International Journal of Agriculture, Environment and Biotechnology
Citation: IJAEB: 11(1): 79-85, February 2018
DOI: 10.30954/0974-1712.2018.00178.10

GENETICS AND PLANT BREEDING

STMS Markers Proved Effective in Establishing Identity and Determining Genetic Purity in Commercial Seed Lots of 'Pusa Rice Hybrid 10'

Praveen K. Singh[1*], Chandra Prakash[2], S. Pandey[3], K.K. Singh[1], S.S. Parihar[1] and A.K. Singh[4]

[1]Division of Seed Science and Technology, Indian Agricultural Research Institute, New Delhi, India
[2]National Research Centre on Plant Biotechnology, New Delhi, India
[3]National Bureau of Plant Genetic Resources, New Delhi, India
[4]Division of Genetics, Indian Agricultural research Institute, New Delhi, India

*Corresponding author: praveen.agrbhu@gmail.com (ORCID ID: 0000-0001-8059-3974)

Paper No. 653 Received: 10-09-2017 Accepted: 09-01-2018

ABSTRACT

The identification of variety has attained paramount importance in the context of plant variety protection as well as to harness the maximum yield potential of any crop. Molecular fingerprinting is one of the most important tools to authenticate the identity and purity of seed lots of crop variety/hybrids. The present investigation was undertaken to establish the identity and genetic purity of 12 commercial hybrid lots of Pusa Rice Hybrid 10 (PRH 10) procured from different public and private seed companies including a farmer's seed through molecular fingerprinting. Ten most informative sequence tagged microsatellite markers (STMS) were employed for fingerprinting of PRH 10. These markers together produced a unique fingerprint of 'PRH 10', which was found to be effective in the identification of this hybrid. Variation could not be observed among the twelve commercial seed lots obtained from different sources with respect to molecular profile of PRH 10. The results indicated the practical usefulness of STMS markers in the protection and identification of hybrids and their parental lines. However, plant-to-plant allelic variation was found within hybrid plants using markers RM 206 and RM 228. These two markers revealed the homozygous pattern in one plant of PRH 10. The occurrence of inter-plant variation at unlinked marker confirmed the molecular heterogeneity. This highlighted the importance of STMS markers in maintaining the genetic purity of the parental lines and hybrids which would be quite effective in preventing the fraudulent marketing of the popular hybrid/variety.

Highlights

- STMS markers are very efficient in establishing the hybrid identity of rice hybrids.
- Molecular profiles of 12 commercial seed lots procured from market were identical to PRH 10.
- The inter-plant variation was observed at unlinked STMS marker loci in comparison to no variation at marker locus linked to specific trait of the pollen parent.
- Fertility restorer gene linked STMS markers, RM 6100 and RM 258 are very useful for genetic purity testing of rice hybrid.
- The molecular profile of seed lot produced and marketed by Krishidhan under the name of KSL 810 was found identical to PRH 10.

Key words: STMS marker, rice, genetic purity, hybrid, fingerprinting

Rice (*Oryza sativa* L.) is the principal food crop for a large segment of the Asian population. The rice production in India as well as several other Asian countries must be doubled by the year 2025 to meet the requirements of the increasing population (Paroda 1998). Therefore, Asiatic rice

growing countries including India need to step up their rice production particularly in view of decreasing land and water resources. Hybrid rice cultivation offers an opportunity to increase rice yields and thereby ensures steady supply of rice (Virmani and Kumar 2004). At present, hybrid rice is commercially cultivated in China, India, Vietnam, Bangladesh and Philippines. In India 78 rice hybrids have been released for commercial cultivation including world's first superfine grain aromatic rice hybrid PRH 10 developed by IARI, New Delhi. These hybrids have recorded a yield advantage of 15-20% over semi dwarf high-yielding varieties (HYVs) at the farmers' fields (Rangaswamy and Jayamani, 1996; Mishra *et al.* 2003). The success of hybrid rice technology for harnessing maximum yield depends upon the production and timely supply of genetically pure seeds to the farmers at an affordable price, besides other factors. 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 becomes necessary. In order to popularize PRH 10; IARI has licensed this hybrid to several companies under public-private partnership. However, it has been suspected that several companies without license are marketing PRH 10 in different names. Therefore, it is necessary to establish the identity of such suspected brands.

