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Marker-assisted selection of potential restorers among red kernel rice (*Oryza sativa* L.) genotypes and evaluation of test cross progenies for their pollen and spikelet fertility

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Abstract
Commercial exploitation of heterosis in rice has been made possible by the use of cytoplasmic genetic male sterility and fertility restoration system. Development of red kernel hybrids for micro-nutrient rich food requires potential restorers which are adapted to particular situation. Conventionally, the process of screening for the trait of fertility restoration is by tedious testcross progeny evaluation. In this study, earlier reported SSR marker RM6100 targeting *Rf*4 gene and candidate gene- based functional markers, RMS-PPR9-1 targeting to *Rf*4 gene and RMS-SF21-5 targeting to *Rf*3 gene have been utilized to screen red kernel rice genotypes. Twenty-four, fifteen, and fourteen genotypes were identified as restorers, respectively, based on molecular screening results of RM6100, RMS-PPR9-1, and RMS-SF21-5 markers. Among these, a set of eleven restorer lines with various *Rf*3 and *Rf*4 combinations were selected and utilized to produce testcross progenies in combination with WA-based CMS lines, and their fertility restoration was assessed using pollen and spikelet fertility. The pollen parent MSN-15-15 and NSN-1-298 yielded testcross progenies with highest pollen fertility being 100% each, amongst all crosses attempted using CMS lines KCMS 57A and KCMS 53A as the female parents respectively. The spikelet fertility of the test cross progenies involving CMS lines KCMS 59 A and KCMS 59 A was found maximum in combination with pollen parent MO-21 (96.98%) and MSN-10-3 (94.70%) respectively. The red rice genotypes, MSN-10-3 and MSN-15-15 were classified as effective restorers for all the nine CMS lines used and these lines can be utilized in hybrid red rice breeding as potential restorers.

Keywords: CMS lines, red rice, restorers, pollen fertility, spikelet fertility, SSR markers

1. Introduction
Rice (*Oryza sativa* L; 2n=24; estimated genome size = 430Mb) is most important food crop of the world where more than 90% of the world's rice is grown and consumed in Asia alone and it has become a synonymous of food. In India, rice is grown in an area of 43.77 million hectares with the production and productivity levels of 111.75 million tonnes and 2.57 tonnes per hectare, respectively. In Karnataka, rice is grown in an area of 0.93 million hectares with the production and productivity levels of 2.6 million tonnes and 2.36 tonnes per hectare, respectively (Indiastat, 2019-20) [5].

In the traditional growing areas of Asia, rice varieties of various colors such as red, purple, black, brown, yellow and green have been cultivated and consumed. Among which, red rice is traditional pigmented rice grown in Southeast Asia for its rich in nutritional values, cultural values, fine aroma and medicinal properties (Ahuja *et al.* 2007) [1]. However, their yield level needs to be increased to meet the demand owing to recent increase in awareness about the benefits of coloured rice. Future food security of major rice growing countries lies in the development of hybrid rice varieties which have potential to increase production and productivity with good grain quality. To achieve targeted food production of increasing population and to address the malnutrition problem of people where rice is major food grain, hybrid rice technology is one of the best options.

Commercial exploitation of heterosis in rice has been made possible by the use of cytoplasmic genetic male sterility (CGMS) and fertility restoration system. Development of red kernel hybrids for micro-nutrient rich food requires restorers which are adapted to particular situation. Therefore, there is need to identify restorer lines to develop red kernel hybrids. Marker based screening for *Rf*3 and *Rf*4 fertility restorer genes can be helpful in rapid selection of restorer lines while dealing with the large quantity of genetic materials.

