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Development of Molecular IDS for the Elite Indian Rice Varieties and Marker-Based Assessment of Seed Purity

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A B S T R A C T

The study intended to characterize fifteen elite Indian rice varieties with the help of molecular markers, to develop molecular ID and to analyze the genetic purity of four selected varieties *viz.*, MTU 1010, MTU 7029, BPT 5204 and Improved Samba Mashuri in different seed lots through Grow-Out Test (GOT) and molecular marker-based assays. The results of both assays were also compared. A total of 48 SSR markers distributed across the twelve rice chromosomes were employed producing 69 alleles which amplified among the fifteen rice genotypes. The Polymorphic Information Content (PIC) value ranged from 0.03 to 0.64. The primers RM24888 with an allele size of 200 bp uniquely identified the variety MTU 1010 from others. The marker combination of RM206 and ESSR12-20.2 with an allele size of 450 bp and 170 bp respectively, identified the variety MTU 7029 uniquely. The marker combination of RM10001 and RM206 with allele size of 480 bp and 450 bp respectively, identified the variety BPT 5204 uniquely. The marker combination of JGT06-6.81 and RM22266 with allele size of 450 bp and 200 bp respectively, identified the variety Improved Samba Mashuri uniquely. Thus, these marker or marker combination served as molecular IDs. On assessing foundation and certified seed lots (truthfully labeled in case of Improved Samba Mashuri) for genetic purity through GOT and molecular markers, impurity percentage detected through molecular markers was 0.75 to 2.75%. Also, there was a marked decrease in the genetic purity in subsequent classes of seed *viz.*, foundation, certified and truthfully labeled seeds which can be assessed rapidly and efficiently by molecular marker-based assays. The results suggested that molecular markers can be used complementary to GOT assays.

Introduction

Rice (*Oryza sativa* L.) is the most important staple food of more than 3.5 billion human populace and serves as a source of livelihood to one fifth of it. More than 90 % of the world's rice is grown and consumed in Asia, where 60 % of the earth's population live and two third of the world's poor thrive. Ranking first in area (44 m ha), India is the second largest rice producing country in the world next to China (IRRI Rice Almanac, 2014) with an annual production of 103.36 million tonnes from an area of 439.49 lakh hectare (Directorate of Economics and Statistics, Ministry of Agriculture, 2014-15). Since India is a signatory to the World Trade Organization (WTO) and 'Protection of Plant

Varieties and Farmer's Rights (PPV & FR) Act, 2001' came into existence, the varietal identification, characterization and testing based on Distinctiveness, Uniformity and Stability (DUS) became more significant and mandatory (Shoba Rani *et al.,* 2004). The breeding efforts in rice have been thoroughly exploited giving rise to the release of more than 1000 HYVs including hybrids to suit varied climatic conditions across the country, but there has been a lack of compilation of key diagnostic characters of the varieties, which is essential to carry out scientific seed production and certification, endorse proper quality control and to promote the seed trade. It has been estimated that even 1% impurity in seed lot may decrease the potential yield of varieties and hybrids by about 100kg/ha (Mao *et al.,* 1996). Traditionally, the seed certification has been based on GOTs based on morphological and floral characters. But they are time consuming, space demanding and do not allow the unequivocal identification of genotypes. Sometimes, a new cultivar may also arise naturally from hybridization between members of elite but genetically close varietal groups, due to which the amount of genetic variability among the newly developed cultivars is likely to become even smaller. With the advent of molecular techniques, it is easier to characterize varieties rapidly and with greater accuracy (Sundaram *et al.,* 2008). The use of DNA markers to obtain a genotype specific profile has distinct advantages over morphological and biochemical markers as they cover a whole genome, offer more polymorphism and are not influenced by the environmental fluctuations and developmental stages. Simple Sequence Repeats (SSRs) are well known for high level of polymorphism and versatility and are preferred due to their reproducibility and amenability to automation (Mc Couch *et al.,* 2002). In rice, SSRs are abundant and well distributed throughout the genome (Mc Couch *et al.,* 1997). Recently, the emphasis in crop genetics has been shifting towards a special class of SSRs, called Expressed Sequence Tag derived SSRs (EST-SSRs) which belong to the transcribed region of the genome and are expected to be more conserved showing higher rate of transferability across species as compared to genomic SSRs. In view of the above, the present investigation was undertaken to develop molecular IDs and assessment of genetic purity of elite Indian rice varieties and to compare the results of GOT assay and molecular marker-based assay.