The ability to distinguish and clearly identify the varieties of cultivated species is fundamental for the operational aspects in the seed trade. The new varieties developed in agriculture and horticulture crops should be distinct from other varieties. Unambiguous identification of elite crop varieties and hybrids is essential for their protection and to prevent unauthorized commercial use (Nandkumar *et al*. 2004). In India, this is highly relevant especially in rice because the hybrid seed production and marketing of public sector bred hybrids is largely taken up by the private sector. A set of qualitative and quantitative characters known as descriptors are currently in use for varietal identification and description. Some of these characters particularly those showing quantitative inheritance, interact with the environment in which the variety is grown and thus make the process of varietal identification subjective. This limitation can be managed effectively by employing the molecular markers because of their insensitivity to environment and their ability to provide an unbiased means of identifying crop varieties.

The biochemical and molecular techniques group of the International Union for the Protection of New Varieties of Plants (UPOV) is evaluating different DNA markers prior to its routine use in establishing distinctness, uniformity and stability (DUS) of plant varieties (Bredemeijer *et al.* 2002; Roder *et al.* 2002; UPOV-BMT, 2002). Molecular markers such as RAPD, ISSR, SSR, SRAP, AFLP and RFLP have been used in cultivar fingerprinting, seed purity testing and germplasm identification for many species such as maize (Wu *et al.* 2006), cabbage (Liu *et al.* 2007), rice (Nandkumar *et al.* 2004; Hashemi *et al.* 2009), sunflower (Pallavi *et al.* 2011), cotton (Dongre *et al.* 2011) and castor (Gourishankar *et al.* 2013). Among the various available DNA based markers, genetically mapped sequence tagged microsatellite sites (STMS) are the markers of choice in rice because of their abundance, co-dominant nature and uniform distribution throughout the genome (McCouch *et al.* 1997). Further, the nature of chromosome specificity, greater levels of allelic diversity, high power of resolution, operational ease and low cost (Chen *et al.* 1997; Garland *et al.* 1999) makes the STMS markers more suitable for fingerprinting and hybrid identity. The objective of the present study was to access the hybrid identity and purity in commercial seed lot of a superfine grained aromatic rice hybrid 'PRH 10'.

MATERIALS AND METHODS

Plant material

PRH 10 is the first superfine grain aromatic rice hybrid released for commercial cultivation in the basmati growing regions of India during 2001. This hybrid matures 20 days earlier with higher yield when compared to the best basmati check variety, 'Pusa Basmati 1' and has excellent grain and cooking quality. Considering the commercial significance of PRH 10 and its suspected unauthorized marketing as detailed earlier, this hybrid was selected for the study. Parental lines of PRH 10 i.e., Pusa 6A (male sterile line) and PRR 78 (restorer line) were obtained from Division of Genetics, IARI, New Delhi and seed sample of PRH 10 was collected from 12 different companies involved in trading of PRH 10 including a seed sample produced by a farmers under supervision

of IARI, New Delhi (Table 1). Young tender and healthy leaves from five phenotypically identical plants were collected separately from each of the twelve plots grown for grow-out test at Seed Production Unit, IARI, New Delhi and used for DNA isolation.

PCR analysis

Plant DNA was isolated following the procedure of Doyle and Doyle (Doyle and Doyle, 1990). For hybrid identity, DNA from the leaf samples of individual plant was used. Quantification of DNA was accomplished by analyzing the DNA on 0.8% agarose gel using diluted uncut lambda DNA as standard. DNA was diluted in $Tris_{10}$: $EDTA_1$ buffer to a concentration of approximately 25 ng/μL for PCR analysis. Based on earlier study of fingerprinting of rice hybrids using STMS markers, nine most informative markers *viz*., RM 250 (LG I), RM 263 (LG II), RM 234 (LG VII), RM 201 (LG IX), RM 216 (LG X), RM 228 (LG X), RM 258 (LG X), RM 206 (LG XI) and RM 247 (LG XII) along with one additional marker RM 6100 (LG X) were selected for fingerprinting of 'PRH 10' (the figures in the parenthesis refers to the respective linkage group). The sequence information for the primer pairs were obtained from Wu and Tanksley (1993); Chen *et al.* (1997); Temnykh *et al.* (2000) and were synthesized from Life Technologies Inc., U.S.A. DNA amplification was carried out in a 10 μL reaction mixture containing 1× PCR assay buffer (50 mMKCl, 10 mMTris-Cl, 1.5 mM $MgCl_2$), 200 μM of each of dNTPs, 1.25 μM of forward and reverse primers, 0.2 units of Taq DNA polymerase (Bangalore Genie Pvt. Ltd., Bangalore, India) and 25 ng of genomic DNA. The amplification was carried out in a thermal cycler (Perkin Elmer, Model 9600). The first stage consisted of initial denaturation of template DNA for 5 min, followed by 35 cycles of denaturation of 1 min at 94°C, primer annealing of 1 min at 55°C and primer extension of 2 min at 72°C, with a final extension for 7 min at 72°C. PCR products were separated on 3% Metaphor agarose gel prepared in 1×TAE buffer containing ethidium bromide. The size of the amplified fragments was determined by using size standards (50 bp DNA ladder, MBI Fermentas, Lithuania). The gel was documented with Biorad gel documentation system.

