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Short Communication

Fertility restorer (*Rf*) gene linked STMS marker: An efficient tool for testing the genetic purity of hybrid rice (*Oryza sativa* L.) seed

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Abstract

A fertility restorer (*Rf*) gene linked STMS marker, RM 6100 as well as Grow Out Test (GOT) were used for testing the genetic purity of hybrid seed of eight popular rice hybrids. The genetic purity ranging from 96.00% to 100.00%, could be determined through marker, whereas, it varied from 97.00% to 99.50% in GOT. The comparative assessment of both methods exhibited that molecular marker was more efficient than GOT, as it could detect 0.25-2.50% more impurities very precisely as compared to GOT.It suggests that a single *Rf* gene linked co-dominant STMS marker appeared to be a simple, precise and quick option over GOT for testing the purity of hybrid seed lots in short span of time.

Key words: STMS marker, fertility restorer gene, rice, genetic purity, hybrid

Hybrid rice technology with an average yield advantage of 15-20% over semi dwarf high yielding varieties offers an opportunity to increase rice yields. Besides timely supply of genetically pure seeds to the growers at affordable price, the genetic purity of the hybrid seeds and parental lines is of paramount importance as it directly influences the yield of the hybrid. It is estimated that for every 1% impurity in hybrid seed, the yield reduction is 100 kg/ha (Mao et al. 1996). Hence, monitoring of genetic purity at each stage of seed production programme is inevitable.Conventionally, genetic purity of hybrid seed is tested through GOT, which involves visual evaluation of set of diagnostic morphological and floral characters of plants throughout its growing period.However, the reliability of GOT is limited by the subjective nature of the entire procedure and significant genotype × environment interactions. Further, GOT requires one full growing season and thus the hybrid seeds are not available for immediate cultivation. The limitation can be effectively mitigated by employing the molecular markers as they provide an unbiased means of identifying genetic impurities in crop varieties within a short span of time.

The reliability of *Rf* gene linked STMS marker for determining the purity of hybrid seed lots has been earlier demonstrated on limited scale (Nandakumar et al. 2004; Garg et al. 2006). However, this concept needs to be validated with more number of hybrids and larger sample size. Therefore, the present investigation was undertaken to assess the genetic purity of eight popular rice hybrids commercially grown in India utilizing STMS marker, RM 6100 linked with *Rf*-4, a major locus for fertility restoration in rice hybrids based on wild abortive (WA) cytoplasm.

The F_1 seeds of eight popular rice hybrids (Table 1) comprising five of private sectors (PA 6444, PA 6129, PHB 71, US 312, and Kushboo) and three from public sectors (Pusa RH 10, DRRH- 3 and KRH- 2) along with their parental lines were obtained from the market and concerned breeder, respectively for testing the genetic purity of seed lots.

For the purpose of marker analysis, a random sample of 100 seeds from each of commercial hybrid seed lots were drawn and germinated under aseptic

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Table 1. Details of rice hybrids used in present study

Hybrids	Duration (days)	Year of release	Developed by
PA6444	135	2001	Bayer Bio-science, Hyderabad
PA6129	120	2007	Bayer Bio-science, Hyderabad
PHB71	130-135	1997	PHI Seeds Pvt. Ltd., Hyderabad
US312	128	2009	Seed Works International, Hyderabad, Andhra Pradesh
KUSHBOO	130-135	NA	Indo-American Hybrid seed co.
DRRH3	131	2009	Directorate of Rice Research, Hyderabad, Andhra Pradesh
KRH2	125-130	1996	Zonal Agricultural Research Station, VC farm, Mandya, University of Agricultural Sciences, Bengaluru, Karnataka
PRH10	110-115	2001	Indian Agricultural Research Institute, New Delhi

condition in the laboratory. The plant DNA was isolated from the leaves of individual seedling following the procedure of Doyle and Doyle (1990). The microsatellite marker, RM 6100 located on chromosome 10 and closely linked at a distance of ~1.2 cM with fertility restorer gene *Rf*-4,a major fertility restoration locus in WA cytoplasm was selected for genetic purity analysis following normal procedures.

The F_1 seeds of the eight hybrids were grown in the nursery and twenty-five days old seedlings were transplanted to the plots of size 6 × 6 m with spacing of 30 cm between rows and 25 cm between plants (Verma, 1996). A sample size of 400 F_1 individuals of each hybrid were selected for observation on some morphological and phenological characters *viz*., pollen sterility, pigmentation on leaf sheath, colour of lemma and awns, presence/absence of awns on the spikelet, panicle exertion, panicle length, nodal pigmentation, flag leaf senescence and grain characteristic, besides plant height and days to maturity. All the plants were visually examined throughout the growing period with special emphasis during the period from flowering to ripening and the plants showing deviation in morphological character were tagged and examined carefully. Such plants were identified as off-type/ impure plants. The number of off-type/ impure plants and the genetic purity (in %) identified in seed lots are presented in Table 2.

The STMS marker, RM 6100 amplified a maximum of two alleles in each hybrid specific to CMS and restorer lines and found to be polymorphic between male and female parental lines. The analysis of PRH 10, PA 6444, PHB 71,KRH 2revealed 2/100 off-types seeds (98 % genetic purity) (Figs. 1 and 2) whereas, rest of the F_1 hybrid seeds were heterozygous and thus showing the presence of both the bands specific to parental lines proving their true hybrid nature.Table 2 indicates the percentage of genetic purity in the remaining hybrid seed lots.

