

Analysis of molecular diversity and fingerprinting of commercially grown Indian rice hybrids

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Abstract Twenty-five commercially grown Indian rice hybrids developed by both the public and private sectors, were analysed for molecular diversity and identification of simple sequence repeat (SSR) marker(s) that can distinguish them from each other. For diversity analysis, a total of fifty eight SSR markers providing genome wide coverage, were used. Forty out of fifty eight SSR markers were polymorphic amplifying a total of 121 alleles with molecular weight ranging from 70 bp – 280 bp. Further, characterisation of these markers was carried out generating parameters of heterozygosity (0.42), polymorphism information content (0.31), probability of identity (4.2×10^{-8}) and probability of exclusion (99.99%). Cluster analysis based on a set of forty highly polymorphic SSR markers generated three groups with dissimilarity index values ranging from 0.0 to 0.8. The hybrids based on common female parent IR58025A grouped together indicating a narrow genetic base of hybrid breeding programme. By combining the rapid and simple method of utilising these unique SSR markers alone or in combination, as molecular tags, identification of all the hybrids was possible even without having their parental lines. Twenty SSR loci produced hybrid specific unique alleles, which will be useful in establishing hybrid's identity. The results have wide prospective in diversifying the genetic base of hybrid breeding programme, identification of rice hybrids, authentication of genetic purity of hybrid seed and protection of IPR.

Keywords Hybrid rice · Hybrid purity · SSR · Molecular diversity

Abbreviations

DUS	Distinctness uniformity and stability
IPR	Intellectual property rights
IRRI	International Rice Research Institute
PBR	Plant breeder's rights
PIC	Polymorphism information content
SSR	Simple sequence repeat
UPGMA	Unweighted Pair Group Method with Arithmetic Mean

Introduction

The rice production in India should reach 124 million tons by 2020 to meet the requirement of the increasing population (Kumar et al. 1995). To increase the yield potential, hybrid rice technology is a feasible and readily adoptable option in the backdrop of declining resources such as land, water, labour and other inputs without adversely affecting the environment (Zhang 2007). India stands next to China in adoption of hybrid rice technology with the official release of 47 hybrids and 1.3 million ha area planted to hybrid rice during *Kharif* 2010 (Hariprasad et al. 2011). However, the growth of area under hybrid rice in India has been rather slow, which accounts for only <3.4% of the total area (44 million ha) under rice. Moderate level of heterosis, unacceptable grain and cooking quality, poor infrastructure for hybrid seed production, low hybrid seed yield, high seed cost and marketing of unscrupulous hybrids of poor seed quality and purity are some of the reasons for less than expected spread of hybrid rice technology. However, in

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recent past these challenges have been adequately addressed with active participation of private sector resulting in development of new generation rice hybrids with substantial yield advantage and improvement of seed production infrastructure. At present, approximately 100 rice hybrids including 47 officially released, most of it by private sector, are under cultivation.

Genetic diversity between combining parents is an essential requirement for realization of heterosis in resultant hybrid. In the first phase of hybrid rice development in India, IR58025A was extensively used as female parent in both public and private sector hybrid breeding programmes. As a result, the hybrids developed uptill 2005, were mostly half-sibs having IR58025A as common female parent. This resulted into narrow genetic base of hybrid breeding programme and thus moderate level of heterosis. It is therefore, absolutely essential to assess the genetic diversity among hybrids under cultivation and devise a breeding strategy to broaden the genetic base of hybrid breeding programme to enhance the magnitude of heterosis.

In addition, molecular fingerprinting of all commercially grown hybrids is essential to create a national database, which could be helpful in checking the marketing of unscrupulous hybrids, protecting intellectual property rights (IPR) and authenticating genetic purity of hybrid seed lots. However, for developing fingerprint of the hybrids, their parental lines are required as control, which are not disclosed particularly in case of private sector hybrids, being a trade secret. Therefore, developing a unique identification system using molecular marker, based on hybrid genotypes *per se* is desirable.