Materials and Methods

Rice genotypes

A total of fifteen rice genotypes were included in the investigation. Selected four elite rice varieties *viz.,* MTU 1010, MTU 7029, BPT 5204 and Improved Samba Mashuri were taken for purity assessment through both Grow-Out Test (GOT) and molecular marker-based assay. Breeder seeds were grown in germination chamber under controlled condition at ICAR-Indian Institute of Rice Research. Seedling samples were collected from 15-20 days old seedlings for the extraction of genomic DNA and further molecular characterization

DNA isolation and PCR analysis for identification of genotype specific SSR markers

For varietal fingerprinting, total genomic DNA was extracted from freshly germinated young seedlings using maxi prep protocol described by Rajendrakumar *et al.,* (2007).The DNA was quantified using Agarose Gel Electrophoresis with standard sample (0.8% agarose). To detect polymorphism among the fifteen rice genotypes a total of 48 SSR markers, distributed across the rice genome were used

for PCR amplification. In the total set of markers, two types of SSRs were utilized *viz.*, 34 EST-SSRs (Expressed Sequence Tag derived-SSRs) and 14 genomic SSRs (SSRs present in non-genic regions). Out of 48 SSRs, 30 were observed to be polymorphic (20 EST-SSRs and 10 genomic SSRs) and 18 to be monomorphic. DNA samples were amplified in 10µl reaction volumes containing 1X PCR buffer [10 Mm Tris-HCL (pH 8.3), 50mM KCL, 1.5Mm $MgCl_2$, 0.01%(v/v) gelatin] (Banglore Genei, India), 0.2 mM of each dNTPs (Banglore Genei, India), 10 pmol of each primer and 1U of Taq polymerase (Banglore Genei, India).The amplified products were resolved in 4% agarose gels (Lonza, USA), stained with ethidium bromide and visualized under UV radiation in a documentation system (Alpha infotech, USA). The sizes of the amplified fragments were estimated with the help of the Alphaease software utility of the gel documentation system using 50 and 100bp DNA ladders (MBI Fermentas, Lithuania) as the molecular size standards. The polymorphism information content (PIC) for each SSR marker was calculated according to the formula PIC= 1- $\Sigma Pi^2 - \Sigma\Sigma Pi^2Pj^2$ where 'i' is the total number of alleles detected for SSR marker and 'Pi' is the frequency of the i^{th} allele in the set of fifteen rice genotypes investigated and j = i + 1(Botstein *et al.*, 1980). If a certain allele with respect to a particular SSR marker was observed uniquely in just one type of genotype under study and absent in rest of the genotypes, it was considered to be specific for that genotype and such SSR marker was considered as genotype specific marker. In the cases where a single marker could not be identified, a unique marker combination was recognized.

Validation of SSR markers for genetic purity assessment through Grow-Out Test

The genetic purity of cultivars was assessed by using morphological traits in rice. The four elite rice varieties namely MTU 1010, MTU 7029, BPT 5204 and Improved Samba Mashuri, were analyzed for Grow Out-Test as per the National Test Guidelines, DUS in order to identify off types in the seed-lots (Shoba Rani *et al.*, 2004). The experimental material was planted in Randomized Block Design (RBD) and one seedling per hill was transplanted. Four hundred plants of each variety in two distinct classes of seed *viz.*, Foundation and Certified seed (Truthfully labeled in case of Improved Samba Mashuri) were examined throughout their growing season. The plants which deviated morphologically from the authentic breeder seed sample grown in adjacent plot were considered as impure plants or off types. Based on the number of off-types and total number of plants studied, the genetic purity percentage was calculated.

Genetic purity assessment through EST-SSR markers

For assessment of genetic purity, leaf samples (5-6 cm) from young plants of 400 individual plants of each of the four selected elite rice varieties viz., MTU 1010, MTU 7029, BPT 5204 and Improved Samba Mashuri, were collected from the experimental field plot of Directorate of Rice Research, Rajendranagar, Hyderabad. DNA was isolated on individual plant basis, using CTAB mini prep DNA isolation protocol described by Rajendrakumar *et al.*, (2007). For this purpose, 2-3 cm leaf pieces from each collected leaf sample of each plant was taken separately and cut into small pieces. They were placed in the well of a spot plate. The seedling pieces were crushed after adding 350µl of extraction buffer (1M Tris (pH-8), 0.5M EDTA (pH-8), 5M NaCl, 2% CTAB and 2% PVP) using a sterilized blunt ended glass rod till it was completely homogenized. After ensuring complete grinding of leaf pieces, 350µl more of extraction buffer was added to the well containing the homogenized

