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Identification of Genotype Specific Marker for Samba Mahsuri (BPT 5204) Variety

K. Aruna Kumari[1*], A. Prasad babu[2], Ch.V. Durga Rani[1] and R.M. Sundaram[2]

[1]Department of Agricultural Biotechnology, College of Agriculture, Rajendranagar, Acharya, N G Ranga Agricultural University, Rajendranagar, Hyderabad 500 030, India
[2]Crop Improvement Section, Directorate of Rice Research, Rajendrangar, Hyderabad 500 030, India

**Corresponding author*

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A B S T R A C T

Samba Mahsuri is an elite, fine grain rice variety, which is grown extensively in India. To finger print and identify a genotype specific marker from 26 other closely related fine grain types, we utilized a 113 SSR markers distributed throughout the rice genome. Twenty six markers found to be polymorphic among the rice varieties analyzed. A twin marker combination RM 19426 +JGT 11-16.3 could unambiguously differentiate samba Mahsuri from other fine grain varieties through multiplex PCR.

Introduction

About 25% area under cultivation of rice in the country is being occupied by different varieties developed by Acharya N G Ranga Agricultural University (ANGRAU), Hyderabad, India and contributing to 1/3rd of country's rice production. Three out of the five best varieties in the country *viz*., Samba Mahsuri (BPT 5204), Swarna (MTU 7029) and Vijetha (MTU 1001) were released by ANGRAU. The variety, Samba Mahsuri is extremely popular amongst rice farmers and consumers because of its high yield, medium-slender, fine-grain type and excellent cooking and eating quality. This variety is being targeted for improvement of multiple traits like biotic and abiotic stress tolerance/resistance. The original variety along with its improved versions needs to be protected in terms of plant breeder's right in the present era of intellectual property protection. There is an imminent need to identify genotype specific markers for cultivar identity and trueness of elite varieties like Samba Mahsuri so that such molecular markers can be utilized to unambiguously identify the variety and purity of seed-lots of

the variety. During the seed production chain, the variety can get contaminated with other medium-slender varieties (some of which are cultivated extensively in India) and such impurities may go on accumulating unnoticed, finally leading to deterioration of the genetic quality of the variety. SSRs have been extensively used in rice for molecular fingerprinting and assessment of genetic purity of hybrids and parental lines [10,18,.20]. Based on the above, we designed the present study with the following objective to identify genotype specific marker(s) for Samba Mahsuri in order to differentiate it from other popular fine-grain varieties.

Materials and Methods

Rice genotypes

A total of twenty seven genotypes including Samba Mahsuri (Table 1), which were developed and released by ANGRAU were analyzed for the identification of genotypic specific molecular finger prints SSR marker.

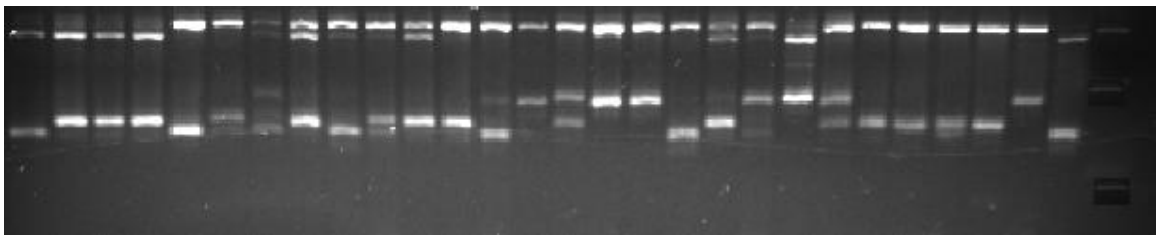
DNA extraction and polymerase chain reaction

