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Parental Polymorphism between Samba Mahsuri and False Smut Tolerant Landraces using SSR and InDel Markers

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Abstract

In the present study, 868 SSR and 475 InDel markers were selected to study parental polymorphism of two different crosses between recipient parent Samba Mahsuri and false smut donor lines IC379047 and IC334233 at ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad. Out of 1343 markers used for parental polymorphism, 100 markers were polymorphic between parental lines Samba Mahsuri and landrace IC379047 and the total polymorphism percentage recorded was 7.44%. Ninety-nine markers exhibited polymorphism between parental lines Samba Mahsuri and IC334233 and 7.37% of total polymorphism percentage was recorded between the parents. The identified polymorphic markers could be further used for QTL mapping studies in false smut resistance.

Keywords: Rice, false smut, polymorphism, SSRs and InDel markers.

Introduction

Rice (*Oryza sativa* L) is a widely cultivated cereal crop all over the world. It is a nutritionally indispensable food crop with carbohydrates being the major fraction along with protein and vitamins; and 21% of energy for more than half of the world's population. The major rice producing countries are China which ranks first in production of 148.3 million metric tons followed by India and Indonesia with production of 122.27 and 35.3 million metric tons respectively (UASD, 2021). To meet the demand of increasing human population, the higher rice productivity can be achieved by developing varieties having stable yielding ability across the areas along with resistance to various pest and diseases. The germplasm consisting of landraces, modern cultivars, breeding stocks, wild forms and wild relatives of the cultivated crop species could be the valuable genetic source to identify the promising donors. Germplasm refers to sum total of genetic material *i.e.,* possible alleles of the various genes present in crop species and its wild relatives, which can be used for exploiting the genes governing various traits for biotic and abiotic stresses; and ultimately in breeding of the new variety. In previous studies at ICAR-IIRR, Hyderabad, landrace IC379047 (Mancha) and germplasm IC334233 were identified as a tolerant source for the false smut disease through artificial screening. The identified lines were crossed to the popular high yielding but disease susceptible cultivar Samba Mahsuri (an elite fine-grain *indica* rice cultivar). The two parental genotypes in crosses

viz., Samba Mahsuri x IC379047 and Samba Mahsuri x IC334233 were phenotypically diverse and genomic diversity was assessed using the marker system.

Molecular markers have wide applications in several genetic research and breeding programme such as genetic diversity assessment, Quantitative Trait Loci (QTL) identification, gene mapping, marker-based gene targeting and characterization of alien introgression lines from wild species of rice. DNA markers like Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphit DNA (RAPD), Sequenced Tagged Sites (STS), Cleaved Amplified Polymorphic Sequence (CAPS) and Simple Sequence Repeats (SSR) have been used in molecular studies over the past few decades. PCR and gel-based markers have several practical utilities for the researchers and breeders. In addition, the recently identified InDel markers are user friendly and also has ease of accessibility and technical simplicity. These markers have been attained great importance over the last two decades in several genotyping studies because of their co-dominant nature, reproducible, high polymorphism, multiallelic nature, widely distributed across the genome, require less DNA quantity and are cost effective (Usman *et al*., 2018 and Chukwu *et al*., 2019). The SSR markers are present in both coding and non-coding genomic region with lower level of mutation rate (10^{-2} and 10^{-4}) per generation. They are used in several studies such as population structure and evolutionary studies, linkage map construction, genetic mapping and marker assisted selection (Edwards and Balley, 2010 and Gonzaga *et al*., 2015). QTL mapping requires mapping population and sufficient number of polymorphic markers identified between the parental lines. Different mapping populations are used for mapping studies like double haploids, F_2 populations, F_2 derived F_3 population, near isogenic lines (NILs), and recombinant inbred lines (RILs). Each mapping population has its own advantages and disadvantages. The polymorphic markers identified between the parental lines are used for the genotyping of mapping population. Phenotypic and genotypic data of mapping population was used for the QTL mapping using mapping tools. The main objective of our research was to identify the polymorphic SSR markers between the parental genotypes for further QTL mapping studies for false smut disease tolerance.