leaf sample. The entire content was transferred in to a 1.5ml eppendorf tube. Equal volume of (700µl) chloroform was added; the contents were mixed well by inversion for about 5 minutes and centrifuged at 13000 rpm for 15 minutes at room temperature. The supernatant was transferred into a fresh centrifuge tube without disturbing the intermediate layer of insoluble proteins. Equal volume of chilled isoproponol was added to the supernatant and kept undisturbed for 10 minutes (Isoproponol helps in the precipitation of DNA). The DNA appears as white sediment. (For better precipitation tubes were kept at – 20°C over night). The contents were mixed gently and centrifuged at 13000 rpm for 10 minutes at room temperature. The supernatant was discarded and the DNA pellet was washed with 200µl of 70% ethanol by tapping gently so that the pellet is disturbed. The contents were centrifuged at 13000 rpm for 5 minutes at room temperature. **(**Ethanol helps in removal of other salts left over).The supernatant was discarded and the DNA pellet was air-dried at room temperature for 2-3 hours. The DNA pellet was dissolved in 50-100µl of TE buffer (10 m MTris-HCl (pH-8.0) and 1mM EDTA (pH 8.0) prepared using sterile double distilled water. 2-3µl of DNA was used per Polymerase Chain Reaction (PCR).

Results and Discussion

The establishment of cultivar identity and genetic purity assessment are the pre-requisites for protection of farmers and breeder's rights and ensuring seed quality. Genetic purity has been assessed by Grow-Out Test (GOT), which have technical, spatial and time limitations. DNA fingerprinting methods based on Polymerase Chain Reaction (PCR) have become methods of choice for germplasm characterization, cultivar identification and genetic purity assessment (Sundaram *et al.,* 2008). Simple Sequence Repeats (SSRs), a special class of DNA markers are more reliable among the markers available because of their ability to produce high-fidelity profiles as a result of their co-dominant nature and chromosome specificity (Nandkumar *et al.,* 2004).In the present study the results of GOT assay and molecular marker-based assay were compared and the utility of molecular markers in the assessment of purity in seed lots has been demonstrated.

Molecular characterization of rice varieties

Characterization with the marker RM24888 generated a maximum number of four alleles, while many other markers exhibited three polymorphic allele's *viz.,* RM10001, RM16150, JGT04-11.5, RM206, JGT06-6.9, JGT06-6.81, RMES6-1, JGT07-22.8, RM23819, and RM24888. Rest of the markers generated two alleles each.

Identification of genotype specific molecular marker and/or marker combination and development of molecular IDs

Molecular marker characterization was done with an objective of identifying genotype specific markers and/or marker combinations for the fifteen elite rice varieties. These markers and/or marker combination acted as the "Molecular IDs" (molecular identity). A list of genotype specific markers for fifteen rice varieties is furnished in Table 1 and the amplification pattern of some such markers are presented in Plates 1 and 2.

The SSR marker RM24888 with an allele size of 200 bp uniquely identified the variety MTU 1010, which was different from others. The same marker also amplified a unique allele of 400 bp size in Mahsuri. The marker combination of JGT01-16.2 and RM17148 with allele size of 180 bp and 280 bp respectively, were unique for MTU 1001. The

marker combination of RM206 and ESSR12-20.2 with an allele size of 450 bp and 170 bp respectively, identified the variety MTU 7029 uniquely. Similarly, the marker combination of RM206 and JGT06-6.9 with an allele size of 400 bp and 550 bp was observed to be unique to the variety MTU 1075. The marker combination of JGT07-22.8 and RMES12-1 with an allele size of 180 bp and 320 bp was observed to be unique to the variety NLR 30491. The marker combination of JGT01-16.2 and JGT06-6.9 with an allele size 170 bp and 350 bp was observed to be unique to the variety NLR 33892. The variety NLR 145 was uniquely identified by the marker combination of RMES2-1 and RM16150 with an allele size of 250 bp and 450 bp respectively.

The variety NLR 34449 was uniquely identified by the marker combination of JGT06-6.9 and JGT07-22.8 with allele size of 550 bp and 300 bp respectively. The marker combination of RMES8-1 and RMES12-1 with an allele size of 260 bp and 320 bp respectively, uniquely identified the variety JGL 384. The marker combination of JGL04-11.5 and RMES9-2 with an allele size of 190 bp and 300 bp respectively, identified the variety JGL 11470 uniquely amongst other varieties. The marker combination of RM10001 and RM206 with allele size of 480 bp and 450 bp respectively, identified the variety BPT 5204 uniquely amongst other varieties. The marker combination of JGT01-16.2 and RM24888 with allele size of 180 bp and 400 bp respectively, identified the variety Mashuri uniquely amongst other varieties. The marker combination of RM206 and JGT06-6.9 with allele size of 480 bp and 400 bp respectively identified the variety Tella Hamsa uniquely. The marker combination of JGT01-16.2 and RMES6-1 with allele size of 180 bp and 130 bp respectively, uniquely identified the variety Pusa Basmati-1.