Total genomic DNA was extracted from freshly germinated young seedlings. A total of 87 hyper-variable SSR primer pairs (selected from http://www.gramene.org) and 26 $(GATA)_n$ motif specific SSR primer pairs (Rajendra Kumar *et al.,* 2009) distributed across the 12 rice chromosomes were used for PCR amplification (listed in supplementary Table 1). DNA samples (50 ng) were amplified in 10µl reaction volumes containing 1X PCR buffer [10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.01% (v/v) gelatin] (Bangalore Genei, India), 0.2 mM of each dNTPs (Bangalore Genei, India), 10 pmol of each primer and 1 U of Taq polymerase (Bangalore Genei, India). PCR was carried out in a Thermal cycler (Perkin–Elmer, USA). A PCR profile consisting of was 5 min initial denaturation at 94°C, 35 cycles of 1 min denaturation at 94°C, 1 min annealing at 55°C, 2 min extension at 72°C and 7 min at 72°C final extension was followed. The amplified products were resolved on 4% Metaphor agarose gels (Lonza, USA), stained with ethidium bromide and visualized under UV in a gel documentation system (Alpha Innotech, USA). The sizes of the amplified fragments were estimated with the help of Alphaease software utility of the gel documentation system using 50 and 100 bp DNA ladders (MBI Fermentas, Lithuania) as the molecular size standards. Initially an attempt was made to identify a single SSR marker-which could unambiguously differentiate Samba Mahsuri from the rest of the genotypes. Since no single marker could differentiate Samba Mahsuri from other varieties, combinations of two SSR markers which could be pre-PCR multiplexed were selected based on the amplification data of the SSR markers in order to identify genotype specific marker combinations. Only those markers that did not show significant primer sequence matches and those with amplicon sizes well separated from each other were selected for pre-PCR multiplexing.

Results and Discussion

DNA fingerprinting methods based on polymerase chain reaction have become methods of choice for germplasm characterization, diversity analysis and seed purity assays (Sundaram *et al.,* 2008). A variety of DNA markers are now available for fingerprinting cultivars and for marker assisted selection. Even though the utility of RAPD fingerprints for establishing genotype identity is known in case of rice hybrids (Qian *et al.,* 1996 and Wang *et al.,* 1994), SSR markers are considered more reliable because of their ability to produce high-fidelity profiles as a result of their co-dominant nature and chromosome specificity (Nandakumar *et al.,* 2004).

Figure.1 Amplification pattern generated through multiplex PCR of JGT 11-16.3 and RM 19426



1. BPT 5204 **2**. BPT 3291 **3**. JGL 1798 **4**. JGL 384 **5**. MTU 2077 **6**. MTU 1001
7. MTU 1010 **8**. MTU 7029 **9.** MTU 2067 **10**. MTU 1061 **11.** MTU 1081 **12.** MTU 5249
13. Earlysamba **14**. Satya **15**.Sumati **16**. Rajavadlu **17**. Rajendra **18**.WGL 14
19. WGL 32100 **20**. RNR-C-28 **21**. RNR 23064 **22**.NLR 33359 **23**. NLR 28600 **24**. NLR 27999
25. NLR 9672-96 **26**. NLR 9674 **27**. Tellahamsa **28**. BPT 5204

Table.1 Details of twenty seven rice varieties analyzed in the present study

S No	Variety	Year of Release	Parentage	Grain type
1	Samba Mahsuri (BPT 5204)	1986	GEB 24 / T(N)1 // Mahsuri	MS
2	Sona Mahsuri (BPT 3291)	1982	Sona / Mahsuri	MS
3	Jagtial Sannalu (JGL 1798)	2002	BPT 5204 / Kavya	MS
4	Polasa Prabha (JGL 384)	2002	Kavya / BPT 5204	MS
5	Krishnaveni (MTU 2077)	1989	Sowbhagya /ARC 5984	MS
6	Vijetha (MTU 1001)	1995	MTU 5249 / MTU 7014	MS
7	Cottondora Sannalu (MTU 1010)	2000	Krishnaveni / IR 64	LS
8	Swarna (MTU 7029)	1982	Vasishtha / Mahsuri	MS
9	Chaitanya (MTU 2067)	1988	Sowbhagya /ARC 5984	MS
10	Indra (MTU 1061)	2007	PLA1100/MTU1010	MS
11	MTU 1081	Completed minikit trails	Ajaya/BPT 5204	MS
12	Vajram (MTU 5249)	1986	MTU 4569 /ARC 6650	LS
13	Early Samba (RNR M-7)	2000	Mutant of BPT 5204	MS
14	Satya (RNR 1446)	1987	Tellahamsa / Rasi	LS
15	Sumati(RNR 18833)	2002	Chandan / Pak Basmati	LS
16	Rajavadlu(RNR 99377)	1993	Rajendra / IR30	LS
17	Rajendra (RNR 12329)	1976	IJ52 / TN1	MS
18	Warangal samba (WGL 14)	2005	BPT 5204 /ARC 5984 // BPT 3291	MS
19	Warangal Sannalu (WGL 32100)	2007	Divya / BPT5204	MS
20	RNR –C-28	Completed minikit trails	IR 64 / IET 9994	LS
21	Taramati (RNR 23064)		BPT 5204 / Tellahamsa	MS
22	Shravani (NLR-33359)	2000	Selection from IR 50	LS
23	Simhapuri (NLR-28600)	1991	RP 5-32 / Bulk H9	SB
24	Tikkana (NLR-27999)	1988	RP 31-49-2 / BCP 2	SB
25	Pinakini (NLR-9672-96)	1987	Bulk H 9 / Millekkunning	MS
26	Kottamolagolukulu(NLR-9674)	1982	Bulk HG 9 / Millekkunning	SB
27	Tellahamsa (C10754)	1971	HR 12 / T(N)1	MS