Materials and Methods

The experimental material for the study comprised of false smut donor lines IC379047 and IC334233 and recipient genotype Samba Mahsuri (high yielding variety susceptible to false smut disease), and SSR and InDel markers were collected from the Department of Genetics and Plant Breeding, ICAR-IIRR, Hyderabad.

Young leaf samples were collected from the field and plant genomic DNA was isolated by CTAB method (Doyle and Doyle, 1987). The leaf samples were ground in CTAB buffer using pestle and mortar. The ground samples were transferred into Eppendorf tube and incubated at 65 ^{o}C for 30 min. The samples were centrifuged for 15 min at 13000 rpm, the supernatant (genomic DNA) was transferred to an Eppendorf tube and equal amount of 24:1 chloroform and isoamyl alcohol was added and again centrifuged for 10 min at 13000 rpm. The supernatant was taken into new Eppendorf tube and equal amount of chilled isopropanol was added and incubated at -20 ^{o}C for 15 min. Centrifugation was done for 10 min at 13000 rpm. The pellet formed in the tube was washed with 70% chilled ethanol and kept out overnight to dry and stored at 4 ^{o}C in Tris EDTA (TE) buffer for further genomic study.

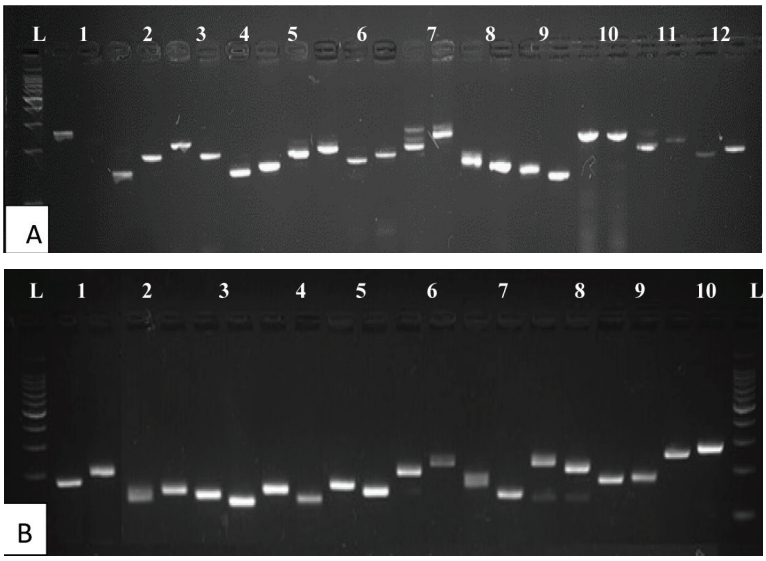
A total of 1343 SSR markers distributed on 12 rice chromosomes were used for the parental polymorphism survey. Among 1343 primers, 868 markers were selected from www.gramene.org. and

remaining 475 were newly developed InDel marker set chosen from the study conducted by Hechanova *et al*., 2021. For PCR analysis, a total 10 µl PCR reaction mixture contained 30-50 ng of 2 µl genomic DNA template, 1 µl of 10X buffer, 5.2 µl of sterilized distilled water, 0.5 µl of 2.5 mM dNTP, 3 units of 0.3 µl Taq polymerase and 1 µl of primer was prepared and PCR amplification was carried out in Bio-Rad PCR machine. The thermal cycles were programmed as follows, mixture was incubated at 94 °C for 5 min; then 35 cycles of 1 min of denaturation at 94 °C, 30 sec of annealing at 58 °C and 1 min of extension at 72 °C; and 10 min of final extension at 72 °C. The PCR products were separated in 3.5% agarose gel with 1X Tris-borate-EDTA (TBE) buffer and the band sizes of the PCR products were detected and visualized through gel documentation unit. Graphical mapping of the markers on all 12 rice chromosome was done using web based tool *Oryza*BASE.