RESULTS AND DISCUSSION

Establishing Identity of Hybrid

Molecular fingerprinting of the hybrid and its parental line assumes utmost importance to protect the Plant Breeder's Rights (PBR) and ensures the genetic purity. Although the utility of RAPD fingerprints for plant variety protection is known in case of rice hybrids (Wang *et al.* 1994), the microsatellite markers are considered more reliable because of their ability to produce high fidelity profile which shows their co-dominant nature and chromosome specificity. In an earlier study conducted on 11 rice hybrids including PRH 10, a set of nine STMS markers

Table 1: Detail of seed producing companies of different commercial seed lots of Pusa RH 10

Sl. No.	Name of seed lots	Seed producing companies	Lot No.	License issued by IARI
1	VNR	VNR Seeds, Raipur	G-127-7071/59	Not licensed
2	SPRIHA	Spriha Bioscience, Hyderabad	29823004	Not licensed
3	MAHYCO	Mahyco Seeds, Jalna	XKE 100013	Licensed
4	MANISHA	Manisha Agribiotech, Hyderabad	May-08-MAB-721(P)-82099	Licensed
5	DHAANYA	Dhaanya Seeds, Bangalore	C-194705	Not licensed
6	BIOSEED	Shriram Bioseed, Hyderabad	PNPG 8708	Not licensed
7	KAMBOJ	Kamboj Export, Karnal	Not Mentioned	Not licensed
8	JAIKISAN	Zuari Seeds, Bangalore	70024449	Licensed
9	JK SEED	JK Seed, Hyderabad	9012-45252	Licensed
10	KRISHIDHAN	Krishidhan Seeds, Jalna	HHR 201133	Licensed
11	KARNAL	IARI Regional Station, Karnal	IARI/RSK/KH-08	Licensed
12	FARMER SEED*	S.P.U, IARI, New Delhi	IARI/SPU/ 08	Licensed

**Farmer produced seed under participatory programme of S.P.U, IARI, New Delhi.*

were identified, which produced unique fingerprint for 11 hybrids and of these only a set of four markers *viz*., RM 206, RM 216, RM 258 and RM 263 unequivocally differentiated all the hybrids from each other and were suggested as referral markers for unambiguous identification and protection of these hybrids (Nandkumar *et al.* 2004). But the entire set of nine markers is still relevant and can be used to differentiate future rice hybrids apart from existing rice hybrids.

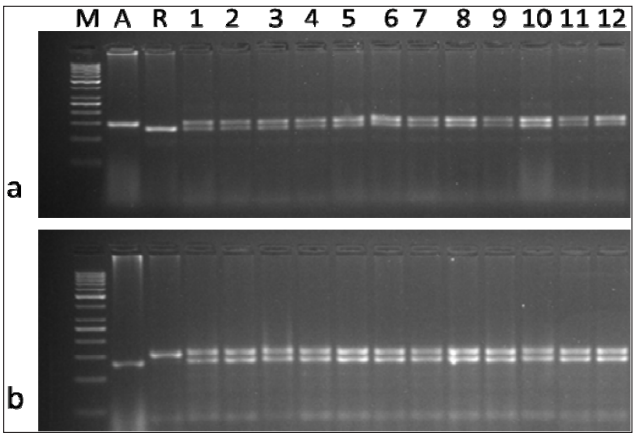

Fig. 1: Molecular profile of hybrid plants as identified by **(a)** RM 258 and **(b)** RM 6100. M: 50bp DNA ladder, A: CMS line Pusa 6A, R: Restorer line PRR 78 and hybrid plants in lane 1-12 (lane 1: VNR, 2: Spriha, 3: Mahyco, 4: Manisha, 5: Dhannya, 6: Bioseed, 7: Kamboj, 8: Jaikisan, 9: JK seed, 10: Krishidhan, 11: Karnal and 12: Farmer's seed).

