Seed Res., Vol. 41(1): 91-96 June 2013

Short Communication

Assessment of genetic purity of rice variety using microsatellite markers

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Rice is the staple food for a large segment of the Asian population. India is the second largest producer of rice in the world. The population of India is drastically increasing day by day, while cultivable land is decreasing and it will difficult to feed the ever-increasing population of India. Hence, it becomes necessary to increase production of food grains through development and release of high yielding varieties. Amongst 800, rice varieties released in India so far; variety, Karma Mahsuri is extremely popular because of high yield and quality produce. Quality seed i.e. genetically and physically pure seed is the cornerstone of achieving good agricultural production. Assessment of seed purity is one of the most important quality control aspects in seed production programme. Traditionally, it has been the practice to carry out a Grow-out test (GOT), based on morphological traits for the assessment of genetic purity of seeds. However, GOT is time consuming, require large area of land and often does not allow the unequivocal identification of genotypes. The use of DNA marker is a modern approach based on DNA polymorphism among tested genotypes and thus applicable in biological research. Simple Sequence repeat (SSR) are well known for high level of polymorphism, versatility and are preferred due to their reproducibility and amenability for automation [1]. Furthermore, DNA markers are 'neutral' and they have no effect on phenotype, no epistatic effect, and are also not influenced by environmental conditions at various developmental stages.

Therefore, these markers have been applied widely in the varietal identification, registration of plant variety and in monitoring of the seed purity due to authenticity with high accuracy, high reliability and low cost [2]. Thus, the major objective of this study to assess purity in seed lot of Karma Mahsuri by using microsatellite markers.

Rice variety released from IGKV, Raipur, Karma Mahsuri was taken for the present study. The nucleus seed of this variety was taken for developing fragment size by using different microsatellite markers.

DNA of 94 plants from certified seed lot of Karma Mahsuri and two seed from nucleus seed as a check was used for molecular studies. Based on morphological characters, true to type and off-type plants were tagged. DNA was isolated by mini prep method [3]. 1-2 g of leaf material from one individual plant was collected. Leaves were cut into small pieces and transferred to 2 ml centrifuge containing 500 µl of extraction buffer along with stainless steel beads prior to DNA isolation according to instructions. Four rice microsatellite primer pairs were used in the analysis (Table 1). Polymerase chain reaction was done in a volume of 20 µl for SSR for amplification of DNA. DNA was visualized by five percent polyacrylamide gels electrophoresis, followed by staining with ethidium bromide [4].

An attempt was made in order to validate the utility of Karma Mahsuri specific fragments of size 250bp, 195bp, 275bp and

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Received: March 2012 Revised: January 2013 Accepted: March 2013

130bp, amplified by the genotype specific microsatellite markers *viz.*, RM 19, RM 169, RM 164 and RM 206 respectively for assessment of seed purity of Karma Mahsuri. A total of 96 plants, including two of nucleus seed from National Seed Project, Raipur were used to determine the genetic purity of Karma Mahsuri based on microsatellite markers. Based on morphological markers, six contaminants were identified. When this population was subjected to molecular test by SSR markers, more off-type plants were diagnosed in addition to tagged off-type plants. Markers RM 19, RM 169, RM 164 and RM 206 showed 21, 15, 16 and 15 off-type plants, respectively. Besides, these molecular markers also diagnosed contaminants other than off-types identified on morphological basis in GOT. Hence, the application of molecular markers are helpful in distinguishing off-types in a more refined way. The development of molecular markers as non-conventional methods can overcome the problem encountered in GOT, which is, tedious and influenced by environment factors to some extent (Table 2, Fig. 1).

The variants identified on the basis of morphological characters also showed variation on molecular basis. Similar observations were also made in rice [5]. The percentage of contaminants detected based on SSR marker analysis was higher than those detected by conventional GOT assay because GOT requires a particular environment, space and time *i.e.* it is environmental dependent and molecular markers are free from these limitations [2]. Molecular markers detected some additional impurities, which were not detected during analysis of morphological characters [6]. This demonstrated the better discriminatory power and efficiency of SSR markers in genetic purity assessment and these markers could even accurately detect residual impurities in the seed. Similar results have been reported by other workers [7-8]. Based on the results obtained from this study, a simple procedure for detecting impurities has been standardized in a pure line variety, which could be used for detection of off-type in varietal seed lots. The cost effectiveness of marker based assay in testing genetic purity of rice varieties is one of the most important considerations while recommending its adoption to the seed industry as an alternative to GOT. Besides being quick and accurate, the use of a single marker would be cost effective as compared to combination of markers in testing genetic purity of seed samples.

ACKNOWLEDGMENTS

Authors would like to thank Rastriya Krishi Vizyan Yojna (RKVY), New Delhi for providing grants.