MS: medium slender, LS: Long slender, SB: Short Bold

Supplementary table.1 List of SSR markers used for identification of genotype specific marker for BPT 5204

S. No	Marker	Chromosome	Forward primer sequence	Reverse primer sequence
1	RM 10936	1	ACGGTTTGGAAGTGTTCGTAGG	TGGTACTGCATAATCTCAGCATCG
2	RM 11229	1	TGACAGAAACAAAGCGGAAGG	TCCAAACCGCTATTCTTGTAGC
3	RM 3917	1	CGGACGACTCCGACAACACG	CGAACGAACGAGGACGAACG
4	RM 17600	1	CAGCATCAAGGGTCGCTACACG	CGATGGCGTGGGATAATTACGG
5	RM 11278	1	ACTTCTTGTAGCACTGCACCTTCG	CCTCGGCAACTGCTTCAAGG
6	RM 10649	1	GACCAATATGGAGTGGTCTCTTGG	ACGCTCCGCCTGCTATTTAGG
7	RM 8126	1	TGGGCCTCTTTGTTTCATACTCC	TCCTCATCTCTCTCCGTGTCTCC
8	RM 11865	1	CAACTCATTCGGCTTCTCTTTCC	GCAATTGCATGAGTTGGAATGG
9	RM 6942	2	CGAACTCCCAATTACAGATCACG	TCATCCAAGACTTAGGTGGAGAAGC
10	RM 13155	2	TACCAACAGGGAGTTGTCTCTCG	AGCGACGGTGTAAAGAATAAGTCG
11	RM 6933	2	AATGCCTAGCACTCATCCTTGC	AGGCACCCTACGATGAAATAGTGG
12	RM 12469	2	ACTCCATCGAACCCTGTTAGAGC	GTCCATGTTTGCTTACGTGTTTGC
13	RM 13154	2	GGTACTTAGCGTGCAACTTTAACC	TAGGTAACTAGACGAAGCGATAGAGG
14	RM 13812	2	AACACTAACCGGGACTAAAGATCG	CTGAGATAACGACTACTGCTACATCG
15	RM 6938	2	CCGATTAGCGATTGATATGGAGTAGG	AGTGCACAGCCATGGAATTATGC
16	RM 14140	2	CCTCCCTCTCCAAACACATTGC	TCATCAGCAGACAGAATGTTGACC
17	RM 15679	3	TAGATGTATGAGTCGGAATGGAGTCG	CAGACGCAGTGTGTGTATGAAGTTCC
18	RM 15630	3	AACTCGAAGGATCTCGCCCAACC	ACCCACCTCCTCACGCTGTACG
19	RM 14250	3	GATTACTGCCGATTCGATAGC	AAATGGGACATGTTCTCTCG
20	RM 14931	3	GCTTCACGCACATATTGCCTTCC	ATCCTCCCACTCCCAAATTATCTCC
21	RM 15331	3	GGTTCGGTGCTTTCTCTTTCAGC	AGCGATGCCGTCCTTACACC
22	RM 5928	3	CTTACCTTCTGAAATGGAGGTAGC	CAAGGTGAAAGACGAAGAATGC
23	RM 15831	3	AACACAATTCACCGGTCCTTAGC	ACGTGGGTGATCGTGTCTGC
24	RM 15727	3	ATCTGTGCGGACCACCATGC	CGCTACGAGCAGCGTACTTAAAGC
25	RM 15466	3	CGGAGTGATCATCAGAGTCG	GTAGAATCGCATGTAGAGTCTTAGGG
26	RM 16153	3	TGGTTGTGGTATAGCACGGTAAGC	TGACCCAAGGAGATACTAGGTTGC
27	RM 15288	3	GAGAGGCTCCTACTGCGGTTGC	CCTTTCCATCTCCTTGTTTCTCTGC
28	RM 6759	3	CCCGAGTCTTCATAGAGATATTCC	ATCCCTAGCTAGCCTTCCTTCC
29	RM 14582	3	ATAACCAAGATCTCGGCCCATCC	CCCATGTCACCGACAACTATCTAGC
30	RM 14320	3	CACCTGTAAATTAGGACACTGG	CAGTGTACTTTGAACTGCCTAGC
31	RM 14860	3	GGAAGGTGATTTCATCCGGTAGC	TGGCATGTTTAATGCTGGTTCG
32	RM 16606	4	TGCACTTCTTTAGAGTAGGAGGAAGC	CATGCATGTGTCCAAAGATTCG
33	RM 16592	4	CTTAGCACGGACACTCATATTTGG	CACAATACGTTTGATGGCTTGC
34	RM 16801	4	CGTTCAAGGAGCTTGTGTTGATCC	GGACCGATTTAAGTGAACGTTGATGG
35	RM 17127	4	CTCAATGTTTCCCACAGTTACCG	TGTGTTATGTGTGCGTGATGAGC
36	RM 16652	4	TGACATTAGTTGTGGCAGATCC	CCTAGAATCTCATCTGTCTTCTGG
37	RM 16649	4	CTCCCTTCATGCGTAAGCTCTCC	GCAAACAGGATCCTCCACAAAGG
38	RM 16343	4	AGAACCAGCAGTTTCTTTGC	TTCTATCCCTACAGTCTTGACAGC
39	RM 16913	4	GTGTACGTGTTGGCTCTCTGTACG	GATGTTGCTTGTGCTGCAACC
40	RM 18222	5	TGATTCCTCTATATGCAGCCTTGG	TATCGTGGTTTCATCGTGTGTGC
41	RM 5592	5	CTCGCTCTTACAACTTTCAAGC	CACTTACCTCCACTTCTCAACC
42	RM 18857	5	ACCGGTCCTTAGCTTCTGAGC	ACCAACCGGGACTAAAGATCG
43	RM 18888	5	GGTGCAAATGCTTGTAGTCCTATGC	GCACCACCAACCTAACAGTGAGC
44	RM 18065	5	CGATGGTGAGTGGTGATTCATGC	ATCATCCGCGCATTAGCATTTCC

