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MOLECULAR FIDELITY ASSESSMENT IN HYBRID RICE (*ORYZA SATIVA* L.) : SSR-BASED ASSURANCE OF TRUE F_1 HYBRIDS

Akanksha Gupta*, Jagdev Kar, Boda Srividhya, Tikeshwar Markande, Nishat Parveen, Prabharani Chaudhari, Ritu R. Saxena and Sunil Kumar Verma

Department of Genetics and Plant Breeding, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur 492012 (C.G.), India

*Corresponding author E-mail: gakanksha156@gmail.com

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ABSTRACT

One of the milestones in the recent history of rice breeding which have contributed to the increase in global output is hybrid rice breeding. It involves the exploitation of heterosis through the development of high-performing F_1 hybrids, derived from genetically divergent and strategically selected germplasm resources. In the present investigation, selected parental lines from diverse rice germplasm pool of IGKV were hybridized to generate experimental F_1's. To validate the hybridity and genetic integrity of these hybrids, Simple Sequence Repeat markers were employed. Out of the twenty markers screened, nine showed clear polymorphism between parents and were subsequently used to assess molecular fidelity in the F_1 plants. A total of forty-four putative F_1 plants, derived from twelve elite × elite crosses were analyzed, out of which fourteen plants were confirmed as true hybrids based on the presence of heterozygous SSR banding profiles. These findings underscore the effectiveness of SSR markers for precise hybridity confirmation, contributing to improved genetic purity maintenance and selection efficiency in rice hybrid breeding pipelines.

***Key words*:** Hybrid rice breeding, Genetic purity, Hybridity testing, Molecular marker, Simple Sequence Repeats (SSR).

Introduction

Rice (*Oryza sativa* L.) is the fundamental component of global caloric intake, nourishing over half of the world's population. Furthermore, recent projections estimate that global food production must increase by approximately 70% by 2050 to meet rising consumption demands (Varshney *et al*., 2011). To address the escalating demand and ensure food security, hybrid rice technology has emerged as a promising approach for enhancing yield potential through the exploitation of heterosis. Hybrids typically demonstrate a yield advantage of 15-20% over high-yielding inbred lines (Huang *et al*., 2020). The success of this approach largely depends on the genetic diversity of parental lines and the genetic purity of hybrid rice seeds supplied to the farmers. It is estimated that for every 1 % impurity in the hybrid seed, the yield reduction is 100 kg per hectare (Mao *et al*., 1998). Hence, verifying genetic integrity is vital to ensure the authenticity of F_1 hybrids.

Conventionally, genetic purity has been assessed through Grow-out test (GOT), which involves field evaluation of plants based on morphological descriptors, typically conducted in the off-season prior to commercial seed distribution. Morphological characterization for quality seed production is not only resource intensive and time taking, but also environment responsive, often resulting in inconsistent and unreliable outcomes due to hindering effect of G × E interaction (Li *et al*., 2002).

Given these limitations, molecular marker-based techniques offer an unbiased, robust, and environment-independent approach for identifying true hybrids and ensuring genetic purity with greater precision and reliability. Among the DNA-based markers, SSR markers commonly referred to as rice microsatellites are extensively employed for hybridity confirmation in rice owing to their co-dominant inheritance, reliability and ability to distinguish homozygous parental alleles from

Table 1: List of crosses attempted.

S.No.	Female	Male
1	Swarna	Piwari Luchai
2	Swarna	Gadursela
3	Swarna	Mahulata
4	Swarna	Nariyal Chudi
5	Swarna	Cross-116
6	Swarna	Kala Mali
7	Swarna	Kariya Jhini
8	Swarna	G-447
9	MTU- 1153	Cross-116
10	MTU- 1153	G-447
11	CG Dhan -1919	Gadursela
12	CG Dhan -1919	G-447

Table 2: Temperature profile used for PCR amplification.

Stages	Temperature (°C)	Duration (min)	Cycle
Denaturation	95	5	1
Denaturation	94	1	
Annealing	55	1	35
Extension	72	1	
Final extension	72	7	1
Storage	4	∞	

heterozygous F_1 alleles. (Bora *et al.*, 2016; Kannan *et al.*, 2017). Subsequently, such validated hybrids can be advanced to segregating generations for bulking and potential deployment in commercial cultivation or utilization in breeding programmes.