In rice, SSRs are distributed widely throughout the genome (McCouch et al. 1996; McCouch et al. 1997; Temnykh et al. 2000). SSR markers, which are co-dominant in nature and detect high levels of allelic diversity, are considered ideal molecular marker for cultivar identification. Nandakumar et al. (2004) established the DNA fingerprint of 11 rice hybrids and their parental lines with 10 SSR markers and found four informative SSR markers differentiating all the hybrids from each other. Xin et al. (2005) used SSR markers for fingerprinting rice hybrids and suggested their wide prospects in analysis of genetic purity and variety identification. Sundaram et al. (2008) suggested utilisation of SSR markers using bulked DNA samples for detection of impurities in parental lines and contaminants in hybrid seed lots. In addition, robustness of fertility restorer gene linked marker in testing genetic purity of hybrid seed lot in rice has been demonstrated by Garg et al. (2006). In the above backdrop, the objectives of present study were to analyse the molecular diversity among commercially grown Indian rice hybrids and to develop molecular tag for their identification using unique SSR markers.

Materials and methods

Plant material

The true F₁ seeds of 25 hybrids (Table 1) including 12 from public and 13 from private sector were obtained from the Directorate of Rice Research, Hyderabad and evaluated following standard agronomic practices at Division of Genetics, Indian Agricultural Research Institute, New Delhi. Observations on phenological (days to 50% flowering and days to maturity) and on morphological traits were recorded on ten randomly chosen plants from each hybrid. Each hybrid was grown in plot size of 5 m² keeping row to row and plant to plant distance at 20 cm and 15 cm, respectively.

Molecular analysis

Total DNA was extracted from the bulked leaf samples collected from 10 F₁ seedlings in each hybrid following the procedure of Prabhu et al. (1998). The quantification of DNA was carried out with 0.8% agarose gel using uncut λ DNA as standard and diluted to concentration of approximately 25 ng/ μ l for PCR analysis.

A total of 58 SSR primers (Table S1) including 49 SSR from the GCP panel of microsatellite markers developed by International Rice Research Institute, Philippines (IRRI) for diversity analysis in rice and 9 most informative markers based on our earlier study on hybrid rice (Nandakumar et al. 2004) were used in the present study. PCR reactions were performed in thermocycler (G-storm, KAPA Biosystems, UK). Template DNA was initially denatured at 94°C for 5 min followed by 35 cycles of PCR amplification with the following parameters: 1 min denaturation at 94°C, 1 min annealing at 55°C and 2 min of primer extension at 72°C followed by final extension of 72°C for 7 min. Reactions were carried out in a volume of 10 μ l reaction mixture consisting of 10x PCR assay buffer (Bangalore Genei Pvt. Ltd., India), 200 μ M of each dNTP (MBI Fermentas, Lithuania, USA), 12 ng (1.8 picomole) each of forward and reverse primers (Sigma), 0.5 units of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., India) and 25 ng template DNA. The PCR products were separated on 3.5% Agarose gel (Bangalore Genei Pvt. Ltd., India) using 1X TAE buffer. The size of the amplified fragments was determined by using 50 bp DNA ladder (Fermentas). Since, the allelic amplicons differed in size by 20 bp–50 bp, across markers, it was easy to resolve them on simple agarose. Forty out of 58 SSR markers used for diversity analysis, were found polymorphic. Therefore, the fingerprint generated by polymorphic markers was used for profiling ten randomly selected F₁ plants from each hybrid to check the consistency in amplification pattern and to eliminate the