The marker combination of JGT06-6.81 and RM22266 with allele size of 450 bp and 200 bp respectively, identified the variety Improved Samba Mashuri uniquely amongst other varieties. Thus, through the present study, unique markers and/or marker combinations were identified for all the fifteen elite rice varieties. The objective of developing a unique molecular ID is fulfilled only when they are capable of detecting even minor impurities and variation in seed lots which cannot be detected by morphological characterization. Keeping this in view, the main focus was on developing molecular IDs for four elite rice varieties *viz.*, MTU 1010, MTU 7029, BPT 5204 and Improved Samba Mahsuri for analysis through GOT and molecular marker assay.

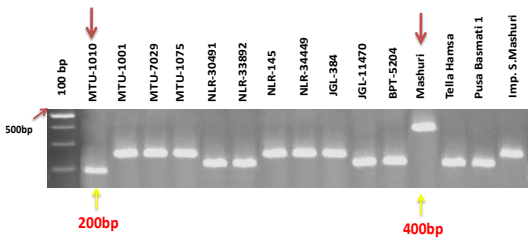

Plate 1: Amplification pattern of the SSR marker RM24888 among the elite rice varieties analyzed. MTU 1010 and Mashuri amplified a unique fragment of size 200 bp and 400 bp respectively (indicated by an arrow).

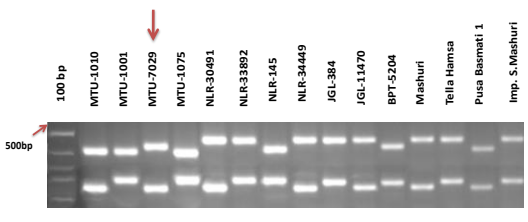

Plate 2: Amplification pattern of the SSR marker combination of RM206 and ESSR12-20.2 amplified a unique fragment of size 450 bp and 170 bp respectively, creating a molecular ID for MTU 7029 (indicated by an arrow).

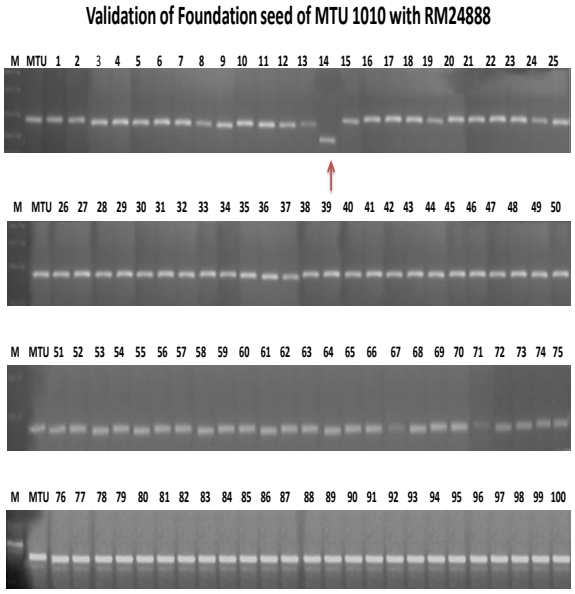

Plate 3: Purity analysis of MTU 1010 (F/S) with RM24888 with allele size of 200 bp (impurities indicated with an arrow). Serial number 1-100 are plants 1-100 from left to right