Results and Discussion

Landraces are reported as genetic reservoirs of many useful genes, which could be introgressed into the cultivars. With respect to rice false smut disease, resistance source has not been identified till date and the disease has been severely affecting the rice production and can become the great cause for the huge yield loss in future. Hence, there is a need to identify the resistance source against false smut. An attempt is made to identify the tolerant sources against false smut through artificial screening at ICAR-IIRR farm, Hyderabad. The tolerant sources for false smut disease were used as donors and high yielding but false smut susceptible variety Samba Mahsuri was used as the recipient parent. The polymorphism survey between two parental genotypes in two separate crosses *viz.,* Samba Mahsuri x IC379047 and Samba Mahsuri x IC334233 was carried out using 1343 SSR markers. The per cent of polymorphism was calculated by formula *i.e.,* the number of polymorphic markers to the total number of markers for each chromosome multiplied by 100. Among the 1343 markers used in polymorphic study for the Samba Mahsuri x IC379047, 100 SSR markers were found to be polymorphic **(Table 1)**. The total polymorphism percentage between parents Samba Mahsuri and landrace IC379047 recorded was 7.44%. The highest percentage of polymorphism was observed on chromosome 1 (14.08%) followed by chromosome 2 and 7 (10.71%) and lowest polymorphic percentage value was recorded on chromosome 12 (1.26%).

Among the total 1343 markers used for the polymorphism study for cross Samba Mahsuri x IC334233, and out of 868 rice markers chosen from Gramene, only 27 SSRs were found as polymorphic and were distributed on all rice chromosomes except on chromosome 7. Among 475 SSRs chosen from InDel marker set designed, 72 markers were found to be polymorphic and were also distributed among all the 12 rice chromosomes. It was found 99 out of 1343 markers as polymorphic between the two genotypes in the study **(Table 1).** The total polymorphism percentage between parents Samba Mahsuri and germplasm IC334233 recorded was 7.37%. The highest percentage of polymorphism was observed on chromosome 6 (10%), which means out of 142 markers positioned on chromosome 6, 13 markers were found to be polymorphic followed by chromosome 1 (9.15%) and lowest polymorphic percentage value was recorded on chromosome 9 (4.30%). Polymorphism percentage for all the 12 rice chromosome for two crosses are represented in **Table 2.** The banding pattern of the polymorphic markers for two crosses has shown in the gel image **(Figure 1).** The frequency distribution and representation of the polymorphic markers on rice chromosomes for two crosses has shown in **Figure 2** and **Figure 3.**



S. No	Marker	S. No	Marker
1	A09P06588	L	Ladder
2	A09P12377	1	RM23946
3	A09P21225	2	RM216
4	A10P08920	3	RM25031
5	A10P09104	4	RM21749
6	RM6470	5	RM28766
7	RM5543	6	RM5479
8	RM17624	7	RM17377
9	RM1204	8	RM18182
10	RM142	9	RM15981
11	RM3394	10	RM22763
12	RM427		

Figure 1: Identified polymorphic SSR and InDel markers between A. Recipient parent (Samba Mahsuri) and donor (IC379047); B. Recipient parent (Samba Mahsuri) and donor (IC334233)