Microsatellite markers are useful tools that could be used as surrogates to track target traits of interest. Polymorphism was detected by SSRs to identify a marker linked to fertility restorer locus. In this context, the reported SSR marker RM6100 linked to fertility locus (*Rf*4) located at 2.3 cM distance (Singh *et al.*, 2005) [12] was used to screen sixty-two red kernelled genotypes with IR 58025B and KMR-3 as standard checks for maintainer and restorer allele, respectively. In this line, Sheeba *et al.* (2009) [11] constructed a linkage map of the genomic region around *Rf*4 and RM6100 was observed to be very close to the gene at a distance of 1.2cM. The accuracy of this marker in predicting fertility restoration was validated in 21 restorers and 18 maintainers. RM6100 amplified the *Rf*4 linked allele in a majority of the restorers with a selection accuracy of 94.87%. Therefore, RM6100 marker was utilized in marker-assisted selection for identification of restorers while screening red kernel rice genotypes rice for their fertility restoration ability.

Recent studies have reported potential candidate nuclear genes, *Rf*3 and *Rf*4, known to be associated with fertility restoration of wild-abortive cytoplasmic male sterility (WA-CMS) in rice. Apart from gene-based marker, the red kernelled genotypes were also screened with the help of candidate gene- based functional markers, RMS-PPR9-1 targeting to *Rf*4 gene and RMS-SF21-5 targeting to *Rf*3 gene reported by Pranathi *et al.* (2016) [9]. These markers were used to ascertain maintainer and restorer lines for the trait of fertility restoration and demonstrate the utility of candidate gene-specific marker for accurate identification of maintainers and restorers.

To ensure rice farmers' livelihoods and the nutritional security of the population, there is a need to develop red rice hybrids utilizing potential restorers which have potential to yield better than open pollinated genotypes and good nutrient profiles so that people consuming rice diets are supplied with adequate minerals, vitamins, proteins, carbohydrates and other health promoting agents that would benefit the farming community by increasing farmer's income and also provides health benefits. In this context, this research program was envisaged to evaluate the available red rice genotypes with the objective to identify potential restorers for future red rice breeding programme.

2. Materials and Methods

The experimental material included 62 red kernelled genotypes (Table 1), as well as IR58025B and KMR 3 as standard checks for maintainer and restorer alleles, which were utilized to select potential restorers using reported SSR markers, respectively. The molecular analysis was accomplished at the MAS lab, department of Genetics and Plant Breeding, UAS, GKVK, Bangalore. Elven red kernel rice genotypes were chosen based on molecular data analysis and utilized as pollen parents to cross with nine wild-abortive based CMS lines to produce ninety-nine testcross progenies. List of CMS lines and selected pollen parents from red kernel rice genotypes used for development of test cross progenies included in the Table. 2. During summer 2019, these testcross progenies were evaluated at College of Agriculture, V. C. Farm, Mandya. University of Agricultural Sciences, Bengaluru, it is located at latitude of 12º 30 'N, longitude of 76º 50'E and altitude of 694.65 m above mean sea level (MSL) with red sandy loam soil for pollen fertility and spikelet fertility as the indices of fertility restoration. For evaluation of new experimental hybrids, seeds of ninety-nine hybrids along with their parents were sown on 30th January 2019. Seedlings of all lines were transplanted on 5th March 2019 with two rows each with spacing of 15 x 15 cm in two rows with a single seedling per hill in Randomized Complete Block Design (RCBD) with two replications. All the recommended package of practices as per UASB were followed timely to ensure good crop establishment.

Pollen fertility of the 99 testcross progenies was tested using potassium iodide (KI) as staining agent (Rosamma and Vijayakumar, 2005) [10]. Three spikelets from individual plants in both the replications representing lower, middle and top portion of the panicle were put together on a glass slide and squashed in 1% iodine solution (1% iodine in 2% KI solution) and pollen grains were examined under light microscope. Deeply stained, fully developed and round pollen grains were counted and the percentages of pollen fertility were estimated. Pollen fertility was computed in percentage according to the formula given by Choudhary *et al*. (1981) [4].

$$\text{Pollen fertility (\%)} = \frac{\text{Number of fertile pollen}}{\text{Total number of pollen}} \text{ x } 100$$

Five main panicles of five randomly selected plants were taken to assess the spikelet fertility in test cross progenies and the numbers of filled and unfilled spikelets were counted in each of the panicles. Spikelet fertility was computed as percentage using the formula given by Choudhary *et al.* (1981) [4].

$$\text{Spikelet fertility (\%)} = \frac{\text{Number of filled spikelets}}{\text{Total number of spikelets}} \text{ x } 100$$

On the basis of pollen fertility and spikelet fertility percentage of testcross progenies, the pollen parents were classified as maintainers, partial maintainers, partial restorers and restorers based on the criteria proposed by Virmani *et al.* (1997) [14] (Table 3).