Table 1 Details of rice hybrids used in present study

Name of Hybrid	Duration (days)	Year of release	Notification No.	Date of notification	Developed by
Pusa RH10	110–115	2001	1134(E)	15.11.2001	Indian Agricultural Research Institute, New Delhi, India
DRRH-2	115	2005	1566 (E)	5.11.2005	Directorate of Rice Research, Hyderabad, Andhra Pradesh, India
PA-6129	120	2007	1703 (E)	5.10.2007	Bayer Bio-Science, Hyderabad, India
CORH-3	115	2006	1178 (E)	20.7.2007	Tamil Nadu Agricultural University, Coimbatore, India
Sahyadri-2	115–120	2006	122 (E)	6.2.2007	Regional Agricultural Research Station, Karjat (BSKVV), Maharashtra, India
Sahyadri-3	125–130	2006	122 (E)	6.2.2007	Regional Agricultural Research Station, Karjat (BSKVV), Maharashtra, India
GK-5003	128	2008	454 (E)	11.2.2009	Ganga Kaveri Seeds Pvt Ltd, Hyderabad, Andhra Pradesh, India
US-312	128	2009	NA	NA	Seed Works International, Hyderabad, Andhra Pradesh, India
NK-5251	128	2009	NA	NA	Syngenta India Ltd, Secundrabad
Indam-200-017	NA	NA	NA	NA	Indo-American Hybrid seed co.
DRH-775	97	2009	2187 (E)	27.8.2009	Metahelix Life Sciences Pvt Ltd, Hyderabad
PA-6201	125–130	2000	92 (E)	19.7.2000	Bayer Bio-Science, Hyderabad, India
PA-6444	135	2001	1134 (E)	5.11.2001	Bayer Bio-Science, Hyderabad, India
PHB-71	130–135	1997	647 (E)	9.9.1997	Pioneer Overseas Corp, Hyderabad, India
Suruchi	130	2004	122 (E)	2.2.2005	Mahyco Ltd, Aurangabad, Maharashtra, India
Sahyadri-1	125–130	1998	821 (E)	13.9.2000	Regional Agricultural Research Station, Karjat, Maharashtra, India
JRH-8	NA	2008	449 (E)	11.2.2009	Jawahar Lal Nehru Krishi Viswa Vidyalaya, Jabalpur, Madhya Pradesh, India
JKRH-401	140	2007	122 (E)	6.2.2007	JK Agri Genetics Ltd, Hyderabad, India
PAC-835	102	2009	2187 (E)	27.8.2009	Advanta India Ltd, Hyderabad, India
PAC-837	97–103	2009	2187 (E)	27.8.2009	Advanta India Ltd, Hyderabad
KRH-2	125–130	1996	401 (E)	15.5.1998	Zonal Agricultural Research Station, VC farm, Mandya, University of Agricultural Sciences, Bengaluru, Karnataka, India
DRRH-3	131	2009	NA	NA	Directorate of Rice Research, Hyderabad, Andhra Pradesh, India
Sahyadri-4	115–120	2008	454 (E)	11.2.2009	Regional Agricultural Research Station, Karjat, Maharashtra, India
Indrasona	125–130	2006	1178 (E)	20.7.2007	Indira Gandhi Krishi Vishwa Vidyalaya, Raipur, Chattisgarh, India
PSD-3	125–130	2004	599(E)	25.4.2006	Govind Ballabh Pant University of Agricultural & Technology, Pant nagar, Uttarakhand, India

NA Not available

chance error due to presence of pollen shedder or off-type plants. These F₁ plants were observed for their phenotype till maturity.

Statistical analysis

Genetic diversity was estimated from the matrices generated using Jaccard's dissimilarity coefficient. The data entry was done into binary data matrix as discrete variables (present-1

and absent-0). A dendrogram was generated based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA) through the software Darwin 5.0 (Perrier and Jacquemoud-Collet 2006). The summary statistics including the number of alleles per locus, major allele frequency and polymorphism information content (PIC) values were determined using PowerMarker version 3.25 (Liu and Muse 2005, <http://statgen.ncsu.edu/powermarker/>).

The unique markers distinguishing rice hybrids were further analysed for the following parameters: 1) probabil-

ity of genetic identity (J) (Paetkau et al. 1995), which corresponds to the probability of two random individuals displaying the same genotype and 2) probability of exclusion (Q) (Weir 1996), by using the software GenAlEx 6.41.

Results and discussions

DNA markers offer many advantages over morphological and biochemical markers. They are present in abundance, simple, quick and experimentally reproducible. Among different types of DNA markers used, SSR markers are most preferred ones in rice due to their abundance, genome wide coverage and co-dominant nature (McCouch et al. 2002) and have been frequently used for diversity analysis, mapping, MAS and identification of plant varieties/hybrids.

The 40 polymorphic SSRs markers used in the study, amplified a total of 121 alleles with an average of 3.02 alleles per locus. The polymorphism information content (PIC) of 40 primers ranged from 0.03 for RM237 and RM201 to 0.64 for RM228 with average of 0.31 (Table 2). The marker RM237 and RM201 exhibited maximum major allele frequency (0.98), while it was least for RM171 (0.36). Explanation of the heterozygosity using micro-satellite markers ranged from 0.00 (RM277) to 1.00 (RM284), with the mean of 0.42. The variability parameters including PIC, heterozygosity and major allele frequency indicated the suitability of markers chosen for diversity analysis and molecular characterization of hybrids.