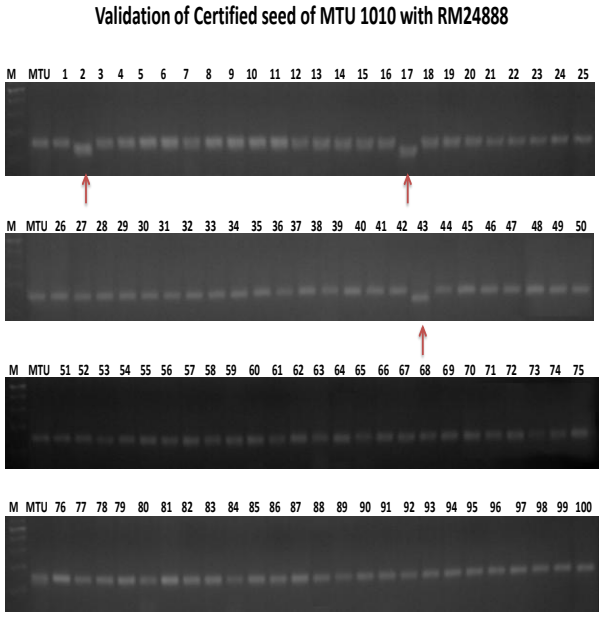

Plate 4: Purity analysis of MTU 1010 (C/S) with RM24888 with allele size of 200 bp (impurities indicated with an arrow). Serial number 1-100 are plants 1-100 from left to right

Graph.1 Comparison of GOT assay and molecular marker-based assay in different seed lots of four elite rice varieties

