

Identification of SSR Markers for Genetic Purity Assessment of Rice Hybrid (CORH 4) and its Parental Lines

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Rice is an important food crop with wide adaptation to a range of environments [1]. With the burgeoning population, increasing the food grain production has become the need of the hour. The hybrid rice technology has proven a yield advantage of 15-20 percent over the best high yielding varieties to meet out the shortage of food grain production. Therefore, hybrid rice cultivation offers an opportunity to increase the rice yield and thereby ensures a steady supply of good quality seeds. Genetic purity maintenance is crucial and very much essential to maintain the quality of seeds. The genetic purity of the rice hybrids is assessed by Grow out test (GOT) which becomes time consuming and expensive, requiring one full season, large areas of land and finally it is subjected to environmental fluctuations. The biochemical and molecular techniques group of the International union for the Protection of new varieties of plants (UPOV) is evaluating different DNA marker parameters prior to its routine use in establishing Distinctness, Uniformity and Stability (DUS) of plant varieties [2]. Accordingly, clear-cut identification of elite crop varieties and hybrids is essential for protection and prevention of unauthorized commercial use [3]. On the other hand, purity of hybrid seeds supplied to farmers must surpass 95% [4].

DNA marker is a new approach based on DNA polymorphism among tested genotypes and thus applicable to biological research. It offers many advantages over other categories of markers such as morphological and biochemical DNA marker which can cover the whole genome and therefore is much larger in quantity. There is more polymorphism in DNA markers, which are able to reveal the variation and allelism. Among the DNA markers, the SSR or microsatellite markers and STS markers are effective for rapid assessment of genetic purity of hybrid and parental lines [5]. Microsatellites are abundant and well distributed throughout the genome of

rice [6]. Microsatellites are tandemly repeated nucleotide units of 1 to 6 base pairs and alleles usually differ in the number of repeated units. These are generally co-dominant and highly polymorphic DNA based markers. Genetically mapped microsatellite markers cover the whole rice genome with at least one microsatellite of every 16 to 20 cM [7].

Rice hybrid CORH 4, released from Tamil Nadu Agricultural University, Coimbatore for commercial cultivation and its parental lines; COMS 23A (CMS line) and CB 174R (Restorer line) were taken for this study. The seeds were collected from Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore for testing their genetic purity through DNA marker studies.

Total DNA was extracted from fresh leaves by the Cetyl Tri-methyl Ammonium Bromide (CTAB) method (Murray and Thompson, 1980). The quality and concentration of extracted DNA was estimated by using a Nanodrop spectrophotometer. DNA was diluted in TE buffer for PCR analysis. A total of 30 SSR primers distributed across 12 chromosomes of the rice genome were used. Among these, 2 primers exhibited a complementary banding pattern between CORH 4 rice hybrid and its parental lines, whereas co-primers were monomorphic (Table 1). The primer sequence information was obtained from the published sequence data [8].

DNA amplification was carried out in a 15 μ L reaction volume containing 0.2 μ M of each primer, 200 μ M of deoxyribonucleotides, 50 mM KCl, 10 mM Tris HCl (pH 8.3), 1.5 mM MgCl₂, 0.1% gelatin, 40 ng of DNA and 0.5 unit of Tag DNA polymerase. The temperature profile used for PCR amplification comprised 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 2 min and ending up with 5 min at 72°C for the final extension. The annealing temperature was adjusted

based on the specific requirements of each primer combination. The PCR products were electrophoresed in 3% agarose gels at 100 V for 2 h. the gels were next stained in ethidium bromide for 30 min, de-stained for 15-30 min and then observed under a UV transilluminator.

Table 1. SSR marker alleles identified as molecular tags for rice hybrid CORH 4 and its parental lines

SSR marker	Size of allele (bp)			Amplification
	COMS 23A	CB 174R	CORH 4	
RM 515	210	250	210 and 250	Polymorphism
RM 570	230	270	230 and 270	Polymorphism
RM 480		200		Monomorphism
RM 17		180		Monomorphism
RM 420		170		Monomorphism
RM 528		230		Monomorphism
RM 297		70		Monomorphism
RM 457		230		Monomorphism
RM 1002		150		Monomorphism
RM 1124		176		Monomorphism
RM 5951		97		Monomorphism
RM 5341		155		Monomorphism

A total of 30 SSR primers distributed across 12 chromosomes of the rice genome were used to identify the suitable polymorphic markers for the rice hybrid CORH 4 and its parental lines. Among the used primers, 2 primers exhibited a complementary banding pattern between CORH 4 rice hybrid and its parental lines, whereas 10 primers were monomorphic. The SSR primers viz., RM 515 and RM 570 (Figure 1) were found to be polymorphic primers and RM 480, RM 17 (Figure 2), RM 420, RM 528 (Figure 3), RM 297, RM 457 (Figure 4), RM 1002, RM 1124 (Figure 5), RM 5951 and RM 5341 (Figure 6) were found to be monomorphic primers. The polymorphic markers viz., RM 515 and RM 570 were considered to be the most informative for fingerprinting of rice hybrid CORH 4 and its parental lines viz., COMS 23A and CB 174R.

