Indian J. Genet., 73(4): 443-445 (2013)
DOI: 10.5958/j.0975-6906.73.4.067

Short Communication

Finger printing and purity testing of rice hybrids using microsatellite markers

Showkat A. Waza*, N. K. Rastogi, S. B. Verulkar and D. A. Bano

Department of Genetics and Plant Breeding, College of Agriculture, IGKV, Raipur 492 012

(Received: January 2013; Revised: August 2013; Accepted: September 2013)

Abstract

Four rice hybrids *viz*., KRH-2, Sahyadri, Indira Sona and Pusa RH-10 along with their parental lines were tested for genetic purity and their identity using microsatellite markers. Out of 53, eight microsatellite markers distributed on six chromosomes showed polymorphism between the parental lines of one or more hybrids. The highest frequency of polymorphism was observed in KRH-2, which exhibited distinguishing banding pattern for markers, RM9, RM17, RM212, RM256, RM335, RM444 and RM1384. Hybrid Sahyadri exhibited polymorphism for RM260 and RM444, whereas, Indira Sona showed differentiating banding pattern for RM260 and RM1384. Similarly, Pusa RH-10 exhibited the polymorphic banding pattern on five microsatellite loci *i.e.,* RM17, RM212, RM335, RM444 and RM1384.

Key words: Genetic purity, grow out test, microsatellite markers, polymorphism

Ever since the report of Jones [1] on exploitation of heterosis has been contemplated as a potential strategy for yield enhancement in rice, which has successfully been demonstrated by China after the commercial release of hybrids. The full potential of any hybrid can be exploited by ensuring the supply of genetically pure seed to farmers. Conventionally, hybrid identification and seed purity testing is done through grow out test (GOT). Being land and labor intensive, time consuming and influenced by the environment, there is an immense need to replace GOT with a simple, rapid, unbiased and cost-effective DNA based assay. Molecular markers have the potential to serve the purpose. Among the available molecular markers, microsatellites or simple sequence repeats (SSRs) have gained considerable importance in plant genetics and breeding owing to many desirable genetic attributes including hypervariability, multiallelic nature, codominant inheritance, reproducibility, relative abundance, extensive genome coverage including organellar genomes, chromosome specific location and amenability to automation and high throughput genotyping [2]. In the present study, the polymorphic survey of several microsatellite loci was conducted to identify SSR markers that can distinguish parental lines of rice hybrids. The identified molecular markers can be used to assess the genetic purity of seed lot and to distinguish different hybrid varieties.

Two CMS lines and four fertility restorer lines in a set of four hybrid combinations were considered for molecular evaluation (Table 1). Genomic DNA was isolated from young succulent diseases free leaves of 8-10 days old rice seedlings as per the protocol of Xu *et al.* [3]. The DNA samples were quantified, diluted, amplified (using thermocycler) and the electrophoresis of the amplified DNA was carried out for 90 minutes at 140 volts on 6% polyacrylamide gel. The staining of the polyacrylamide gels was done with the help of Ethidium Bromide solution containing 10 ml of 1% EtBr in 300 ml of distilled water.

The banding pattern of parental lines and their hybrids was observed with the aim to detect the polymorphic bands between parental lines and to detect both the types of banding patterns in their hybrids. Fifty-three microsatellite loci were analyzed,

*Corresponding author's e-mail: sahmad777@gmail.com
Published by Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
Online management by indianjournals.com

out of which eight *i.e.,* RM9, RM17, RM212, M256, RM260, RM335, RM444 and RM1384 were found to be polymorphic for one or more hybrid combinations. Primers exhibiting polymorphism were screened again to confirm the results. Generally, only one allele was detected in a hybrid when the parents were monomorphic for a particular microsatellite locus and two alleles (one allele per parent) were present in a hybrid when polymorphism was detected between the CMS and restorer lines (Fig. 1).

The frequency of polymorphism in parental combination ranged from 3.77 (2/53) to a maximum of 13.21% (7/53) with an average frequency of 7.55 percent. In general, the level of polymorphism observed was relatively lower than that observed by Yashitola *et al.* (2002) [4], Nandakumar *et al*. (2004) [5] and Sundaram *et al*. (2008) [6]. Among the hybrids studied, the highest frequency of polymorphism was observed in KRH-2 (7/53) while as, lowest frequency of polymorphism was observed in Sahyadri and Indira Sona (2/53 each). Pusa RH-10 exhibited an intermediate frequency (5/53) of polymorphism. The relatively low and high recovery of parental polymorphism under the present study may be attributed respectively to the narrow and broad genetic variation between the parents.

The two microsatellite markers RM444 and RM1384 together differentiated all the four hybrids at least with a single marker allele difference. The microsatellite marker RM444 amplified alleles specific to differentiate parental lines of KRH-2, Sahyadri and Pusa RH-10. Likewise, RM1384 can be used to differentiate the parental lines of KRH-2, Indira Sona and Pusa RH-10. Apart from RM444 and RM1384, the rice hybrid KRH-2 was also distinguished from other hybrids by using markers RM9, RM17, RM212, RM256 and RM335. Similarly, Pusa RH-10 also exhibited the polymorphic banding pattern on RM17, RM212 and RM335. The primer RM260 exhibited polymorphism for parental lines of Sahyadri and Indira Sona with the same allelic pattern.