Table 1: List of red kernelled breeding lines used to select restorers during *Kharif* -2018

Sl. No.	Genotypes	(Parentage/source)	Sl. No.	Genotypes	(Parentage/source)
1	MO-13	Surekha x MO-5	34	Bramavara-7	ZAHRS, Brahmavar
2	MSN-15-15	Mandya source nursery	35	Bramavara-8	ZAHRS, Brahmavar
3	CR-2652-14	Mandya source nursery	36	Bramavara-11	ZAHRS, Brahmavar
4	AISHWARYA-5	Jyothi x BR 51-46-1	37	MSN-39-1	Mandya source nursery
5	ME-19	Mandya source nursery	38	MSN-20-3	Mandya source nursery
6	IET-14214	Mandya source nursery	39	IET-16902	Mandya source nursery
7	NSN-1-298	Mandya source nursery	40	MSN-33-1	Mandya source nursery
8	MSN-98	Mandya source nursery	41	IET-14757	Mandya source nursery
9	CR-2711-144-1	Mandya source nursery	42	IET-16750	Mandya source nursery

10	IET-19260	Mandya source nursery	43	MSN 20C	Mandya source nursery
11	MSN-13-4	Mandya source nursery	44	CBO5-022	Mandya source nursery
12	RL-102	Mandya source nursery	45	MSN 29	Mandya source nursery
13	RL-106	Mandya source nursery	46	MSN-5	Mandya source nursery
14	RL-109	Mandya source nursery	47	Jenugudu	Traditional rice variety
15	MSN-10-3	Mandya source nursery	48	Dodda Byranellu	Traditional rice variety
16	IET-14790	Mandya source nursery	49	Kari Kagga	Traditional rice variety
17	IET-19778	Mandya source nursery	50	Dunda	Traditional rice variety
18	AISHWARYA-1	Jyothi x BR51-46-1	51	Krishnaleela	Traditional rice variety
19	RP-1310-326	Mandya source nursery	52	Hola Batta	Traditional rice variety
20	4-1-2-1	Mandya source nursery	53	Rajamudi	Traditional rice variety
21	MUKTHI	Released variety from Mandya	54	Duddoge	Traditional rice variety
22	CHAMPAKA	Released variety from UAHS, Shimoga	55	Kempudaddi Gidda	Traditional rice variety
23	MSN-17	Mandya source nursery	56	Ugibatta	Traditional rice variety
24	MO-4	IR 8 x Ptb 20	57	Kartha	Traditional rice variety
25	MO-21	MO-1 x MO-6	58	Navara	Traditional rice variety
26	AC39000	Mandya source nursery	59	Sanna Mallore	Traditional rice variety
27	KCMS29A/MSN78A	Mandya source nursery	60	Mallige-1	Traditional rice variety
28	BMR-MS-1-2-4	ZAHRS, Brahmavar	61	Rajamani	Traditional rice variety
29	IRGA-318-11-6-9 2B	Mandya source nursery	62	Mahaveer	
30	MSN-15-16	Mandya source nursery	Checks		
31	KAJEJAYA	Indigenous variety	63	Jyothi	Ptb-10 x IR-8
32	BRAMAVARA-2	ZAHRS, Brahmavar	64	MSN-100	Mandya source nursery
33	BRAMAVARA-4	ZAHRS, Brahmavar			

Table 2: List of CMS lines and selected pollen parents from red kernel rice genotypes used for development of testcross progenies