Genetic diversity is the foundation of genetic improvement in any crop and also fundamentally important for hybrid development. The genetic diversity among the hybrids estimated as Jaccard's dissimilarity coefficient, ranged from 0.0 between US-312 and GK-5003) to 0.80 Pusa RH10 and Indra Sona. The cluster analysis grouped the hybrids into two groups and one hybrid namely, indra sona remained ungrouped (Fig. 1). The cluster I had as many as 16 hybrids including seven from public sector (Sahyadri-1, Sahyadri-2, Sahyadari-3, Sahyadri-4, PSD-3, PRH-10 and KRH-2) and nine from private sector (PHB-71, DRH-775, JKRH-401, PA-6444, PA-6201, US-312, GK-5003, NK-5251, PA-6129). A perusal of the parentage of the hybrids belonging to this group revealed that all but one (Pusa RH-10) public sector bred hybrids belonging to this group namely Sahyadri-1, Sahyadri-2, Sahyadari-3, KRH-2, Sahyadri-4 and PSD-3 were developed using a common female parent i.e. IR58025A, a CMS line carrying WA cytoplasm developed at IRRI during late nineteen eighties. Therefore, the genetic relationship among these hybrids is that of half-sibs. The only exception, Pusa RH-10 was also based on Pusa 6A, as a female which was developed using a sister line of IR58025B, thus grouping of PusaRH-10 in cluster I is understandable. Although the

Table 2 Variability parameters of forty polymorphic SSR markers

Chrom No.	Marker	MLA	NAO	NRA	H	PIC
1	RM495	0.70	3	0	0.52	0.33
1	RM1	0.92	3	1	0.16	0.14
1	RM312	0.88	2	0	0.24	0.18
1	RM237	0.98	2	1	0.04	0.03
2	RM250	0.84	4	2	0.32	0.25
2	RM263	0.58	6	0	0.40	0.54
2	RM154	0.90	2	0	0.20	0.16
3	RM514	0.72	3	0	0.56	0.37
3	OSR13	0.78	4	0	0.20	0.31
4	RM307	0.52	3	0	0.40	0.37
5	RM413	0.78	4	0	0.36	0.33
5	RM334	0.70	3	0	0.60	0.41
6	RM510	0.76	3	1	0.44	0.31
6	RM162	0.96	2	0	0.08	0.07
7	RM234	0.54	4	0	0.76	0.42
7	RM125	0.52	2	0	0.96	0.37
7	RM11	0.84	3	1	0.32	0.24
7	RM118	0.42	3	0	0.60	0.58
8	RM152	0.52	4	0	0.72	0.52
8	RM408	0.92	3	1	0.12	0.14
8	RM25	0.92	3	0	0.08	0.13
8	RM44	0.96	2	0	0.08	0.07
8	RM284	0.50	2	1	1.00	0.40
8	RM433	0.70	2	0	0.60	0.33
8	RM447	0.84	3	0	0.24	0.23
9	RM201	0.98	2	1	0.04	0.03
9	RM316	0.80	4	2	0.40	0.29
9	RM105	0.64	2	0	0.72	0.35
10	RM258	0.74	5	0	0.44	0.39
10	RM216	0.46	4	0	0.84	0.56
10	RM228	0.38	7	0	0.92	0.64
10	RM6100	0.52	3	1	0.96	0.40
10	RM271	0.94	2	0	0.12	0.10
10	RM171	0.36	2	0	0.72	0.58
11	RM144a	0.56	3	0	0.80	0.37
11	RM552	0.94	2	0	0.12	0.10
11	RM287	0.74	5	0	0.44	0.40
12	RM247	0.92	3	1	0.04	0.14
12	RM19	0.70	3	0	0.52	0.40
12	RM277	0.56	2	0	0.00	0.37
Mean		0.72	3.02	0.35	0.42	0.31