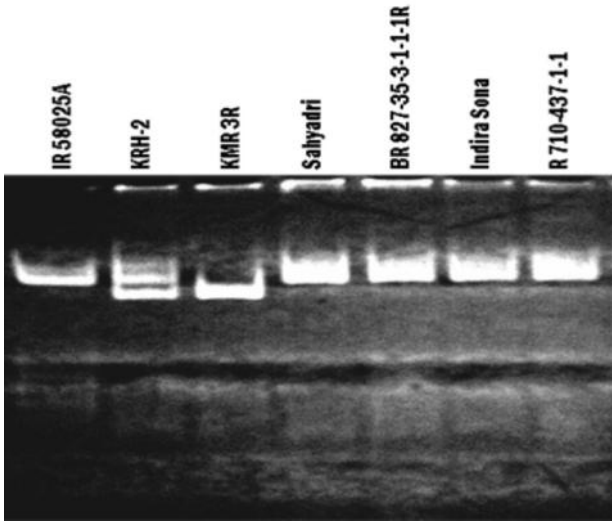

Fig. 1. Amplification pattern obtained using SSR marker RM17 and showing the polymorphic banding pattern among the parental lines of KRH-2

The SSR marker RM1384 was used for accessing the genetic purity of the hybrid seed lot of Pusa RH-10. In a random sample of 100 seeds the marker identified 2 off types indicating 98% genetic purity of the seed lot. The result was confirmed with the help of another marker RM260 using same DNA for amplification. For purity assessment, the DNA was isolated from the single seedlings of Pusa RH-10.

The complete exploitation of heterosis in rice hybrids can be achieved only by ensuring the availability of genetically pure and good quality seed to farmers. But, the only legally recognized traditional method of genetic purity assessment for seed certification purpose in the countries like India is based on grow-out tests. The GOT involves only the morphological characteristics of a variety, which are often inadequate for its identification and purity testing. They require replications of observations, trained personnel and suitable land [7]. Moreover, in India, seed production of hybrid rice is generally done in *Rabi* season (January–April), while hybrid rice is mostly cultivated in the *Kharif* season (June–October). The hybrid seed produced can't be used immediately for raising the crop without passing GOT, which requires one full season. This leads to locking up of the capital invested on hybrid seed production and additional expenditure incurred on storage of seed, ultimately raising the cost of hybrid seed. Furthermore, in India the grow out test for assessment of varietal genetic purity is conducted by seed certification agencies in off-season of the crop which does not allow the varieties to fully express their morphological features as expressed in their normal growing season. Thus, we need to develop the methodology for testing the hybrid seed purity that is accurate, cheap and faster, so seed produced in the *Rabi* season can be used for commercial cultivation in the oncoming *Kharif* season. The DNA-based markers have the potential to be applied for this purpose as they can act as ideal tools

Table 1. Rice hybrids and their parental lines used in the study

S.No.	CMS line	Restorer line	Hybrid
1.	IR 58025A	KMR 3R	KRH-2
2.	IR 58025A	BR 827-35-3-1-1-1R	Sahyadri
3.	IR 58025A	R 710-437-1-1	Indira Sona
4.	Pusa 6A	PRR 78	Pusa RH-10

for precisely assessing the genotype of a plant within short period of time. The SSR markers have the features like reproducibility, stability over different environments, non specificity for growth stage of a plant, multiallelic nature, co-dominant inheritance, relative abundance, good genome coverage, which can serve them as convenient and reliable tools for varietal identification according to the international norms. The studies conducted by different workers have shown that SSR markers are useful in identification of rice hybrid, testing their genetic purity and can also be used for assessment of plant to plant variation within the parental lines of rice hybrids [4-7]. The genetic purity analysis through SSR marker will be a useful tool for resolving the problems arising in seed certification programmes.

The marker(s) used for testing the hybrid seed purity should exhibit similar banding pattern between the CMS line and potential rogue donors but polymorphic banding pattern between CMS and restorer lines. These polymorphic markers can be identified in polymorphism surveys conducted by the random use of either microsatellite or STS primers on CMS, restorer, potential rogue donor and hybrid lines.

Use of single polymorphic marker is generally sufficient to ascertain the hybrid seed purity, but more than one marker can be used to confirm the polymorphic results subject to that additional cost should be kept in consideration. Multiplex PCR using two different primers simultaneously can be used to reduce the expenditure. Using molecular markers will lead to the considerable savings for seed industry, especially in India where huge amount of capital is locked in the form of stored seed. The cost of seed storage as well as cost of acquiring land and growing the crop for the GOT can also be avoided.

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