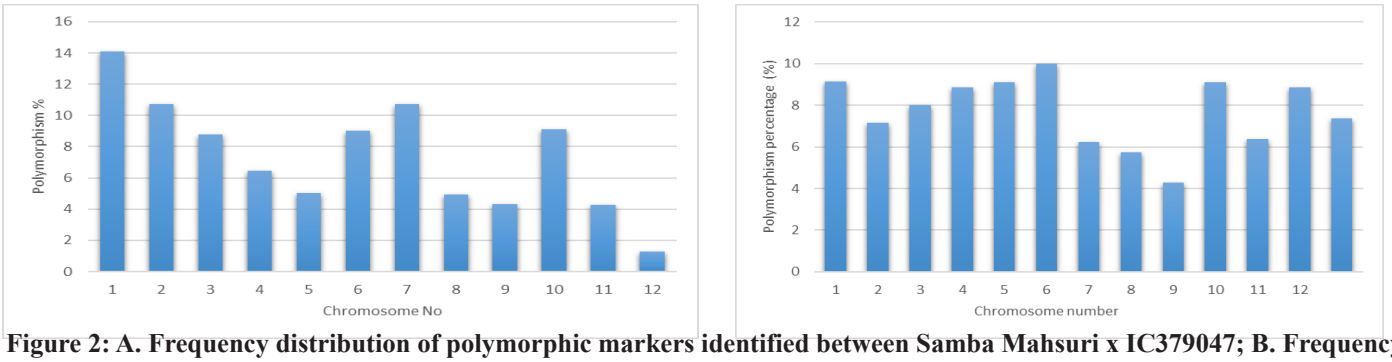


Figure 2: A. Frequency distribution of polymorphic markers identified between Samba Mahsuri x IC379047; B. Frequency distribution of polymorphic markers identified between Samba Mahsuri x IC334233

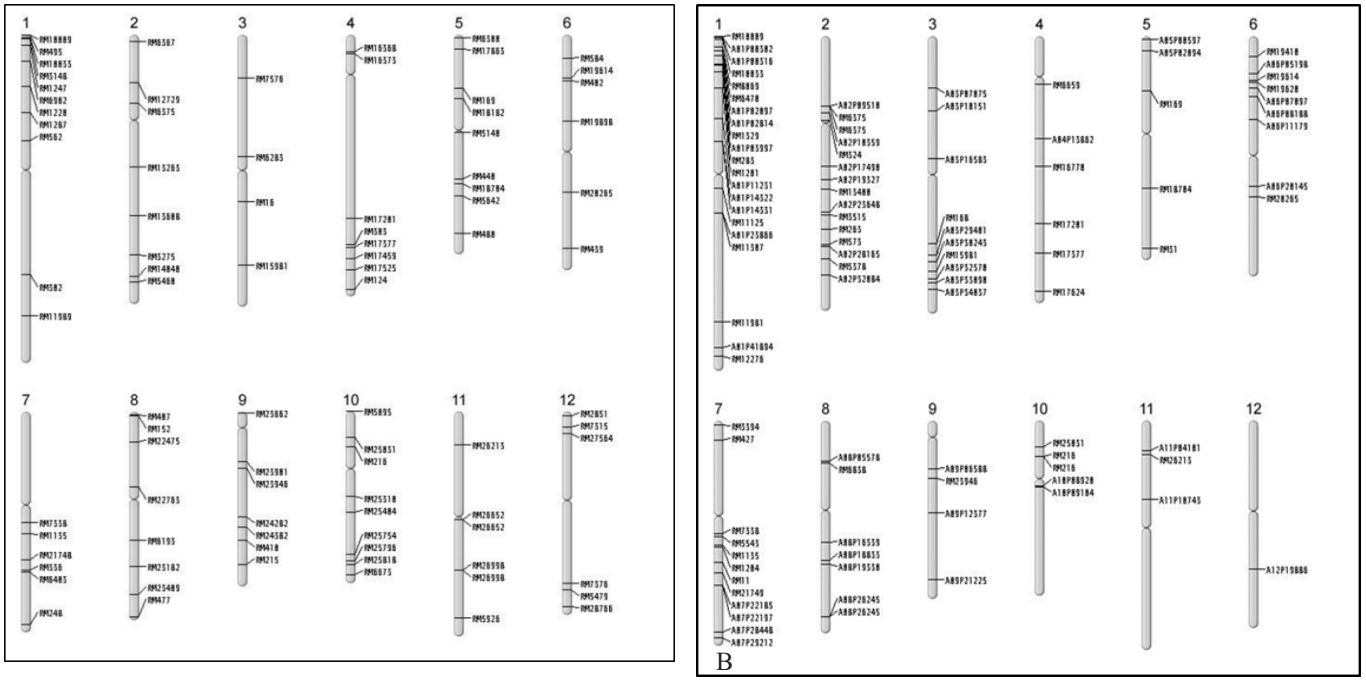


Figure 3: A. The distribution of the polymorphic markers on 12 rice chromosome for cross Samba Mahsuri x IC379047; B. The distribution of the polymorphic markers on 12 rice chromosome for cross Samba Mahsuri x IC334233