Materials and Methods

Plant Materials

The experimental material constituted twelve elite rice crosses (Table 1) developed using three popular varieties Swarna, MTU-1153, and CG Dhan 1919 as female parents. The male parents were selected from a diverse germplasm pool maintained at Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur. The hybridization program was executed during the *Kharif* 2023 season at the Research-cum-Instructional Farm, IGKV, Raipur. The resulting F_1 plants were grown during *Kharif* 2024, and hybridity confirmation was performed through SSR marker profiling at the MAS Laboratory, RRL, IGKV, Raipur.

DNA Extraction and Quantification:

Genomic DNA was extracted from young leaf tissues using the modified CTAB method as described by Doyle and Doyle (1990). The concentration of the extracted DNA was quantified using a NanoDrop spectrophotometer (NanoDrop 2000), and the purity was assessed based on the absorbance ratio at 260 nm and 280 nm (A260/A280).

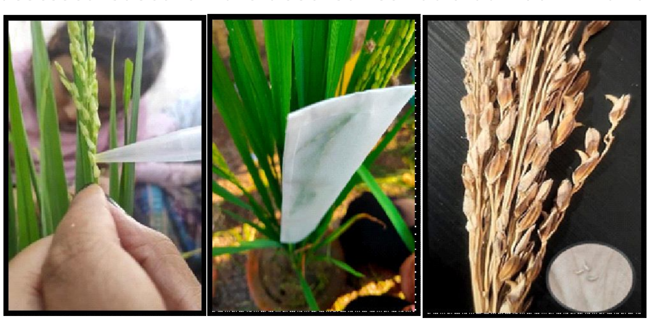

Fig. 1: Crossing procedures and production of F_1 seeds.

PCR Amplification and Gel electrophoresis:

A total of twenty randomly selected SSR primers (Table 3) were employed for screening polymorphism between the parental lines. PCR was performed with a reaction volume of 10/ µL containing 1.5/ µL genomic DNA, 1/ µL of forward and reverse primers, 3.5/ µL Tag polymerase, and 4/ µL nuclease-free water. The PCR temperature regime is summarized in Table 2. The amplified PCR products were resolved on 5% PAGE, stained with ethidium bromide (500/ mL solution), and visualized under UV light using a Gel Doc imaging system.

Results and Discussions

Out of the twenty SSR primers screened, nine (RM 19, RM 152, RM 212, RM 236, RM 259, RM 413, RM

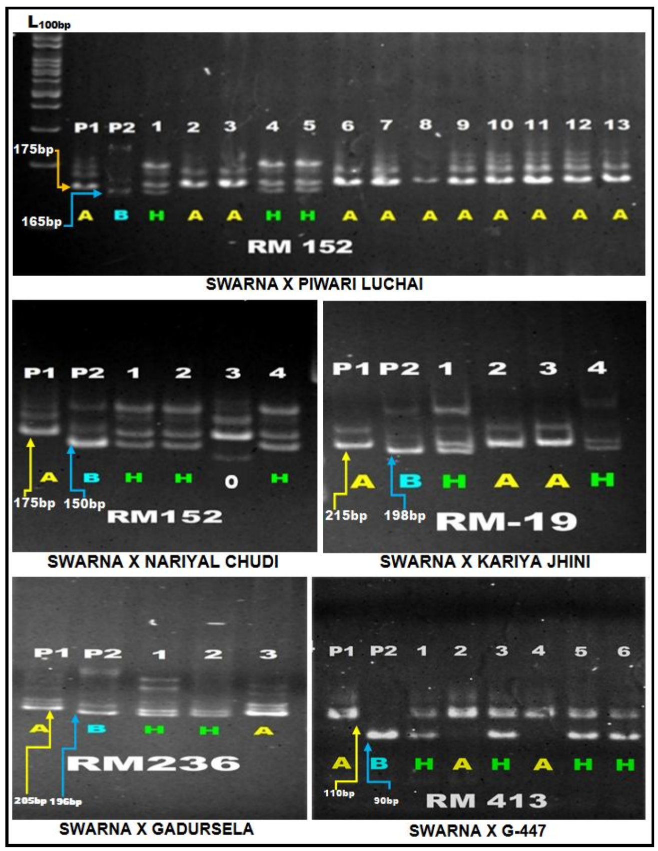

Fig. 2: SSR marker profiling of F_1 population. (**A-** Female Parent; **B-** Male parent; **H-** True hybrid; **O-** Off type).

Table 3: List of SSR markers used for screening parental polymorphism.