The above monomorphic primers could not distinguish the rice hybrid CORH 4 and its parental lines whereas; the polymorphic primers viz., RM 515 and RM 570 distinguished the rice hybrid CORH 4 and its parental lines. The polymorphic primers RM 515 amplified an allele size of 210 bp in COMS 23A and 250 bp in CB 174R. Another polymorphic primer RM 570 amplified and allele size of 230 bp in COMS 23A and 270 bp in CB 174R (Table 1).

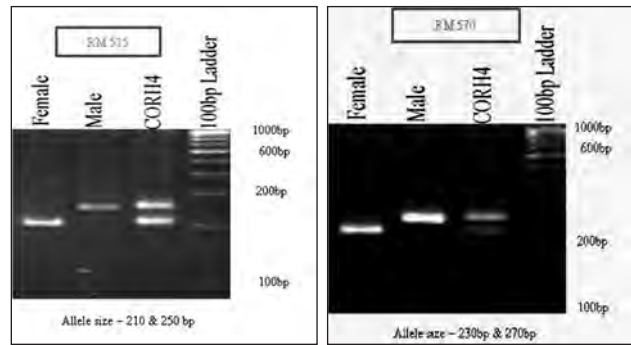


Figure 1. Polymorphic amplification pattern of rice hybrid CORH 4 and its parental lines using RM 515 and RM 570

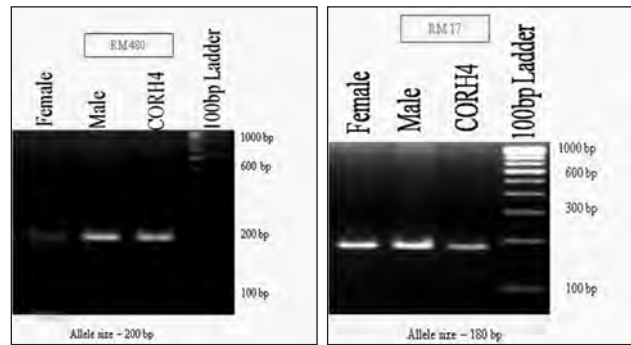


Figure 2. Polymorphic amplification pattern of rice hybrid CORH 4 and its parental lines using RM 480 and RM 17

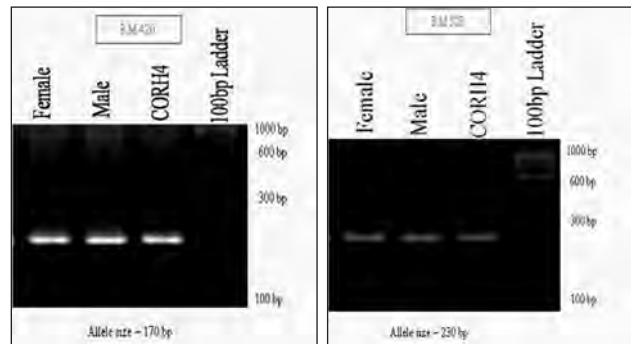


Figure 3. Polymorphic amplification pattern of rice hybrid CORH 4 and its parental lines using RM 420 and RM 528

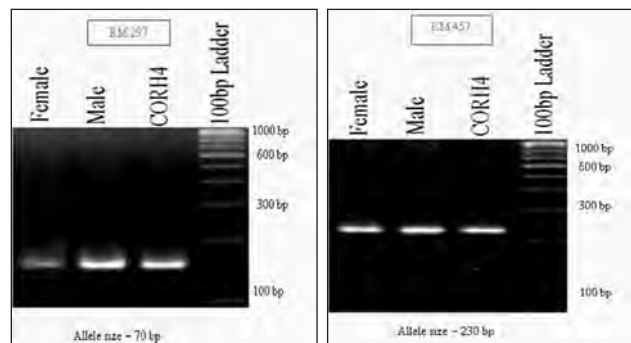


Figure 4. Polymorphic amplification pattern of rice hybrid CORH 4 and its parental lines using RM 297 and RM 457