Table 1: Details of identified polymorphic SSR and InDel markers for the cross Samba Mahsuri x IC379047 and Samba Mahsuri x IC334233

Chromo some No.	Polymorphic marker identified between		Chromo some No.	Polymorphic marker identified between		Chromo some No.	Polymorphic marker identified between		Chromo some No.	Polymorphic marker identified between	
	Samba Mahsuri x IC379047	Samba Mahsuri x IC334233		Samba Mahsuri x IC379047	Samba Mahsuri x IC334233		Samba Mahsuri x IC379047	Samba Mahsuri x IC334233		Samba Mahsuri x IC379047	Samba Mahsuri x IC334233
1	RM11307	A01P00302	2	RM324	RM13608	5	RM31	A04P20855	8	RM6838	A08P05578
	RM11981	A01P11231		A02P09510	RM14040		RM169	A04P22100		A08P05578	A08P16339
	RM10009	A01P23866		A02P10359	RM5460		RM18704	RM303		A08P16339	A08P02005
	RM1329	A01P29533		A02P17490			A05P00597			A08P19330	A08P24236
	RM8069	A01P10284		A02P19327			A05P02094			A08P26245	A08P19324
	RM283	A01P02097		A02P23648		6	RM19620	A05P00597		A08P26245	RM22763
	RM1201	A01P37714		A02P28165			RM19614	A05P01457			RM407
	RM12276	A01P08402		A02P32084			RM19410	A05P00226	9	RM23946	A09P06590
	RM10033	A01P14811	3	RM168	A03P16583		RM20265	A05P22287		A09P06588	A09P11829
	RM6470	A01P17986		RM15981	A03P30243		A06P05198	A05P08866		A09P12377	RM23946
	A01P41894	A01P34148		RM15981	A03P33090		A06P07097	RM169		A09P21225	RM24382
	A01P00302	RM1220		A03P07075	A03P10151		A06P08188	RM440	10	RM216	A10P09104
	A01P00316	RM6902		A03P10151	A03P24625		A06P11179	RM480		RM25031	A10P17146
	A01P02097			A03P16583	A03P29286		A06P20145	RM5140		A10P08920	RM216
	A01P02814			A03P29401	A03P00091	7	RM11	A07P06109		A10P09104	RM25031
	A01P03997			A03P30243	RM15981		RM21749	A07P27655			RM25754
	A01P11231			A03P32570	RM7576		RM7338	A07P13588	11	RM26213	A11P10743
	A01P14322			A03P33090	RM16		RM5543	A07P15254		A11P10743	A11P04101
	A01P14331			A03P34037			RM1204	A07P23358		A11P04101	A11P22653
	A01P23866		4	RM17201	A04P08530		RM3394	A07P19443		Chr.11.8.9	A11P27658
2	RM6375	A02P10359		RM17377	A04P04207		RM427	A07P21651			A11P28918
	RM573	A02P01132		RM17624	A04P31237		RM1135				RM26998
	RM3515	A02P01124		RM142	A04P34324		A07P22185		12	A12P19886	A12P02180
	RM5378	A02P17490		RM6659	A04P11418		A07P22197				A12P23064
	RM263	A02P09510		RM16770	A04P12051		A07P28448				A12P24258
	RM6375	RM3275		A04P13862	A04P12977		A07P29212				A12P22450
	RM13400	RM12729		RM307	A04P13832						RM27564
											RM28766
											RM2851

It was observed that the InDel marker set showed higher base pair difference between the parents compared to the SSR markers used. The polymorphic markers identified from the newly designed InDel marker set showed three times higher polymorphism than RM markers indicating that the InDel marker set exhibited higher allelic diversity between the parental lines. It was also reported that around 27 SSRs were found to be common in both the crosses across the genome except at chromosome 4,7 and 12 **(Table 3).** In addition to the parental lines, the polymorphic markers identified between parents were used for the hybrid confirmation study, in which the markers clearly exhibited both the alleles in F_1s indicating the true hybrid.