Sl. No	Code	CMS lines	Sl. No	Code	Testers (All are red kernel genotypes)
1	L_1	KCMS 57 A (White kernel)	1	T_1	ME-19
2	L_2	KCMS 53 A (White kernel)	2	T_2	NSN-1-298
3	L_3	KCMS 58 A (White kernel)	3	T_3	MSN-10-3
4	L_4	KCMS 59 A (White kernel)	4	T_4	AISHWARYA-1
5	L_5	KCMS 54 A (White kernel)	5	T_5	MUKTHI
6	L_6	KCMS 29A / MSN -78 (Red kernel)	6	T_6	MO-21
7	L_7	KCMS 60 A (White kernel)	7	T_7	BRAMAVARA-8
8	L_8	KCMS 55 A (White kernel)	8	T_8	IET-14757
9	L_9	KCMS 63 A (White kernel)	9	T_9	MAHAVEER
			10	T_{10}	MSN-98
			11	T_{11}	MSN-15-15

Table 3: Classification of fertility restoration (Virmani *et al.* 1997) [14]

Category	Pollen fertility (%)	Spikelet fertility (%)
Maintainer	0.0-1.0	0.0-5.0
Partial maintainer	1.1-20.00	5.1-20.00
Partial Restorer	20.00-80.00	20.00-70.00
Restorer	>80.00	>70.00

2.1 Molecular analysis

Leaf samples of twenty-five to thirty days old seedlings of red kernelled genotypes were used for DNA extraction. DNA was extracted as per the modified Cetyl trimethyl ammonium bromide (CTAB) method (Cao and Oard, 1997). List of SSR marker RM6100 and two candidate gene-based functional markers targeting *Rf*4 and *Rf*3, primer sequence and expected PCR amplicon size for restorers and non-restorers are given in the Table 4.

Table 4: Details of the SSR markers used for identification of restorers from red kernelled genotypes.

Sl. No	Primer name	Primer sequence	Gene	Expected PCR amplicon size (bp)	
				Restorer	Non-restorer
1	RM6100	F: TCCTCTACCAGTACCGCACC R: GCTGGATCACAGATCATTGC	*Rf*4	175	165
2	RMS-PRR9-1	F: GAGTTTTGAATAGATTTACGTGTGGA R: AGTGTCCAGATTCGTAGTAATGC	*Rf*4	114	159
3	RMS-SF21-5	F: GAGTTGGGGGTCGAGAAATC R: CGTACGTGCGGCTAGGATCAA	*Rf*3	172	127

F: Forward primer R: Reverse primer

3. Results and Discussion

3.1 Marker assisted selection of maintainer and restorer lines in red kernelled genotypes of rice

The utility of CMS lines in hybrid rice breeding is determined by the availability of characterized and effective fertility restoration lines. Seventeen alleles for fertility restoration have been identified in rice and all except *rf*17 are dominant in rice. Among these, at least two genes, *viz.*, *Rf*3 (located on chromosome 1) and *Rf*4 (located on chromosome 10), are known to control fertility restoration of WA cytoplasm (Zhang *et al.*1997) [15]. Various attempts have been made to fine-map, characterize and validate the candidate genes underlying *Rf*3 and *Rf*4 (Sheeba *et al*, 2009; Ngangkham *et al.* 2010; Balaji *et al.* 2012) [8, 11, 2]. Ngangkham *et al.* (2010) [8] proposed that a gene encoding a pentatricopeptide repeat (PPR) motif-containing protein, named PPR3, located on the long arm of chromosome 10 is the candidate gene for *Rf*4, while recently, another study (Tang *et al.* 2014) [13] identified another candidate, PPR9-782 (M, I), located in the same region as PPR3 as the candidate for *Rf*4 gene. Concerning *Rf3*, Balaji *et al.* (2012) [2] reported that a gene, named SF21, encoding a pollen-specific protein to be a putative candidate for the gene.