Table 2. Comparison of Genetic purity analysis between Molecular markers and Grow-Out test

	No. of Off-type/ Impure plants		Genetic Purity (%)	
Hybrids	GOT	Marker	GOT	Marker
PA6444	3	2	99.25	98.00
PA6129	3	1	99.25	99.00
PHB71	10	2	97.50	98.00
US312	2	0	99.50	100.0
KUSHBOO	6	4	98.50	96.00
DRRH 3	12	3	97.00	97.00
KRH 2	4	2	99.00	98.00
PRH10	5	2	98.75	98.00

The study further revealed that *Rf* gene linked STMS marker, RM 6100 is highly useful for genetic purity assessment of rice hybrids as compared to time consuming GOT. A single STMS marker linked to specific trait of pollen parent such as fertility restorer (*Rf*) gene may be sufficient enough to identify off-types plant(s) in commercial hybrid seed lots and assays based on such markers can be deployed for reliable estimation of genetic purity of the hybrid cultivars (Garg et al. 2006). Whereas, testing of the genetic purity of hybrid seeds using a combination of markers (Yashitola et al. 2002), would be laborious and costly when compared to the use of a single, restorer gene linked co-dominant DNA marker (Nandkumar et al. 2004; Garg

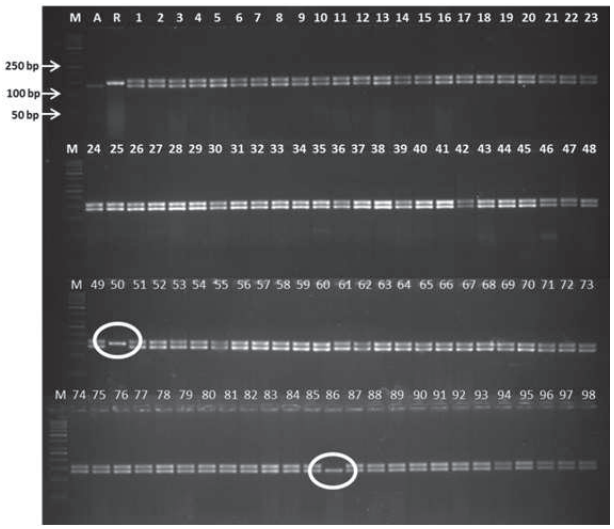

Fig. 1. Genetic purity assessment of hybrid seed lot of PRH 10 by using STMS marker RM6100. M: 50bp DNA ladder, A: CMS line Pusa 6A, R: Restorer line PRR 78 and hybrid seeds (lanes 1- 100). White circle indicates impurities in the test samples as detected by the marker

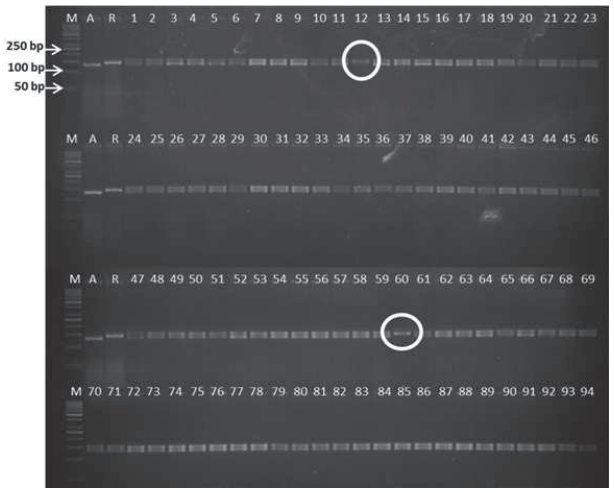

Fig. 2. Genetic purity assessment of hybrid seed lot of KRH 2 by using STMS marker RM6100. M: 50bp DNA ladder, A: CMS line IR58025A, R: Restorer line KMR 3R and hybrid seeds (lanes 1-100). White circle indicates impurities in the test samples as detected by the marker

et al. 2006). The concept of using single fertility restorer (*Rf*) gene linked STMS marker, RM 6100 for testing genetic purity of hybrid seed lots is employed on large scale across several rice hybrids for the first time.

The comparative assessment of both data obtained from the experiments involving STMS marker and GOT analyses were found to be similar *vis-a-vis* comparable (Table 2). However, the percentage impurity detected in rice hybrids through molecular marker analysis was 0.25-2.50 % higher as compared to that detected in GOT except for DRRH 3, where the result of genetic purity testing through marker analysis was in highly accordance with that from GOT. In general, the percentage impurities detected through molecular marker analysis was relatively higher as compared to GOT. The observation of lower impurity per cent in few hybrids by marker analysis may be due to small sample size analyzed. Similar results have been also reported earlier by several workers (Yun et al. 2005; Sundaram et al. 2008; Moorthy et al. 2011; Bora et al. 2016). The present finding indicated that the selected molecular marker is indeed highly informative and useful in marker based seed genetic purity assessments in rice hybrids.

Thus, *Rf* gene linked STMS markers can be effectively used for testing the genetic purity of hybrid seed in other crops where hybrid seed production is based on CGMS system.The cost effectiveness of marker based assay in testing genetic purity of hybrid seed is one of the most important considerations while recommending the adoption of this technology to the hybrid seed industry as an alternative to GOT. Besides being quick and accurate, the use of a single *Rf* gene linked marker would be cost effective as compared to use of a combination of unlinked markers in testing genetic purity of hybrid seeds.

Authors' contribution

Conceptualization of research (SKY, NKS, SKC); Designing of the experiments (SKY, PKS, SKC); Contribution of experimental materials (SKY, SS, PKS); Execution of field/lab experiments and data collection (PKS, CP, SS); Analysis of data and interpretation (PKS, CP, SKY); Preparation of manuscript (PKS, SKY).

Declaration

The authors declare no conflict of interest.

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