For establishing the hybrid identity, the genomic DNA isolated from leaf samples of 12 commercial lots of 'PRH 10' procured from different private and public sector organizations/seed companies were analyzed with 10 informative STMS markers (RM 201, RM 206, RM 216, RM 228, RM 234, RM 247, RM 250, RM 258, RM 263 and RM 6100) to develop fingerprint of PRH 10 seed samples obtained from different sources. Molecular profile of 12 commercial seed lots of 'PRH 10' along with A and R lines generated by STMS markers RM 258 (Fig. 1a), RM 6100 (Fig. 1b), RM 247 (Fig. 2a), RM 263 (Fig. 2b), RM 206 (Fig. 3a) and RM 228 (Fig. 3b) are presented. All the polymorphic markers amplified a maximum of two alleles. The marker RM 234 was found monomorphic. There was no variation among the 12 commercial seed lots obtained from different source with respect to molecular profiling of 'PRH 10' originally done by Nandkumar et al. (2004) using same nine informative STMS markers except RM 6100. The molecular profile of one of the seed lot marketed under the name of 'KSL 810' by 'Krishidhan' was found identical to that of 'PRH 10'. Thus, it was clear that seed samples collected from different public and private seed companies were indeed of 'PRH 10'. IARI has signed MOU with different private seed companies for production and marketing of PRH 10. But out of ten commercial seed lots of private seed companies analyzed in this study, only five companies have signed MOU with IARI for marketing of seed of PRH 10 (Table 1). The efficacy of the STMS markers in establishing the hybrid identity of various seed lots of 'PRH 10' has been clearly brought out by the present study.

At present, the registration and protection of new plant varieties is based on DUS testing. The DUS testing based on phenotypic characterization often suffers from limited number of target traits and environment variation when the new variety/hybrid is evaluated across the environments. Therefore, the use of DNA marker for establishing DUS of plant varieties/ hybrids has been advocated by several workers (Bredemeijer *et al*. 2002; Roder *et al*. 2002; Singh *et al*. 2004). The DNA-marker-based DUS testing will augment the process of identification and protection of candidate varieties and hybrids.

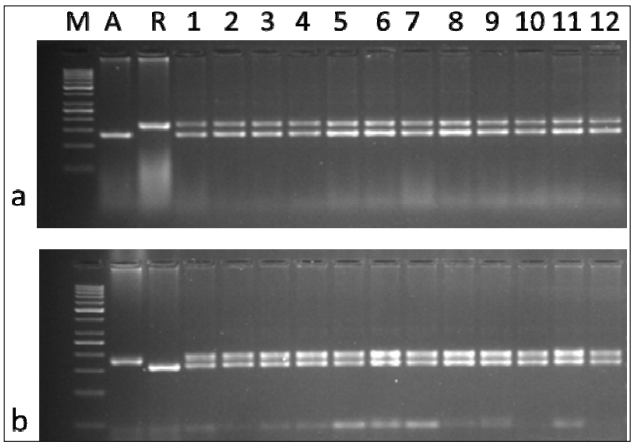

Fig. 2: Molecular profile of hybrid plants as identified by **(a)** RM 247 and **(b)** RM 263. M: 50bp DNA ladder, A: CMS line Pusa 6A, R: Restorer line PRR 78 and hybrid plants in lane 1-12 (lane 1: VNR, 2: Spriha, 3: Mahyco, 4: Manisha, 5: Dhannya, 6: Bioseed, 7: Kamboj, 8: Jaikisan, 9: JK seed, 10: Krishidhan, 11: Karnal and 12: Farmer's seed).

Testing Genetic Purity of Hybrid Seed Lots

In the present investigation 12 putative F_1 plants were analyzed with 10 informative markers as detailed earlier. STMS marker RM 258 linked with '*Rf*' gene amplified a product of 160 and 140 bp in the CMS

line Pusa 6A and restorer line PRR 78, respectively (Fig. 1a).