In WA-CMS-based hybrid breeding, identification of potential restorers among the diverse rice germplasm lines is of significant importance, as genetically diverse restorer lines can be helpful in breeding hybrid with a higher magnitude of heterosis. The conventional method of screening for trait of fertility restoration by breeders is tedious and time consuming as it involves test crossing the prospective lines with selected WA-CMS lines and evaluating the testcross progenies for pollen and spikelet fertility. Lines with progenies showing 80% pollen fertility and 70% spikelet fertility are designated as restorers (Govindaraj and Virmani 1988) [5].

3.2 Validation of reported SSR marker and candidate gene-specific markers for major fertility restorer genes

The use of molecular markers linked to *Rf* genes can enhance the selection efficiency, save time and avoid the complications associated with phenotype-based screening. The genetic linkage analysis indicated that the SSR markers RM6100 reported by Singh *et al.* (2005) [12], on the long arm of chromosome 10, linked with the *Rf*4 gene at a distance of 1.2 cm has been utilized for the identification of restorers. Molecular markers targeting the candidate gene associated with the trait are more efficient in accurate identification of restorers among rice germplasm (Sheeba *et al.* 2009) [11]. The candidate gene-based functional markers targeting major fertility restoration genes, *Rf*4 and *Rf*3, are efficient in distinguishing restorers and maintainers in the present study. Kumar *et al.* (2017), reported that, the efficiency of gene-based markers, DRCG-RF4-14 and DRRM-RF3-10 for *Rf*4 (87%) and *Rf*3 (84%) genes was higher than respective gene-linked SSR markers RM6100 (80%) and RM3873 (82%).

In the present study, the red kernelled genotypes (listed in Table 1) used to develop testcross progenies were screened for earlier reported SSR marker RM6100 and two candidate gene-based functional markers targeting *Rf*4 and *Rf*3 listed in Table 4. The amplification pattern of the same is presented in the fig. 1, 2 and 3. Scoring of red kernelled genotypes of rice using these three SSR markers associated with fertility restorer (Rf) locus is also presented in Table 5. For SSR marker RM6100, out of sixty-two red kernelled genotypes screened, twenty-four lines showed the presence of *Rf*4 by amplifying 175- bp size fragment, and thirty-four lines showed the absence of *Rf*4 by amplifying 165-bp size and four showed the heterozygous amplification pattern (Fig. 1). Based on these results, we could confirm that, out of sixty-two lines, twenty-four are restorers, thirty-four are non-restorers and four lines may be partial restorers. Apart from gene-based marker, these red kernelled genotypes were also screened with the help of candidate gene- based functional markers, RMS-PPR9-1 targeting to *Rf*4 gene and RMS-SF21-5 targeting to *Rf*3 gene reported by Pranathi *et al.* (2016) [9].