45	RM 20060	6	CACACATGAGTGGTTAGGTAAGATGC	CAGTGACAAGAGCGAAATGATCC
46	RM 20458	6	GTGTGGTGGTCTTGGGATGTTGG	TATAGCCCAGCAAGGGTGGTACG
47	RM 20196	6	GTGGACCCACTACACAACATGG	TGTTCTACACTACGCCATTAGGC
48	RM 19697	6	AACAACCTGAGAACACCTCTTGG	GGACAAACACATGGTGATCTGC
49	RM 20096	6	CGGTAAGCCATAAATAGATCCCAAGG	TTTGAACAGCGACACGGTTTCC
50	RM 19682	6	CCCACTGATTGACCAAGAGC	TTTGCTAAAGGAGCACTACGC
51	RM 19426	6	CGGTGTCTTCTTTAAACAGC	ATGGATAAGCGGTATGTTCC
52	RM 400	6	TTACACCAGGCTACCCAAACTCG	TTGCTGAGTTCCCTCGTCTATCC
53	RM 19660	6	TTTGTCCCTGCCGTACTTGC	AGCCACGTTGGGTGAAATTAGC
54	RM 20818	7	AGATGCAGATAGATGCATGTCACG	ACCGATCATCCACGATCCTACG
55	RM 6697	7	TATTCCCGGGAGATCCAACAGC	AAGATCCAGTCGATTTGGTTCAGG
56	RM 21069	7	ATCTAGTACCGGATGTGACACG	AGACAGAGGCATGACAGAAAGG
57	RM 5720	7	GACTCGTCACTGACACTGATACG	CTTGTTAGGAAGAGCATTCTGC
58	RM 21258	7	TATCATTCCGGTCCAAAGTGTCG	TCCGGTCCAAAGTCTCATTTGC
59	RM 21564	7	CGACGGAAACACAATTCATAGG	ACCAACCGGGACTAAAGATCG
60	RM 21813	7	NA	NA
61	RM 23237	8	TAAAGCATTTGGACGGTGGATGG	GAGGTGGGTGTGACCCTTGG
62	RM 22622	8	TAGGCCGTTCTGACGTAATACCC	CAGTGATGGTGATGCGATTTAGC
63	RM 22585	8	CACCGATTATTGTCGTATGG	AGTGAGGAAGGGAAGAATACG
64	RM 5933	8	AGCGATTCAGAACGAATCAACG	TGCCAAAGCTACACAAATCTGACC
65	RM 6699	8	TGGTATCACCATTCACCAACACC	TCCTTACGCTACCACGACTTCC
66	RM 24542	9	ATCCACAAGAGCACCGATGAGG	TGACCTGGTAGTGGTGAGTGTGC
67	RM 24038	9	GTCCGGGCAACAATAACAAAGC	CACCAACCGGGACTAAAGATGG
68	RM 24780	9	GACTAGCCAGCCAAGGTTTGAGC	TGCTGCATGTGTGTATGTGACTACG
69	RM 25368	10	TATAGTTAAGGGAGCCACGCAAGC	CCACCTCGTAAGAACATGGAGAACC
70	RM25328	10	CATTTGATCTGTCCGGCTCTAGG	ATTGCTAGTGGGTGATGATGTGG
71	RM 6745	10	GAAGCCTCCTTGATGTTGACTGG	CAACGATTATGCGTCCTTAGATGC
72	RM 26329	11	TAACCGGGACTAAAGATAGAGC	CTACGTCGAAATCGTAACTAGC
73	RM 26086	11	CAGCGCTTTGTACATCTCACTCC	GCATCCTCTGCACCTATTCTTGG
74	RM 25970	11	TGTGAATCTGACTTGCCAATCG	AAGGCTGTTATGTGTCTTGCAACC
75	RM 27311	11	TTACCAACCGGGACTAAAGATCG	CAATTCATAACGTCGGTCCTTCC
76	RM 27034	11	AGGCCCTCGCGTGTACATACC	ATCCGACCCACGGTAATCTGAGG
77	RM 27101	11	CGGGCACCATGGTATCTAATGC	ATGCTCGGTCCACAGAGAATAGG
78	RM 28157	12	GCTTAATTTCTGACAGACCAGTGC	GATCTAAACACAGCCTTCCTTGG
79	RM 27840	12	TTTGCGTGCTAGGGAGATTAGC	CATTATGTACTTACTCCCTCCCTTCC
80	RM 27406	12	TGGTAGGTGTGCAATAGAAGTAGG	AATGCATGCAAACACAGTGG
81	RM 3103	12	CTGGAGTGGAGAAGAGAGAACAGG	TCTCCGCTCGGTTTCATCTAGG
82	RM 28024	12	CCGGGTGTTTGACCTAGTTTCC	CACGATGGTCGTCTTGTTACTGC
83	RM 27864	12	NA	NA
84	RM 28464	12	CTATATCCGCACCAATGGAAACC	TAGGAATTGCGACGAGTTTGTGG
85	RM 27861	12	TGCTAGCATGCTCATCCACTTGC	AATTGCCTCTGTAATACCGCCCTTCC
86	RM 27905	12	AACACAATTCACCGGTCCTTAGC	ATCGATCTTTAGTCCCGGATTGG
87	RM 27882	12	NA	NA
88	JGT 01-16.2	1	TGTTGGCTGAATGATTGATGGATAGA	GGACCTATGTGGCACGAATTTGAA
89	JGT 01-23.1	1	CGGCGTAGGAGTATTTGTAGGAAGTT	GCGGCAATGATGATGGTGGA
90	JGT 01-29.9	1	TCGATACTTTCGGAAATGGGAAGG	GCTAGCTCTAACATCCAAGCTTCC
91	JGT 02 -35.2	2	CGGGGCGAATCGAGGTCAG	TCGCGGTGGATTAGATGCCTC
92	JGT 02-9.8	2	GAGCGTTTTAAGGAAGCCACATG	CACCGAGTCCCAAGCTGATGA