Whereas, another '*Rf*' gene linked STMS marker RM 6100 amplified a product of 135 and 150 bp in the CMS line Pusa 6A and restorer line PRR78, respectively (Fig. 1b). The 12 putative F_1 plants analyzed were heterozygous at RM 258 and RM 6100 loci showing the presence of both the bands and thus their hybrid nature (Fig. 1). The plants identified as hybrid using markers RM 258 and RM 6100 were further genotyped using the rest of eight markers, the expected heterozygous pattern was not obtained in one of the plant. At RM 206 and RM 228 locus, one plant (in lane 11) was found homozygous for the restorer PRR 78 specific allele (Fig. 3a and 3b).

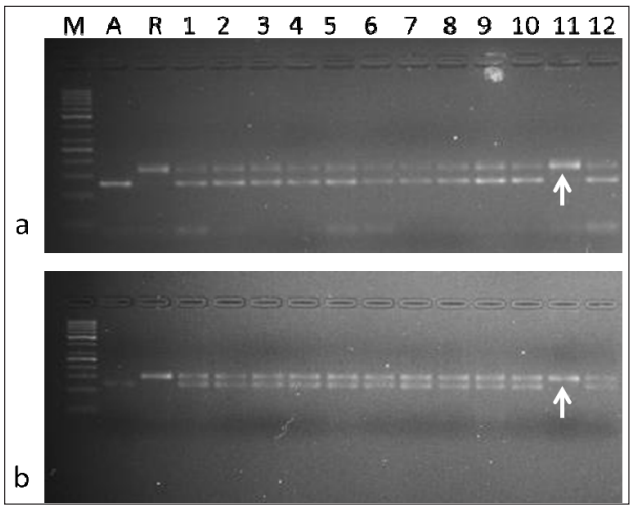

Fig. 3: Molecular profile of hybrid plants as identified by **(a)** RM 206 and **(b)** RM 228. M: 50bp DNA ladder, A: CMS line Pusa 6A, R: Restorer line PRR 78 and hybrid plants in lane 1-12 (lane 1: VNR, 2: Spriha, 3: Mahyco, 4: Manisha, 5: Dhannya, 6: Bioseed, 7: Kamboj, 8: Jaikisan, 9: JK seed, 10: Krishidhan, 11: Karnal and 12: Farmer's seed).

The remaining 11 plants were heterozygous showing the allele specific to Pusa 6A and PRR 78 (Fig. 3a and 3b). While the same plant (in lane 11) was found heterozygous at other marker loci (RM 201, RM 216, RM 247, RM 250, RM 258, RM 263 and RM 6100) showing the allele specific to Pusa 6A and PRR 78 and thus the hybrid nature of this plant was confirmed with these markers. The deviation from heterozygous pattern was obtained at two unlinked marker loci (RM 206 and RM 228) due to inter-plant variation that might have existed in parental lines.

The results had shown plant-to-plant allelic variation at unlinked STMS marker loci within hybrid plant in comparison to no variation at marker locus linked to specific trait of the pollen parent. The occurrence of inter-plant variation at unlinked marker RM 206 and RM 228 among the 12 plants of PRH 10 confirmed the molecular heterogeneity among plants of parental lines of PRH 10. The STMS markers RM 206 and RM 228 were not linked to '*Rf*' gene (Mishra *et al.* 2003) and thus were less efficient when compared to the markers RM 258 and RM 6100 in identifying the true hybrids. Testing with these unlinked markers resulted in some parental genotypes getting identified as hybrids (heterozygous) or *vice- versa* probably owing to the existence of random genomic heterogeneity in the regions (Garg *et al.* 2006). This observation further emphasized the significance of selecting the '*Rf*' gene linked markers for unambiguous identification of true hybrid plants. Markers unlinked to the '*Rf*' gene or specific traits of the pollen parent have lower efficiency due to persistent heterogeneity at unlinked marker loci within parental lines (Garg *et al.* 2006).