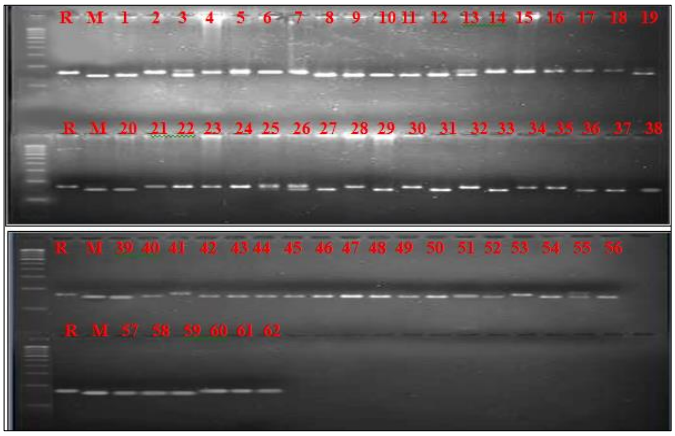

Fig 1: Amplification pattern of the SSR marker, RM6100 targeting the *Rf4* gene

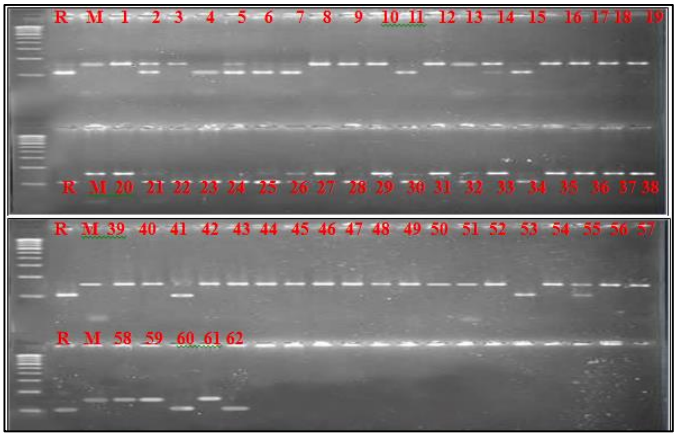

Fig 2: Amplification pattern of the functional marker, RMS-PRR 9-1, targeting the candidate gene for *Rf4*

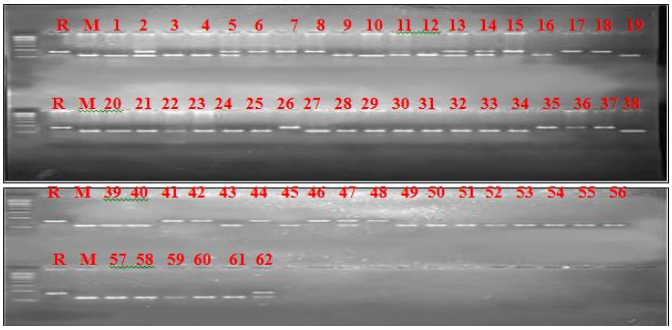

Fig 3: Amplification pattern of the functional marker, RMS-SF 21-5, targeting the candidate gene for Rf3

Out of sixty-two lines screened, fifteen showed the presence of *Rf*4 by amplifying 114-bp size fragment, forty-one showed the absence by amplifying 159- bp product size and six showed heterozygous amplification pattern for RMS-PPR9-1

(Fig. 2). Based on molecular screening results of RMS-PPR9-1, we could assume that out of sixty-two genotypes, fifteen are restorers, forty-one are non- restorers and six are partial restorers. Similarly, for another candidate gene-based functional marker, RMS-SF21-5, fourteen lines showed presence of *Rf*3 by amplifying 172-bp size fragment, forty-one showed the absence by amplifying 127-bp product size and seven showed heterozygous amplification pattern (Fig. 3). Based on molecular screening results of RMS-SF21-5 targeting *Rf*3, we could assume that out of sixty-two genotypes, fourteen are restorers, forty-one are non- restorers and seven are partial restorers. Among these, a set of eleven restorer lines with various *Rf*3 and *Rf*4 combinations were selected and utilized to produce testcross progenies in combination with WA-based CMS lines, and their fertility restoration was assessed using pollen and spikelet fertility.

3.3 Phenotypic confirmation of fertility restorability of male parents: Using pollen fertility and spikelet fertility as the indices of fertility restoration, the pollen parent of test cross progenies was categorized as a restorer (R) and partial restorer (PR). The data on pollen and spikelet fertility of test cross progenies have been presented in Table 6. The pollen and spikelet fertility in the testcross progenies ranged from 11.82 to 100.0% and 7.39 to 96.98%, respectively. The pollen parent MSN-15-15 and NSN-1-298 yielded testcross progenies with highest pollen fertility being 100% each, amongst all crosses attempted using CMS lines KCMS 57A and KCMS 53A as the female parents respectively. Similarly, MO-21 for KCMS 57A, MSN-15-15 for KCMS 58A, NSN-1-298 for KCMS 60A, ME-19 for KCMS 58A and MO-21 for KCMS 55A produced testcross progenies with highest pollen fertility being, 99.81, 98.34, 97.37, 97.34 and 97.13%, respectively, amongst all crosses derived from these CMS lines. It was observed that for each CMS line, the same pollen parent did not yield testcross progenies with highest pollen and spikelet fertility, although pollen fertility exhibited a significant positive association with spikelet fertility.