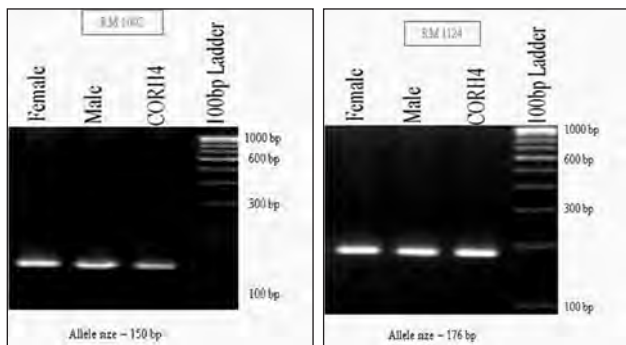


Figure 5. Polymorphic amplification pattern of rice hybrid CORH 4 and its parental lines using RM 1002 and RM 1124

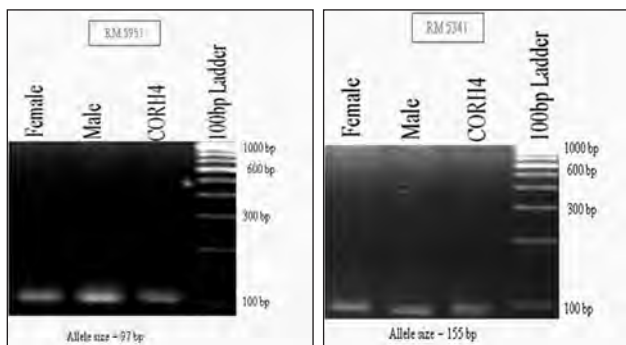


Figure 6. Polymorphic amplification pattern of rice hybrid CORH 4 and its parental lines using RM 5951 and RM 5341

DNA fingerprinting is one of the novel techniques which can be used to identify a variety or hybrid based on its banding pattern over other varieties or hybrids. In DNA fingerprinting, marker identification for a particular hybrid is very important. There are number of molecular markers and some of them are Simple Sequence Repeats (SSR), Random amplified polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and Sequence Tagged Sites (STS). All these markers have lower power of discrimination compared to SSR marker. Among these, the SSR markers are characterized as co-dominant among different markers and they can be detected by PCR (non-hybridization) based assay and the data obtained are highly informative [9]. Besides that, the fingerprints are easy to score. In plant genome analysis, microsatellites are frequently used for cultivar fingerprinting [10].

In the present study, among the 30 SSR primers evaluated, RM 515 and RM 570 exhibited polymorphism between the parental lines viz., COMS 23A and CB 174R. The SSR marker RM 515 amplified the PCR product at 210 bp for female parent (COMS 23A) and at 250 bp for male parent (CB 174R). Similarly, the RM 570 amplified

the PCR product at 230 bp for female parent and at 270 bp for male parent. Out of 30 SSR primers viz., RM 480, RM 17, RM420, RM 528, RM 297, RM 457, RM 1002, RM 1124, RM 5951 and RM 5341 were found to be monomorphic primers. The other primers did not produce any amplification. Hence, RM 515 and RM 570 can very well be used for the genetic purity assessment of rice hybrid CORH 4 and its parental lines, since these SSR primers exhibited a complementary banding pattern between the CORH 4 hybrid rice and its parental lines viz., COMS 23A and CB 174R.

The microsatellite marker, RM234 amplified alleles specific to differentiate parental lines of CORH3 likewise RM 276 for KRH2, RM258 for PRH10, RM202 for AJAY and RM204 for RAJALAXMI used to differentiate parental lines of respective hybrids. The unique microsatellite marker, RM234 was used for testing the genetic purity of CORH3 seeds [11]. The use of SSR markers for assessing seed purity has been reported in rice [12]. Simple Sequence Repeats (SSRs) are the most suitable markers for hybrid purity assessment as the heterozygosity of the hybrids can be easily determined by the presence of both the parental alleles. Development of SSRs is prohibitively expensive, but once the SSR have been developed for a crop, they are cost-and time effective in comparison to RAPD [13]. Microsatellites are considered appropriate for variety identification because of their ability to detect large numbers of discrete alleles repeatedly, accurately and efficiently [14] and then they are very much useful in genetic purity assessment of seeds.

The success behind in hybrid rice technology besides other factors depends on the production and timely supply of genetically improved seed to farmers. The present study establishes the microsatellite marker, RM 515 and RM 570 for genetic purity assessment of CORH 4 hybrid rice and its parental lines.

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