Similar studies on parental polymorphism have also been carried out by various researchers. Hable *et al*., (2020) found the highest polymorphism percentage of 40.96% for chromosome 4 and on chromosome 9 recorded lowest polymorphic percentage (9%). Polymorphism percentage between the parents

Table 2: Polymorphism percentage for all the 12 rice chromosome for cross Samba Mahsuri x IC379047 and Samba Mahsuri x IC334233

S. No.	Chromo-some No.	Total No. of markers used		No. of polymorphic markers obtained		Polymorphism (%)	
		Samba Mahsuri x IC379047	Samba Mahsuri x IC334233	Samba Mahsuri x IC379047	Samba Mahsuri x IC334233	Samba Mahsuri x IC379047	Samba Mahsuri x IC334233
1	1	142	142	20	13	14.08	9.15
2	2	140	140	15	10	10.71	7.14
3	3	125	125	11	10	8.80	8.00
4	4	124	124	8	11	6.45	8.87
5	5	99	99	5	9	5.05	9.09
6	6	100	100	9	10	9.00	10.00
7	7	112	112	12	7	10.71	6.25
8	8	122	122	6	7	4.91	5.74
9	9	93	93	4	4	4.30	4.30
10	10	55	55	5	5	9.09	9.09
11	11	94	94	4	6	4.25	6.38
12	12	79	79	1	7	1.26	8.86
Total markers		**1343**	**1343**	**100**	**99**	**7.44**	**7.37**

Table 3: Common polymorphic SSR and InDel markers identified between Samba Mahsuri x IC379047 and Samba Mahsuri x IC334233

Sl. No.	Markers	Chromosome No.	Sl. No	Markers	Chromosome No.
1	A01P00302	1	15	RM169	5
2	A01P11231	1	16	A06P05198	6
3	A01P23866	1	17	A06P08188	6
4	A01P02097	1	18	A06P11179	6
5	A01P37714	1	19	A06P20145	6
6	A02P10359	2	20	A08P05578	8
7	A02P17490	2	21	A08P16339	8
8	A02P09510	2	22	RM23946	9
9	A03P16583	3	23	A10P09104	10
10	A03P30243	3	24	RM216	10
11	A03P33090	3	25	RM25031	10
12	A03P10151	3	26	A11P10743	11
13	RM15981	3	27	A11P04101	11
14	A05P00597	5			

Rajendrakasturi and URG-30 reported was 29.02%. Chandu *et al*., (2020) using 800 SSR markers observed 20. 75% of total polymorphism percentage between Samba Mahsuri and *O. rufipogon* WR119 parents. The highest polymorphism percentage for chromosome 6 (26.67%) and lowest for chromosome 10 (8.93%) was also reported by Rathi *et al.,* (2021) with total percentage of polymorphism (16.67%) between Improved Samba Mahsuri and local landrace Badshabhog using 576 random SSR markers. Kulkarni *et al.,* 2020 found total polymorphism percentage of 6.93 between the parents IR58025A and KMR-3R using 1904 genomic SSR markers**.**

Conclusion

The identified polymorphic markers on all 12 rice chromosomes are useful for linkage analysis and QTL mapping for the traits of interest in the biparental mapping populations derived from the two crosses *viz.,* Samba Mahsuri x IC379047 and Samba Mahsuri x IC334233. Genotyping and QTL mapping using these markers for false smut resistance will help in mapping QTLs for resistance to false smut disease. Development of the false smut resistant rice varieties through marker assisted breeding method is feasible using the detected polymorphic markers flanking the QTLs.

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