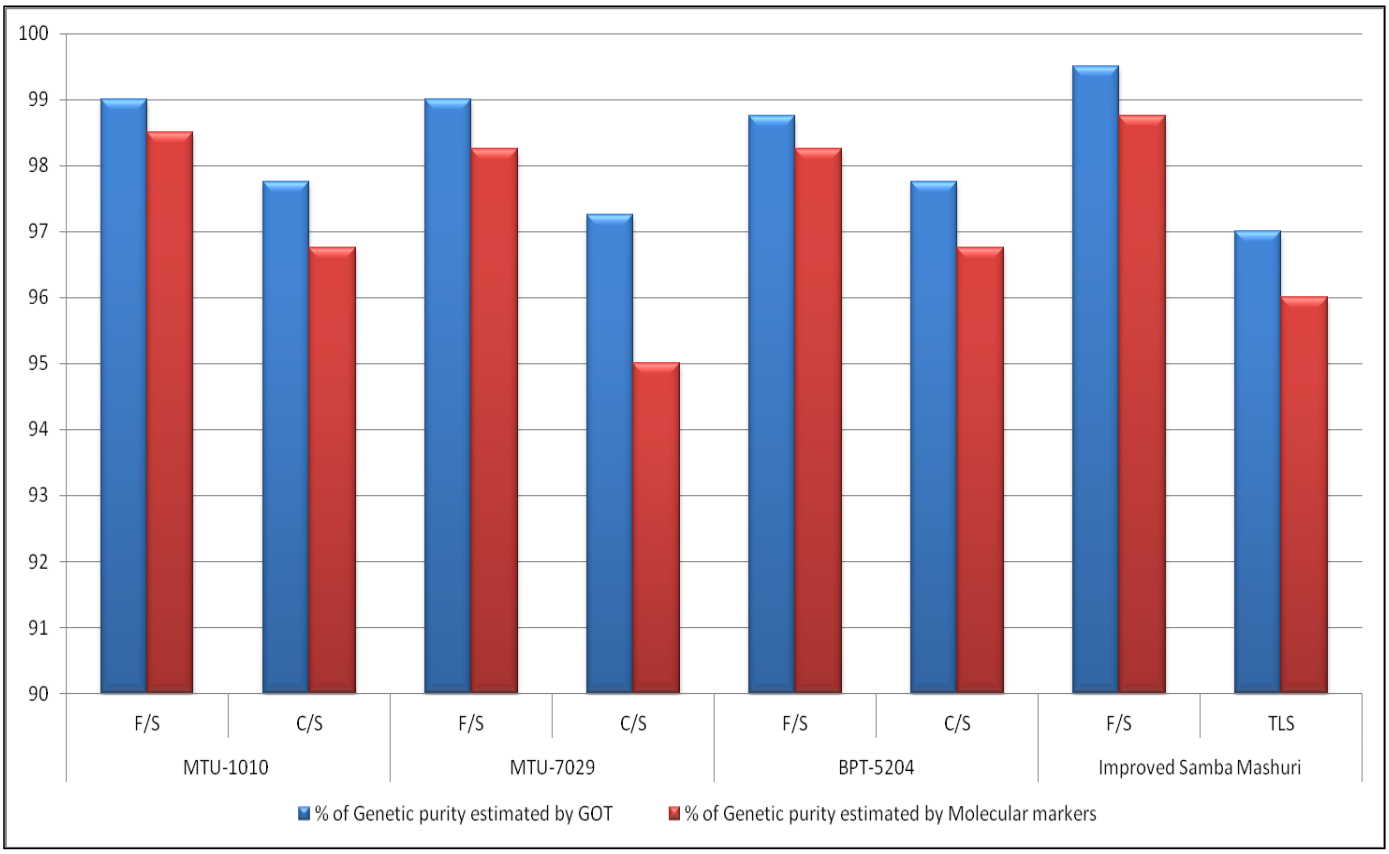

Table.1 List of genotype specific markers for fifteen elite rice varieties

S. No.	Rice variety	Marker(s) and/or marker combinations specific for the genotype and the allele size amplified (given in parenthesis in bp)
1	MTU 1010	RM24888 (200bp)**
2	MTU 1001	JGT01-16.2 (180bp) + RM17148 (280bp)
		RMES2-1 (280bp) + ESSR6-7.1 (170bp)
		RM13584 (200bp) + ESSR6-7.5 (150bp)
		RM16150 (450bp) + RMES11-2 (100bp)
3	MTU 7029	RM10001 (450bp) + RMES2-1 (250bp)
		JGT01-16.2 (180bp) + RM17632 (300bp)
		JGT04-11.5 (130bp) + RM206 (450bp)
		JGT04-28.5 (450bp) + RM17632(300bp)
		RM206 (450bp) + RMES9-2(300bp)
		RM206 (450bp) + ESSR12-20.2 (170bp)**
		RMES12-1 (300bp) + RM17632 (300bp)
4	MTU 1075	RM16150 (400bp) + RM206 (400bp)
		RM17632 (250bp) + RM17148 (300bp)
		RM206 (400bp) + JGT06-6.9(550bp)
		JGT06-18.1 (450bp) + JGT06-6.9 (550bp)
5	NLR 30491	RM13584 (250bp) + RMES12-1 (320bp)
		RMES6-1 (200bp) + RM22266 (200bp)
		JGT07-18.6 (300bp) + RMES8-1 (280bp)
		JGT07-22.8 (180bp) + RMES12-1 (320bp)
6	NLR 33892	JGT04-11.5 (450bp) + JGT01-16.2 (170bp)
		JGT01-16.2 (170bp) + ESSR06-17.1 (110bp)
		JGT01-16.2 (170bp) + JGT06-6.9 (350bp)
		JGT01-16.2 (170bp) + JGT07-18.6 (320bp)
7	NLR 145	RMES2-1 (250bp) + RM16150 (450bp)
		RMES2-1 (250bp) + JGT04-28.5 (400bp)
		RMES2-1 (200bp) + RM206 (400bp)
		RMES2-1 (200bp) + JGT06-18.1 (450bp)
		RM206 (400bp) + JGT07-18.6 (300bp)
		RM206 (400bp) + RMES9-2 (300bp)
8	NLR 34449	JGT01-16.2 (180bp) + JGT07-22.8 (300bp)
		RM13584 (200bp) + JGT07-22.8 (300bp)
		JGT04-11.5 (150bp) + RMES6-1 (150bp)
		JGT04-11.5 (150bp) +JGT07-22.8 (300bp)
		JGT06-6.9 (550bp) + RMES8-1 (260bp)
		JGT06-6.9 (550bp) + JGT07-22.8(300bp)
9	JGL 384	RMES8-1 (260bp) + RMES12-1 (320bp)

10	JGL 11470	RM10001 (450bp) + JGT04-11.5 (200bp)
		JGT04-11.5 (200bp) + JGT06-6.9 (550bp)
		JGT04-11.5 (200bp) + RMES9-2 (300bp)
		JGT04-11.5 (200bp) + ESSR12-20.2 (200bp)
		JGT06-6.9 (550bp) + JGT07-22.8 (480bp)
11	BPT 5204	RM10001 (480bp) + RM13584 (250bp)
		RM10001 (480bp) + RM206 (450bp)
		JGT01-16.2 (180bp) RM13584 (250bp)
		RM13584 (250bp) + RM16150 (450bp)
		RMES2-1 (280bp) + RM13584 (250bp)
12	Mashuri	RM10001 (450bp) + JGT07-22.8 (180bp)
		JGT01-16.2 (180bp) + RM24888 (400bp)
13	Tella Hamsa	JGT04-11.5 (190bp)+ JGT04-28.5(400bp)
		RMES2-1 (280bp)+ JGT07-22.8(300bp)
		JGT04-11.5 (190bp) + ESSR06-17.1(150bp)
		RM206 (480bp) + ESSR06-17.1(150bp)
		RM206(480bp) + JGT06-6.9 (400bp)
14	Pusa Basmati 1	JGT01-16.2 (480bp) + RMES6-1 (200bp)
		RMES2-1 (280bp) + RMES6-1 (200bp)
15	Improved Samba Mashuri	JGT06-6.9 (550bp) + JGT06-6.81 (450bp)
		JGT06-6.81 (450bp) + JGT07-22.8 (480bp)
		JGT06-6.81 (450bp) + RM22266 (200bp)
		RMES6-1 (150bp) + RM24888 (230bp)
		RMES9-2 (350bp) + RM24888 (230bp)
		RMES9-2 (350bp) + ESSR12-20.2 (200bp)