Table 1: Details of the microsatellite markers used for purity analysis of karma mahsuri

S.No.	Marker Sequence	Annealing Temperature (°C)	Location on Chromosome	Primer (5′ to 3′)
1	RM 19	55	12	CAAAAACAGAGCAGATGAC
				CTCAAGATGGACGCCAAGA
2	RM 206	55	11	CCCATGCGTTTAACTATTCT
				CGTTCCATCGATCCGTATGG
3	RM 169	55	5	TCCCGTTGCCGTTCATCCCTCC
				TGGCTGGCTCCGTGGGTAGCTG
4	RM 164	55	5	GCAGCCCTAATGCTACAATTCTTC
				TCTTGCCCGTCACTGCAGATATCC

Table 2: Genetic purity of seed lot of Karma Mahsuri

S.No.	Morphological Marker	Molecular Markers				Remarks
		RM 19	RM 164	RM 169	RM 206	
1	Nucleus seed	True-to-type	True-to-type	True-to-type	True-to-type	
2	Nucleus seed	True-to-type	True-to-type	True-to-type	True-to-type	
3	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
4	Off-type	Off-type	Off type	NA	Off-type	Identified by morphological markers
5	True-to-type	Off-type	Off-type	NA	True-to-type	Not identified by Morphological markers
6	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
7	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
8	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
9	True-to-type	True-to-type	Off-type	True-to-type	True-to-type	Not identified by Morphological markers
10	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
11	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
12	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
13	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
14	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
15	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
16	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
17	True-to-type	True-to-type	True-to-type	True-to-type	Off -type	Not identified by Morphological markers
18	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
19	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
20	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
21	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
22	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
23	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
24	True-to-type	True-to-type	True-to-type	NA	True-to-type	
25	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
26	True-to-type	True-to-type	True type	True-to-type	True-to-type	
27	True-to-type	True-to-typee	True type	True-to-type	True-to-type	
28	True-to-type	True-to-type	True type	True-to-type	True-to-type	
29	True-to-type	True-to-type	True type	True-to-type	True-to-type	
30	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
31	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
32	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
33	True-to-type	Off-type	Off-type	Off-type	Off-type	Not identified by Morphological markers
34	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
35	True-to-type	Off-type	True-to-type	Off-type	Off-type	Not identified by Morphological markers
36	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
37	True-to-type	Off-type	Off-type	Off-type	Off-type	Not identified by Morphological markers

S.No.	Morphological Marker	Molecular Markers RM 19	RM 164	RM 169	RM 206	Remarks
38	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
39	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
40	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
41	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
42	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
43	True-to-type	Off-type	Off-type	Off-type	Off-type	Not identified by Morphological markers
44	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
45	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
46	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
47	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
48	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
49	True-to-type	Off-type	Off-type	Off-type	True-to-type	Not identified by Morphological markers
50	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
51	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
52	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
53	Off-type	Off-type	Off-type	Off-type	Off-type	Identify by morphological makers
54	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
55	True-to-type	Off-type	Off-type	Off-type	Off-type	Not identified by Morphological markers
56	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
57	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
58	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
59	Off-type	Off-type	Off-type	True-to-type	Off-type	Identify by Morphological markers
60	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
61	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
62	Off-type	Off-type	Off-type	Off-type	Off-type	Identify by morphological markers
63	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
64	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
65	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
66	True-to-type	True-to-type	True-to-type	True-to-type	Off-type	Not identified by Morphological markers
67	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
68	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
69	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
70	True-to-type	Off-type	True-to-type	True-to-type	True-to-type	Not identified by Morphological markers
71	True-to-type	Off-type	Off-type	NA	Off-type	Not identified by Morphological markers
72	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
73	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	

S.No.	Morphological Marker	Molecular Markers				Remarks
		RM 19	RM 164	RM 169	RM 206	
74	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
75	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
76	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
77	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
78	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
79	True-to-type	Off-type	True-to-type	Off-type	Off-type	Not identified by Morphological markers
80	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
81	Off-type	Off-type	Off-type	Off-type	Off-type	Identify by morphological markers
82	True-to-type	Off-type	True-to-type	Off-type	True-to-type	
83	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
84	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
85	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
86	True-to-type	Off-type	Off-type	Off-type	True-to-type	Not identified by Morphological markers
87	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
88	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
89	True-to-type	Off-type	True-to-type	Off-type	True-to-type	Not identified by Morphological markers
90	Off-type	Off-type	Off-type	Off-type	True-to-type	Identify by morphological markers
91	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
92	True-to-type	Off-type	True-to-type	Off-type	Off-type	Not identified by Morphological markers
93	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
94	True-to-type	Off-type	True-to-type	Off-type	True-to-type	Not identified by Morphological markers
95	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
96	True-to-type	True-to-type	True-to-type	True-to-type	True-to-type	
Total	6	21	15	16	15	

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