MLA Major allele frequency; NAO No. of alleles observed; NRA Number of rare alleles; H Heterozygosity; PIC Polymorphism information content

parentage of the private sector bred hybrids is not disclosed, it is quite likely that some of these hybrids may also be based on IR58025A, which being a good general combiner

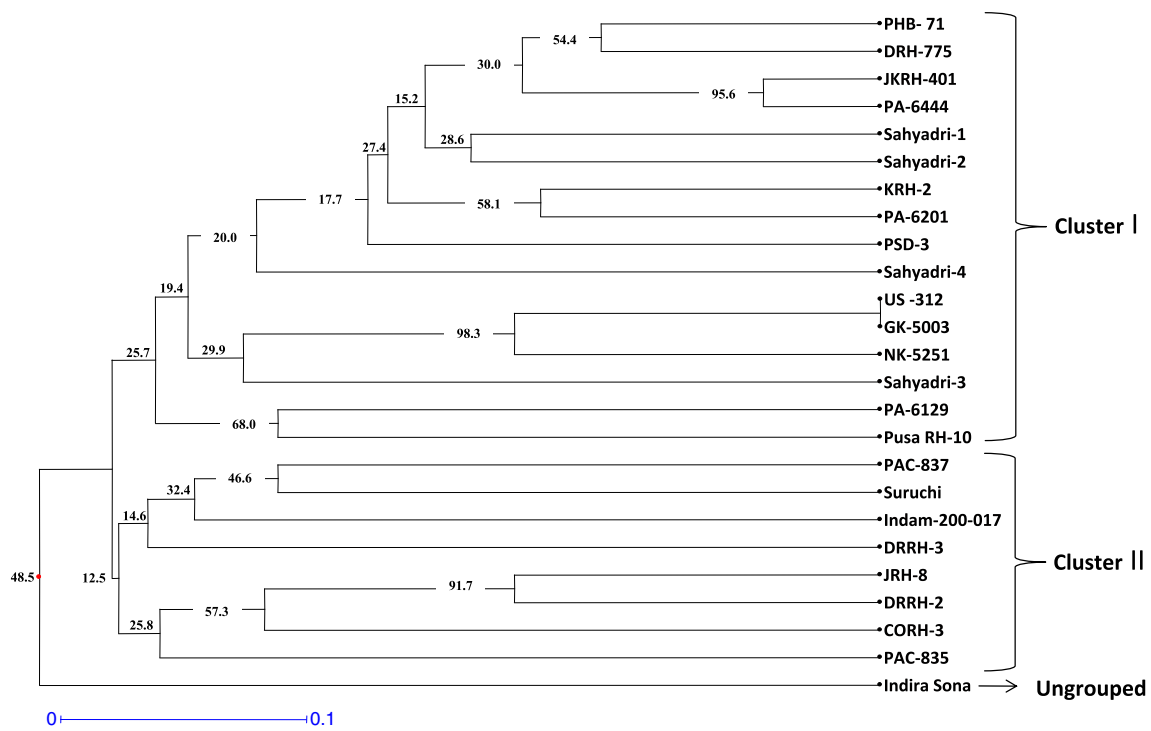


Fig. 1 Dendrogram depicting genetic relationships among rice hybrids based on SSR data (The numbers at the branches representing bootstrap values)

was extensively used in hybrid development in India and in many other countries during first phase of hybrid development. The extensive use of a common female parent reduced the genetic diversity of hybrid rice breeding programme which resulting in only a moderate level of heterosis. It is obvious from the foregoing discussion that the rice hybrids developed during the first phase of hybrid breeding programme have exploited not only a common cytoplasmic source (WA) but also a common nuclear background (IR58025A), thus are potentially vulnerable to outbreak of diseases and pests. In addition, these hybrids provide limited opportunity for enhancing level of heterosis owing to reduced genetic diversity from female side. Genetic uniformity as cause of genetic vulnerability has been well established with the susceptibility of T-CMS based maize hybrids in USA to southern corn blight caused by *Helmenthosporium maydis* (Levings 1990). It is therefore, necessary to diversify the cytoplasmic source as well as nuclear background of CMS lines, in order to prevent outbreak of diseases and pests and also to enhance magnitude of heterosis.