Note: SSR marker or marker combination with ** are presented in Plate 1 and 2

Table.2 List of genotype specific markers or marker combinations for four varieties used for Genetic purity assessment

S. No	Variety	Allele size of specific marker/ marker combination	Allele Size (bp)
1	MTU 1010	RM24888	200
2	MTU 7029	RM206	450
		ESSR12-20.2	170
3	BPT 5204	RM10001	480
		RM206	450
4	Improved Samba Mashuri	JGT06-6.81	450
		RM22266	200

Table 3: List of offtypes identified in the foundation and certified seed lots through molecular marker analysis

Variety and its unique molecular ID	Class of Seed	Plant numbers identified to be offtypes in GOT seed-lot	Marker allele amplified by the offtype		Variety and its unique molecular ID	Class of Seed	Plant numbers identified to be offtypes in GOT seed-lot	Marker allele amplified by the offtype	
			RM24888	NA				RM10001	RM206
MTU 1010 (RM24888 with an allele size of 200bp)	F/S (4 offtypes)	14	250		BPT 5204 (RM10001 with an allele size of 480bp and RM206 with an allele size of 450bp)	F/S (5 offtypes)	76	520	500
		289	250				199	500	320
		304	300				277	520	200
		322	250				388	520	500
	C/S (9 offtypes)	2	220				390	500	300
		17	220			C/S (9 offtypes)	31	520	550
		43	220				56	520	550
		139	300				57	520	550
		274	250				93	520	550
		346	380				101	500	200
		358	220				150	480	400
		385	220				254	500	150
		397	350				256	520	200
			RM206	ESSR12-20.2			352	480	150
MTU 7029 (RM206 with an allele size of 450bp and ESSR12-20.2 with an allele size of 170bp)	F/S(4 offtypes)	42	430	200	Improved Samba Mashuri (JGT06-6.81 with an allele size of 450bp and RM22266 with an allele size of 200bp)			JGT06-6.81	RM22266
		111	480	150		F/S (2 offtypes)	9	510	250
		208	400	200			91	510	250
		399	250	150		TLS (12 offtypes)	6	510	250
	C/S (11 offtypes)	4	430	200			10	510	250
		6	430	200			34	510	250
		33	430	200			43	510	250
		80	430	200			44	510	250
		101	400	190			45	510	250
		108	480	200			103	510	280
		124	400	190			192	500	250
		148	430	230			207	500	280
		244	250	230			270	500	300
		261	350	200			340	510	280
		393	350	190			368	510	250

GOT and molecular marker analysis of seed lots

During the GOT analysis of four hundred plants from each seed-lot, in the field plot of MTU 1010 foundation seed, a total of four off types were observed, while in the field plot of certified seed nine off types were recorded. In the field plot of MTU 7029 foundation and certified seed, a total of four and eleven off types were recorded respectively. Similarly, five and nine off types were observed respectively in the field plots of foundation and certified seed of variety BPT 5204. In the field plot of Improved Samba Mashuri foundation and truthfully labelled seed a total of two and twelve off types were recorded as represented in Table 3.