93	JGT 03- 0.01	3	CCGATGCACCAACACCCTCAC	GACGCATGGTGAGTTTGGAGTGAT
94	JGT 03 -36.1	3	GCGGCAACACGACCAGCTT	CATCCTGAGTTTTGAGAAGCACCATA
95	JGT 03- 29.2	3	CCTGTAACCTGAACGGAGAGAGTA	TGAAAAAAAAAAGTCTCCCAATGTC
96	JGT 04 -11.5	4	AGGGCTCGTAGAAAGTGTCCAATTAG	CCGCTGGGTATTGCCTAACTG
97	JGT 04- 28.5	4	TTGAATAGATCGCACCGTTGAC	AAAAGGGCAGCTACGTCTGAGC
98	JGT 04 -34.9	4	ACGAACTCTAGTCCCTCCGCTC	ACGACATTATCCTAACACCACG
99	JGT 05 -05	5	TGTAGGAAGACATGTGGCAGCT	TACAACTAGATGCAATGGCACC
100	JGT 05-7.8	5	ACGGTGAGATTAGGTATTGCCA	AGTTCGGACAAGGGGGAGTACT
101	JGT 06-18.1	6	GAAGAACGTGGTTTGCATCGT	TGTACCGTCGGCGAAGAACGCACC
102	JGT 07 22.7	7	AAGGGGTTATTGATTTAGTCCATTT	AGGGGTTGAACTTTGATGCTAATATG
103	JGT 07-22.8	7	TGGCGATCTAGGAGCGTCTGT	TGTAAACATTTCAAAAGGGCACTAA
104	JGT 08-19.5	8	TTCTGAAAAAGCTCTGACCAAGC	ACTAGCTACATGCTGCAGTGCAT
105	JGT 08-10.2	8	GTACTAAACAGATGGAGCAGTACG	GTGGTACTGCTAATTAGGGGGTA
106	JGT 08-16.7	8	GAAAGAATTTGTTCCCACGACCTC	GCTACAATTGCATGCTGGATGTCG
107	JGT 09-4.8	9	CGGGATACTAACAGCAAGCAAAT	TCTCGCACCACTAACAGGGAACA
108	JGT 10-0.3	10	TGGCGATTTAGGAGCATTTGTAGAA	GCCAACGACCACTCTCACCCTAC
109	JGT 10-4.3	10	TGGCGACTTAGGAGCGTTTGTAG	GCGGCACTTCCCCAAACAA
110	JGT 11-16.3	11	GGCGGCGTATTAGCGTTGTA	AGGTTCTAGCCCATGTTAAATCTTCT
111	JGT 12-20.2	12	CTTACATGGATATGCACATCGAGC	CAAGGGAGCACACGAACAACAG
112	JGT 12-7.7	12	GAGCGTTTTGAGGAAGTTTAATGGAC	GGCACCGTTTGATTTAGATTATTACC
113	JGT 12-25.9	12	GGCGGCATAGGAGTGTTTGTAG	CGCGGTGGTCGGGATGAG