Cluster II consisted of eight hybrids mostly developed during second phase of hybrid rice programme in India including four each from public (DRRH-3, JRH-8, DRRH-2 and CORH-3) and private sector (PAC-837, Suruchi, Indam-200-017 and PAC-835). In addition to diversity among restorer parents of these hybrids, the most important reason for hybrids in cluster II, being diverse from cluster I

was the fact that these hybrids were based on CMS lines with diverse nuclear background as compared to IR58025A. For instance, DRRH-3, JRH-8, DRRH-2 and CORH-3 were based on CMS lines APMS6A, IR68897A, IR68897A and TNAU-CMS-2A respectively. The hybrid Indrasona, fell apart from other hybrids and remained ungrouped. This hybrid is though based on IR58025A as female parent but had a diverse pollen parent (R710-437-1-1) derived through selection from a landrace Safri- 17.

Further, in order to identify SSR marker combinations that could differentiate all the 25 rice hybrids from each other, the probability of identity (*I*) i.e. the probability that two independent individuals will have the identical genotype for a certain loci in the population was worked out. The combined estimate of probability of identity of all the loci was very low (4.2×10^{-8}), indicating that among the hybrids studied, the probability of any two hybrids being identical by chance is almost nil. In addition, the combined probability of exclusion was greater than 99.99%, indicating that these markers were appropriate in differentiating all the hybrids unequivocally.

List of marker(s) and their combinations along with amplicon size providing unique fingerprint to each hybrid is presented in Table 3. The highest number of 4 alleles were scored for the marker RM228, with the fragment sizes of 120 bp, 130 bp, 140 bp and 150 bp. Based on the allelic profile at RM228 locus, the 25 hybrids were grouped into seven categories via 120 bp (1), 120 bp+130 bp (11),

Table 3 List of marker(s) and their combinations along with amplicon size providing unique fingerprint to each hybrid

Hybrid Name	Unique markers and its amplified allele (bp)
Pusa RH10	RM234 (190 bp), RM263 (190 bp+160 bp), RM201 (145 bp+155 bp), RM6100 (160 bp+140 bp)
DRRH-2	RM152 (170 bp +140 bp)+RM514 (280 bp)
PA-6129	RM6100 (140 bp)
CORH-3	RM125 (130 bp), RM247 (80 bp)
Sahyadri-2	RM247 (100 bp+80 bp)
Sahyadri-3	RM144a (250 bp)
GK-5003 ^a	-
US-312 ^a	-
NK-5251	RM447 (125 bp)
Indam-200-017	RM1 (120 bp+80 bp), RM413 (100 bp), RM228 (150 bp +120 bp), RM258 (145 bp)
DRH-775	RM152 (150 bp +130 bp)+RM228 (140 bp +130 bp)
PA-6201	RM514 (280 bp+240 bp)+RM316 (150 bp)
PA-6444	RM413 (100 bp+75 bp)+RM228 (130 bp +120 bp)+RM152 (150 bp +130 bp)
PHB-71	RM19 (250 bp)+RM263 (210 bp+180 bp)
Suruchi	RM228 (150 bp)
Sahyadri-1	RM263 (180 bp+150 bp)
JRH-8	RM237 (140 bp +130 bp)
JKRH-401	RM152 (150 bp+130 bp)+RM228 (130 bp+120 bp)
PAC-835	RM234 (220 bp)
PAC-837	RM228 (120 bp)
KRH-2	RM514 (280 bp+240 bp)+RM316 (170 bp+150 bp)
DRRH-3	RM250 (180 bp+150 bp)
Sahyadri-4	RM263 (180 bp)
Indra Sona	RM495 (170 bp), RM316 (180 bp+150 bp)
PSD-3	RM287 (120 bp +90 bp)+RM514(280 bp+250 bp)

^a Hybrids could not be differentiated

130 bp+140 bp (4), 120 bp+150 bp (1), 120 bp+140 bp (3), 150 bp (1) and 130 bp+150 bp (4). Likewise, markers RM263, RM287 and RM258 had high discriminating power. The diversity analysis based on four markers, RM228, RM263, RM287 and RM258, grouped 25 hybrids in 3 groups. However, in total a minimum of 20 markers were required to differentiate all 25 hybrids from each other. The number of different alleles and their allelic combinations with respect to other marker can be seen in Table 3. The markers RM250, RM495, RM1, RM125, RM6100, RM247, RM447, RM228, RM263, RM234 singly established identity for hybrids DRRH-3, Indra