S. No.	SSR markers	Ch. No.	Forward sequence	Reverse sequence	PCR Product size(bp)	Annealing T (°C)
1	RM 11	7	TCTCCTCTTCCCCCGATC	ATAGCGGGCGAGGCTTAG	140	55
2	RM 19	12	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGCCAAGA	226	55
3	RM 124	4	ATCGTCTGCGTTGCGGCTGCTG	CATGGATCACCGAGCTCCCCCC	271	67
4	RM 152	8	GAACCACCACACCTCACCG	CCGTAGACCTTCTTGAAGTAG	151	55
5	RM 171	10	AACGCGAGGACACGTACTTAC	ACGAGATACGTACGCCTTTG	328	55
6	RM 212	1	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG	136	55
7	RM 236	2	GCGCTGGTGGAAAATGAG	GGCATCCCTCTTTGATTCCTC	191	55
8	RM 244	10	CCGACTGTTCGTCCTTATCA	CTGCTCTCGGGTGAACGT	163	55
9	RM 259	1	TGGAGTTTGAGAGGAGGG	CTTGTTGCATGGTGCCATGT	162	55
10	RM 277	12	CGGTCAAATCATCACCTGAC	CAAGGCTTGCAAGGGAAG	124	55
11	RM 307	4	GTACTACCGACCTACCGTTCAC	CTGCTATGCATGAACTGCTC	174	55
12	RM 413	5	GGCGATTCTTGGATGAAGAG	TCCCCACCAATCTTGTCTTC	79	55
13	RM 452	2	CTGATCGAGAGCGTTAAGGG	GGGATCAAACCACGTTTCTG	209	55
14	RM 472	1	CCATGGCCTGAGAGAGAGAG	AGCTAAATGGCCATACGGTG	296	55
15	RM 474	10	AAGATGTACGGGTGGCATTC	TATGAGCTGGTGAGCAATGG	252	55
16	RM 519	12	AGAGAGCCCCTAAATTTCCG	AGGTACGCTCACCTGTGGAC	122	55
17	RM 586	6	ACCTCGCGTTATTAGGTACCC	GAGATACGCCAACGAGATACC	271	55
18	RM 5759	1	TTAACGGTCGGGAGTCAAAG	CATCGTCTTTGTCAGATGGC	77	50
19	RM 7195	12	CGCGTGAGAGCTCCTAAAAG	TCCTTGTGTAACTACCGCCC	138	50
20	RM 20331	6	TAGGACAGCTTGTAGATTCAGC	GAGTACGGAAGGAGTAAGTGC	566	55

474, RM 519 and RM 586) exhibited clear polymorphism between the parental lines, with each marker generating distinct allele sizes for the male and female parents. The amplified banding patterns of the parental lines served as reference profiles for evaluating the F_1 individuals. The presence of bands corresponding to both the parental alleles in a single F_1 plant was considered indicative of true hybridity. Similar instances of microsatellite-based hybridity testing in rice were earlier reported by Sundaram *et al*., (2008); Gimhani *et al*., (2014); Ranjitha *et al*., (2017); Hangloo *et al*., (2020); Shashibhushan *et al*., (2021); Pramanik *et al*., (2022); Ahmad *et al*., (2023) and Patel *et al*., (2025).

Out of the twelve rice crosses examined, F_1 plants from five crosses (41.67%) were genetically validated as true hybrids. The remaining seven crosses showed mono-parental banding patterns, suggesting possible selfing or non-hybridization events.

Conclusions

The present study successfully demonstrated the utility of SSR markers for reliable hybridity confirmation in rice. The co-dominant nature of SSR markers enabled the identification of F_1 individuals by detecting the presence of both parental alleles thereby, confirming the true hybrids. Among the twelve rice crosses assessed, three out of four F_1 hybrids (75%) in Swarna × Nariyal Chudi, two out of three (66.67%) in Swarna × Gadursela, four out of seven (66.67%) in Swarna × G- 447, two out of four (50%) in Swarna × Kariya Jhini and three out of thirteen (23%) in Swarna × Piwari Luchai were confirmed as true hybrids. The confirmed hybrids can be advanced to subsequent segregating generations, where phenotypic and genotypic selection will aid in enhancing homozygosity and developing genetically stable lines. These stabilized lines can then be subjected to yield evaluation through multi-location trials (MLTs), and the most promising genotypes may ultimately be released as new cultivars with improved agronomic performance.

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