In the present study, DNA from 27 rice genotypes including Samba Mahsuri and other popular fine-grain type rice varieties developed by ANGRAU, Hyderabad, India were subjected to SSR marker analysis for identification of genotype specific markers/marker combinations for Samba Mahsuri. We have identified genotype specific SSR markers for Samba Mahsuri which can distinguish this variety from other medium-grain type slender grain types. The results obtained in the study are presented and discussed below.

Of the 113 SSR markers utilized in the present study, 26 displayed polymorphism among the 27 rice genotypes analyzed and amplified a total of 74 alleles with an average of 2.84 alleles per marker. The number of alleles detected by the polymorphic SSR markers varied from 2 to 5. These include five alleles with one marker (RM16649), four alleles each with 2 markers (RM12469 and JGT07-22.8), three alleles each with 15 markers (RM14250, RM26329, RM15630, RM6745, RM400, RM 10936, RM16153, RM16592, RM23237, RM14140, JGT01-16.2, JGT03-29.2, JGT 12-20.2, JGT11-16.3, JGT04-11.5) and two alleles each with 8 markers (RM24542, RM27840, RM19426, RM11229, JGT03-36.1, JGT02-9.8, JGT03-0.01 and JGT10-4.3).

The SSR marker JGT11-16.3, targeting a $(GATA)_n$ motif, clearly distinguished Samba Mahsuri from other medium slender varieties except MTU 2077, MTU 2067, Early samba, and WGL 14 by amplifying a 139 bp fragment, whereas another SSR marker RM19426 clearly distinguished Samba Mahsuri from the above mentioned four medium slender rice varieties by amplifying a 272 bp fragment. When these two markers were pre-PCR multiplexed, they could clearly distinguish Samba Mahsuri from all the other rice varieties and hence the fragments amplified by both these markers (i.e. in combination) could be considered as a molecular ID for Samba Mahsuri (Figure 1). This can be considered the first step towards

DNA fingerprinting of elite, quality rice varieties like Samba Mahsuri and thus protect the IPR of the institutions developing such unique and well accepted varieties. Followed (Sundaram *et al.,* 2008) a similar multiplexing approach for checking the purity of hybrid rice parental lines. Based on the results of the present study, it can be concluded that SSR markers can be efficiently used to generate locus specific allelic information which can serve as molecular IDs for elite rice varieties like Samba Mahsuri

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