The commercial success of hybrid rice technology largely depends on the quality i.e., genetic purity of the supplied hybrid seed. Traditionally, it has been practiced to carry out a grow-out test (GOT) based on morphological traits for assessing the purity of seeds. Grow-out test is space demanding, time consuming (takes one full growing season for completion) and often does not allow the unequivocal identification of genotypes. This environmental dependence limitation can be managed effectively by employing the molecular markers. Testing 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 like RM 258 (Nandkumar *et al.* 2004; Garg *et al.* 2006). The present study also indicated the utility of fertility restorer gene linked STMS markers, RM 6100 and RM 258 for genetic purity testing of rice hybrid.

CONCLUSION

STMS markers may be useful for cultivar identification and hybrid purity test in rice especially for Plant Breeder Right and seed quality control programme. In the present study, on the basis of molecular fingerprinting it was found that all the

seed lots procured from market were Pusa RH 10. IARI has signed MOU with different private seed companies for production and marketing of PRH 10. But out of ten commercial seed lots procured from private seed companies only five companies has signed MOU with IARI for marketing of seed of PRH 10. The molecular profile of seed lot produced and marketed by Krishidhan under the name of KSL 810 was found identical to PRH 10. Plant-to-plant variation at unlinked STMS marker loci within plant of PRH 10 was observed. Thus, the assessment of genetic purity of hybrid seeds based on unlinked markers might not be fully reliable. Molecular markers are presently not accepted as a means for registering and protecting plant varieties. However, the STMS approach has all the characteristics to become, in near future, one of the preferred tools for this purpose.

ACKNOWLEDGMENTS

The first author is thankful to the ICAR (Indian Council of Agricultural Research, New Delhi, India) for the grant of junior research fellowship and to Dr. A.K. Singh, Assistant Professor, Department of Genetics and Plant Breeding, I.G.K.V., Raipur, CG, India for his help and suggestions during the study. We would like to thank IARI, New Delhi for providing necessary fund to carry out this research work.

REFERENCES

Bredemeijer, G.M.M., R.J. Cook, M.W. Ganal, R. Peeters, P. Issac, Y. Noordijk, S. Rendell, J. Jackson, M.S. Roder, K. Wendehake, M. Dijcks, M. Amelaine, V. Wickaert, L. Bertrand and B. Vosman. 2002. Construction and testing of microsatellite database containing more than 500 tomato varieties. *Theoretical and Applied Genetics,* **105**: 1019-1026.

Chen, X., S. Temnykh, Y. Xu, Y.G. Cho and S.R.McCouch.1997. Development of a microsatellite framework map providing genome wide coverage in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics,* **95**: 553-567.

Dongre, A.B., M.P. Raut, M.R. Bhandarkar and K.J. Meshram. 2011. Identification and genetic purity testing of cotton F_1 hybrid using molecular markers. *Indian Journal of Biotechnology,* **10:** 301-306.

Doyle, J.J. and J.L. Doyle 1990. Isolation of plant DNA from fresh tissue. *Focus,* **12**: 13–14.

Garg, A., A.K. Singh, K.V. Prabhu, T. Mohapatra, N.K. Tyagi, N. Nandakumar, R. Singh and F.U. Zaman. 2006. Utility of a fertility restorer gene linked marker for testing genetic purity of hybrid seeds in rice (*Oryza sativa* L.). *Seed Science and Technology,* **34**: 9-18.

Garland, S.H., L.Lewin, M. Abedinia, R. Henry and A. Blakeney 1999. The use of microsatellite polymorphisms for the identification of Australian breeding lines of rice (*Oryza sativa* L.). *Euphytica,* **108:** 53-63.

Gourishankar, V., P.V.R. Rao, R.P. Priya, M.V.N. Kumar, A.V. Ramanjaneyulu and A.V. Reddy 2013. Genetic purity assessment of castor hybrids using EST-SSR markers. *SABRAO journal of breeding and genetics,* **45**: 504-509.

Hashemi, S.H., S.A.M. Mirmohammadi-Maibody, G.A. Nematzadeh and G. Arzani. 2009. Identification of rice hybrids using microsatellite and RAPD markers. *African Journal of Biotechnology,* **8**: 2094-2101.

Liu, L., G. Liu and Y. Gong. 2007. Evaluation of genetic purity of F_1 hybrid seeds in cabbage with RAPD, ISSR, SRAP, and SSR markers. *HortScience,* **42**: 724-727.

