mapping and applications in rice genetics and breeding. *Plant Mol Biol,* **35** (1997) 89-99.
5. McCouch SR, Teytelman L, Kono I, Yano M, Fjellstrom RG, Declerck GA, Schneider DJ, Cartinhour SW, Ware D & Stein L, Development of 2240 New SSR Markers for Rice (*Oryza sativa* L.). *DNA Res*, **9** (2002) 199-207.
6. Nagaoka T & Ogihara Y, Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. *Theor Appl Genet,* **94** (1997) 597-602.
7. Rafalski A, The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol. Breed*, **2** (1996) 225- 238.
8. Salimah SS, De Oliveira AC & Godwin ID, Assessment of genome origin and genetic diversity in the genus *Eleusine* with DNA markers. *Genomics*, **38** (1995) 757-763.
9. Murray MG & Thompson WF, Rapid isolation of molecular weight plant DNA, *Nucleic Acids Res,* **8** (1980) 4321-4325.
10. Wu KS & Tanksley SD, Abundance, polymorphism and genetic mapping of microsatellites in rice. *Mol Gen Genet*, **241** (1993) 225-235.
11. Chen X, Temnykh S, Xu Y, Cho YG & McCouch SR, Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). *Theor Appl Genet,* **95** (1997) 553-567.
12. Temnykh S, Park WD, Ayres N, Cartinhour S, Hauck N, Lipovich L, Cho YG, Ishii T & McCouch SR, Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). *Theor Appl Genet,* **100** (2000) 697-712.
13. Rohlf FJ, NTSYS-PC Numerical taxonomy and multivariate analysis system, Version 2.0. Exeter Software, Setauket, New York (1998).
14. Smith JSC, Chin ECL, Shu H, Smith OS, Wall SJ, Senior ML, Michell SE, Kresovick & Ziegle J, An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): Comparison with data from RFLPs and pedigrees. *Theor Appl Genet,* **95** (1997) 163-173.
15. Prevost A & Wilkinson MJ, A new system for comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theor Appl Genet*, **98** (1999) 107-112.
16. Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey & Rafalski A, A comparison of RFLP, RAPD, AFLP and SSR markers for germplasm analysis. *Mol Breed*, **2** (1996) 225-238.
17. Temnykh S, Park WD, Ayres N, Cartinhour S, Hauck N, Lipovich L, Cho YG, Ishii T & McCouch SR, Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). *Theor Appl Genet,* **100** (2000) 697-712.
18. Temnykh S, DeClerck G, Lukashova A, Lipovich L,

Cartinhour S & McCouch SR, Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.), frequency, length variation, transposon associations and genetic marker potential. *Genome Res*, **11** (2001) 1441-1452.

19. Yu SB, Xu WJ, Vijayakumar CHM, Ali J, Fu BY, Xu JL, Jiang YZ, Marghirang R, Domingo J, Aquino C, Virmani SS & Li ZK, Molecular diversity and multilocus organization of the parental lines used in the International Rice Molecular Breeding Program. *Theor Appl Genet*, **108** (2003) 131-140.
20. Ni J, Colowit PM & Mackill DJ, Evaluation of genetic diversity in rice subspecies using microsatellite markers. *Crop Sci*, **42** (2002) 601-607.
21. Cooke RJ, Bredemeijer GMM, Ganal MW, Peeters R & Isaac P, Assessment of the uniformity of wheat and tomato varieties at DNA microsatellite loci. *Euphytica,* **132** (2003) 331-342.
22. Law JR, Donini P, Koebner RMD, Reeves JC & Cooke RJ, DNA profiling and plant variety registration. III: The statistical assessment of distinctness in wheat using amplified fragment length polymorphisms. *Euphytica,* **102** (1998) 335-342.
23. Nandakumar N, Singh AK, Sharma RK, Mohapatra T, Prabhu, KV & Zaman FU (2004) Molecular fingerprinting of hybrids and assessment of genetic purity of hybrid seeds in rice using microsatellite markers. *Euphytica*, **136**: 257-264.
24. Sundaram RM, Naveenkumar B, Biradar SK, Balachandran SM, Mishra B, Ilyas Ahmed M, Viraktamath BC, Ramesha MS & Sarma NP, Identification of informative SSR markers capable of distinguishing hybrid rice parental lines and their utilization in seed purity assessment. *Euphytica*, **163** (2008) 215-224.
25. Zhu YF, Qin GC, Hu J, Wang Y, Wang JC & Zu SJ. Fingerprinting and variety identification of rice (*Oryza sativa* L.) based on simple sequence repeat markers. *Plant Omics J*, **5** (2012) 421-426.
26. Sajib AM, Hossain MM, Mosnaz ATMZ, Hossain H, Islam MM, Ali MS & Prodhan SH, SSR marker-based molecular characterization and genetic diversity analysis of aromatic landraces of rice (*Oryza sativa* L.). *J Biosci Biotechnol*, **1 (2)** (2012) 107-116.
27. Kanawapee N, Sanitchon J, Srihaban P & Theerakulpisut P, Genetic diversity analysis of rice cultivars (*Oryza sativa* L.) differing in salinity tolerance based on RAPD and SSR markers. Electronic J Biotechnol, **14 (6)** (2011).