Sona, Indam-200-017, CORH-3, PA-6129, Sahyadri-2, NK-5251, Suruchi, Sahyadri-1 and PAC-835 respectively (Fig. S1a-i). Therefore, among these 10 hybrids, same hybrid could be unambiguously resolved using a single marker. For instance, a combination of 120 bp+80 bp fragments amplified by RM1 was unique to hybrid Indam-200-017 (Fig. 2), therefore, whether a suspected seed lot is of Indam-200-017 or not, can be ascertained using RM1 alone. In order to differentiate rest of the hybrids from each other a combination of two or more markers was required. The study therefore, resulted in establishing unique identity of 23 rice hybrids using 20 markers. However, with

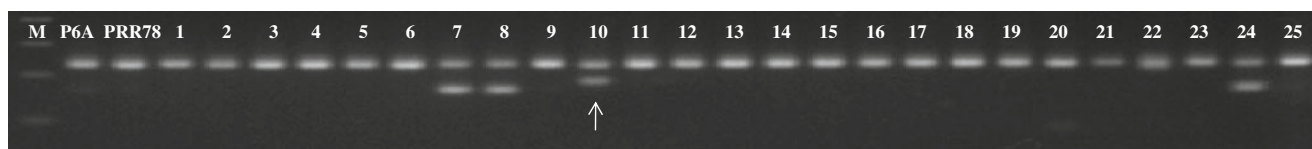


Fig. 2 Amplification profile of hybrids with RM 1 providing unique fingerprint for Indam-200-017. Arrow depict hybrid specific unique fingerprint; M= 50bp ladder; 1= Pusa RH10; 2= DRRH-2; 3= PA-6129; 4= CORH-3; 5= Sahyadri-2; 6= Sahyadri-3; 7= GK- 5003; 8=

US-312; 9= NK-5251; 10= Indam-200-017; 11= DRH-775; 12= PA-6201; 13= PA-6444; 14= PHB-71; 15= Suruchi; 16= Sahyadri-1; 17= JRH-8; 18= JKRH-401; 19= PAC-835; 20= PAC-837; 21= KRH-2; 22= DRRH-3; 23= Sahyadri-4; 24= Indra Sona; 25= PSD-3

expanding list of hybrids more markers need to be added to distinguish the hybrids.

It was noted that the hybrids US-312 and GK-5003 marketed by two different seed companies had identical profile based on amplification of bands by 40 markers. This was possibly a case of same hybrid being marketed in different names by different companies. In order to further confirm if these hybrids were actually same or different, they were further analysed using additional 48 markers. Therefore, including earlier used 40, a total of 88 markers were used to profile US312 and GK5003 with an average density of 7.3 markers/chromosome. Even then, these hybrids could not be differentiated. Since these hybrids could not be differentiated based on morphological characters as well, it is concluded that US-312 and GK-5003 are most likely the same hybrid.

The molecular fingerprint of the hybrid assumes importance in protecting the Plant Breeders' Rights (PBR) and monitoring genetic purity of hybrid seeds. To meet the standard specifications of purity, the parental lines used in hybrid seed production should have a very high (>99%) level of purity (Yashitola et al. 2002). Genetic purity of hybrids is essential for ensuring its agronomic performance and its protection under Plant Variety Protection (PVP) or Plant Breeder's Rights (PBRs), which is based on the criteria of distinctness, uniformity and stability (DUS) characteristics supported by molecular fingerprint. However, at present, DUS guidelines framed in India for the purpose of registration of new plant varieties are primarily based on morphological characters but have not been able to resolve closely related genotypes due to overlapping of descriptor expression in various trait combinations (Joshi et al. 2007). In the present study, SSR markers have produced unique alleles and established discrete identity of rice hybrids except for the two hybrids namely, US-312 and GK-5003. The study clearly demonstrates that it is possible to differentiate hybrids using molecular fingerprint even if the information on parental lines of the hybrids are not available. The results have implications in protection and authentication of the hybrids and analysis of genetic purity of hybrid seed lots.

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