Mao, C.X., S.S. Virmani and I. Kumar. 1996. Technological innovations to lower the costs of hybrid rice seed production. In: Advances in Hybrid Rice Technology, Virmani, S.S. *et al.* (Eds.). DRR., Hyderabad, India, pp: 111-128.

McCouch, S.R., X. Chen, O. Panaud, S. Temnykh, Y. Xu, Y.G. Cho, N. Huang, T. Ishii and M. Blair. 1997. Microsatellite marker development, mapping and applications in rice genetics and breeding. *Plant Molecular Biology,* **35**: 89-99.

Mishra, G.P., R.K. Singh, T. Mohapatra, A.K. Singh, K.V. Prabhu, F.U. Zaman and R.K. Sharma.2003. Molecular mapping of a gene for fertility restoration of wild abortive (WA) cytoplasmic male sterility using a Basmati rice restorer line. *Journal of Plant Biochemistry and Biotechnology,* **12**: 37-42.

Nandakumar, N., A.K. Singh, R.K. Sharma, T. Mohapatra, K.V. Prabhu and F.U. Zaman. 2004. Molecular fingerprinting of hybrids and assessment of genetic purity of hybrid seeds in rice using microsatellite markers. *Euphytica,* **136**: 257-264.

Pallavi, H.M., R. Gowda, Y.G. Shadakshari, K. Bhanuprakash and K. Vishwanath. 2011. Identification of SSR markers for hybridity and seed genetic purity testing in sunflower (*Helianthus annuus* L.). *Helia,* **34:** 59-66.

Paroda, R.S. 1998. Priorities and opportunities of rice production and consumption in India for self-sufficiency. pp. 357-390. In: N.G. Dowling *et al.* (ed.) Sustainability of rice in the global food system. International Rice Research Institute, Manila, Philippines.

Rangaswamy, M. and P. Jayamani. 1996. Hybrid rice research in Tamil Nadu. In: Rangaswamy, M. (ed.) Hybrid rice technology. School of Genetics, Tamil Nadu Agricultural University, Coimbatore, India.

Roder, M.S., K. Wendehake, V. Korzun, G.M.M. Bredemeijer, D. Laborie, L. Bertrand, P. Issac, S. Rendell, J. Jackson, R.J. Cook, B. Vosman and M.W. Ganal. 2002. Construction and analysis of a microsatellite-based database of European wheat varieties. *Theoretical and Applied Genetics***106**: 67-73.

Singh, R.K., R.K. Sharma, A.K. Singh, V.P. Singh, N.K. Singh, S.P. Tiwari and T. Mohapatra. 2004. Suitability of mapped sequence tagged microsatellite site markers

for establishing distinctness, uniformity and stability in aromatic rice. *Euphytica,* **135**: 135-143.

Temnykh, S., W.D. Park, N. Ayres, S. Cartinhour, N. Hauck,L. Lipovich, Y.G. Cho, T. Ishii and S.R. McCouch. 2000. Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics,* **100**: 697-712.

UPOV-BMT 2002. BMT/36/10 Progress Report of the 36th Session of the Technical Committee, the technical working Parties and working group on biochemical and molecular techniques and DNA-profiling in particular, Geneva.

Virmani, S.S. and I. Kumar. 2004. Development and use of Hybrid rice technology to increase rice productivity in the tropics. *International Rice Research Newsletter,* **29**: 10-20.

Wang, G., S. Castiglione, J. Zhang, R. Fu, J. Ma, W. Li, Y. Sun and F. Sala. 1994. Hybrid rice (*Oryza sativa* L.): Identification and parentage determination by RAPD fingerprinting. *Plant Cell Reports,* **14**: 112-115.

Wu, K.S. and S.D. Tanksley. 1993. Abundance, polymorphism and genetic mapping of microsatellites in rice. *Molecular and General Genetics,* **241**: 225-235.

Wu, M., X. Jia, L. Tian and B. Lv. 2006. Rapid and reliable purity identification of F_1 hybrids of maize (*Zea mays* L.) using SSR markers. *Molecular Plant Breeding,* **4**: 381-384.

Yashitola, J., T. Thirumurugan, R.M. Sundaram, M.K. Naseerullah, M.S. Ramesha, N.P. Sarma and R.V. Sonti. 2002. Assessment of purity of rice hybrids using microsatellite and STS markers. *Crop Science,* **42:** 1369-1373.