After the molecular marker assessment of genetic purity of foundation and certified seed lots of MTU 1010, many samples showed molecular weight deviating from the molecular weight of normal plants of the variety. They were considered as off types or contaminants. The base pair size of marker RM24888 observed to be unique to the variety MTU 1010 was 200 bp. An impure plant number 14 (well no 14 on gel image of Plate 3) was identified with an allele size of 250 bp in the foundation seed lot. While, for the certified seed lot of the same variety showed three contaminant plant numbers 2, 17 and 43 with an allele size of 220 bp (Plate 4). For the assessment of genetic purity of foundation and certified seed-lots of MTU 7029, the unique marker combination RM206 and ESSR12-20.2 with an allele size of 450 bp and 170 bp respectively, was utilized and any deviation from this fragment size was considered as off type or contaminant. The impure plant number 42 to amplified at an allele size of 430 bp in the foundation seed lot when subjected to RM206 and 200 bp when subjected to ESSR12-20.2. Impure plant numbers 4, 6, 33, and 80 were observed with allele size of 430 bp when subjected to RM206 and 200 bp when subjected to ESSR12-20.2 in the certified seed-lot. The genetic purity of foundation and certified seed-lots of BPT 5204 was assessed with the unique marker combination RM10001 and RM206 having allele size of 480 bp and 450 bp respectively was utilized. The impure plant number 76, which amplified an allele size of 520 bp were observed with respect to the marker RM10001 and 500 bp for RM206 in the foundation seed-lot. Impure plant numbers 31, 56, 57 and 93 were found with an allele size of 520 bp when subjected to RM10001 and 550 bp when subjected to RM206 in the certified seed-lot. For the variety Improved Samba Mashuri, the genetic purity of foundation and truthfully labelled seed-lots was assessed with the unique marker combination JGT06-6.81 and RM22266 with an allele size of 450 bp and 200 bp respectively. The impure plant numbers 9 and 91 amplifying an allele size of 510 bp were found with respect to JGT06-6.81 and 250 bp for RM2226 in the foundation seed-lot. The plant numbers 6, 10, 34, 43, 44 and 45 were identified as contaminants with an allele size of 510 bp analyzed when subjected to JGT06-6.81 and 250 bp with RM22266 in the truthfully labelled seed-lot.

Comparison of results of GOT and molecular marker-based assay

When the results of GOT and molecular marker assays were compared, the following points were observed.

The foundation seed-lot of MTU 1010 showed 99% genetic purity through morphological assay recorded over one season, while through molecular marker assay, the value was 98.5%. The difference between morphological assay and molecular marker assay was calculated as 0.5%. Similarly, the certified seed lot of MTU 1010

showed 97.75% genetic purity through morphological assay recorded over one season, while 96.75% genetic purity was recorded when analyzed by marker-based assay. The difference was calculated as 1%.In the variety MTU 7029, foundation seed-lot exhibited 99% genetic purity through morphological assay however, in molecular marker-based assay additional impure plants were identified with the genetic purity being 98.25%. The difference was calculated to be 0.75%. Similarly, the certified seed lot of MTU 7029 exhibited 97.25% genetic purity through morphological assay. When analyzed with markers, the genetic purity was lower at 95% and the difference between the two assays was calculated as 2.25%. The foundation seed-lot of BPT 5204 exhibited 98.75% genetic purity through morphological assay however, through molecular marker-based assay the genetic purity was lowered by 98.25%.

The difference between the two assays being 0.5%. Similarly, the certified seed lot of BPT 5204 exhibited 97.75% genetic purity through morphological assay and with respect to marker-based assay, the genetic purity was estimated to be only 96.75% and the difference of 1% was calculated between the two assays. In Improved Samba Mashuri, foundation seed-lot exhibited 99.5% genetic purity through morphological assay recorded over one season. However, it exhibited 98.75% genetic purity by molecular marker-based assay. The difference was calculated to be 0.75%. Similarly, the truthfully labeled seed lot of Improved Samba Mashuri exhibited 97% genetic purity through morphological assay while it showed 96% through molecular marker-based assay. The difference of 1% was calculated between the two assays.

The results have clearly shown that the impurity percentage detected in molecular marker analysis was in the range of 0.75 to 2.25% higher as compared to that detected based upon morphological characters in GOT assay. In accordance with the investigation, Graph 1 has been worked out which is evident of the ability and accuracy of molecular markers over morphological characterization to assess genetic impurities in the seed lots. The graph clearly depicts the genetic purity in seed lots to be higher when assessed through GOT while, the genetic purity is lower when assessed through molecular markers-based assessment.

Thus, highly informative EST-SSRs and genomic-SSR molecular markers used in the present study are able to detect even those minor genetic variations and impurities which were not detected by the morphological or phenotypic characterization of GOT. These minor genetic variations cause deterioration of crop varieties. They go unnoticed in the seed multiplication chain leading to reduction in the genetic purity over subsequent classes of seed as well as within the same class in different locations. They can occur even in varieties appearing phenotypically uniform and homogenous when released.

In conclusion, comparison of GOT and molecular marker analysis clearly establishes the usefulness and efficiency of molecular markers in seed purity assessment, wherein more number of plants were identified as impure in molecular marker analysis as compared to GOT. Although neither International Seed Testing Association (ISTA) nor Indian Minimum Seed Certification Standards has approved DNA markers for ensuring genetic purity of seed lots, they can be used complementarily with GOT assays. The results of the present study unambiguously indicate that molecular markers like EST-SSRs are very robust and rapid methods in comparison with any other methods.

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