Table 5: Scoring of red kernelled genotypes of rice using three SSR markers associated with fertility restorer (*Rf*) locus for maintainer and restorer type allele

Genotype	RM6100 (*Rf4*)	RMS-PRR 9-1 (*Rf4*)	RMS-SF 21-5 (*Rf3*)	Genotype	RM6100 (*Rf4*)	RMS-PRR 9-1 (*Rf4*)	RMS-SF 21-5 (*Rf3*)
MO-13	M	M	M	BRAMAVARA-2	R	R	M
MSN-15-15	R	H	H	BRAMAVARA-4	M	M	M
CR-2652-14	H	M	M	BRAMAVARA-7	R	R	M
AISHWARYA-5	R	R	M	BRAMAVARA-8	R	M	R
ME-19	R	H	H	BRAMAVARA-11	M	M	R
IET-14214	R	R	R	MSN-39-1	M	M	R
NSN-1-298	R	R	R	MSN-20-3	M	M	M
MSN-98	M	M	R	IET-16902	M	M	M
CR-2711-144-1	M	M	M	MSN-33-1	M	M	M
IET-19260	M	M	M	IET-14757	R	R	R
MSN-13-4	M	R	M	IET-16750	M	M	R
RL-102	M	M	M	MSN 20C	M	M	M
RL-106	H	M	H	CBO5-022	M	M	R
RL-109	R	H	H	MSN 29	M	M	M
MSN-10-3	R	R	R	MSN-5	M	M	R
IET-14790	R	M	M	JENUGUDU	M	M	H
IET-19778	R	M	R	DODDA BYRANELLU	M	M	R
AISHWARYA-1	R	M	R	KARI KAGGA	M	M	M
RP-1310-326	M	M	M	DUNDA	M	M	M
4-1-2-1	M	M	M	KRISHNALEELA	M	M	M
MUKTHI	R	R	M	HOLA BATTA	M	M	M
CHAMPAKA	R	R	M	RAJAMUDI	R	R	M
MSN-17	R	R	M	DUDDOGE	M	M	M
MO-4	R	R	M	KEMPUDADDI GIDDA	H	H	M
MO-21	H	R	M	UGIBATTA	M	M	M
AC39000	M	H	R	KARTHA	M	M	M
KCMS29A/MSN78 B	M	M	M	NAVARA	M	M	M
BMR-MS-1-2-4	R	R	M	SANNA MALLORE	M	M	M
IRGA-318-11-6-9 2B	M	M	M	MALLIGE-1	R	R	M
MSN-15-16	R	R	M	RAJAMANI	R	M	M
KAJEJAYA	M	M	M	MAHAVEER	R	M	H

The spikelet fertility of the test cross progenies involving CMS lines KCMS 59 A and KCMS 57A was found maximum in combination with pollen parent MO-21 (96.98%) and MSN-10-3 (94.70%) respectively. Similarly, MSN-10-3 for KCMS 63 A, MO-21 for KCMS 63 A, IET-14757 for KCMS 60 A, MSN-10-3 for KCMS 59 A and MO-21 for KCMS 29A / MSN -78 produced testcross progenies with high spikelet fertility being, 94.00, 92.82, 91.37, 91.34 and 90.26%, respectively.

Based on pollen fertility, sixty-seven hybrids were found to be complete fertile and thirty-two hybrids as partial fertile. Similarly, based on spikelet fertility, sixty hybrids were found to be complete fertile and thirty-nine hybrids as partial fertile. Based on pollen fertility, the CMS line KCMS 54A had the

highest number of effective restorers (10) followed by KCMS 29A / MSN -78, KCMS 55A and KCMS 63 A which had 9 effective restorers each. The CMS lines KCMS 60 A and KCMS 59 A had 8 and 7 effective restorers, respectively. Similarly, the restorers, MSN-10-3 and MSN-15-15 were classified as effective restorers for all the nine CMS lines (Table 7).

Table 6: Classification of crosses into maintainers and restorers based on pollen fertility, spikelet fertility and both

Hybrid	Pollen fertility		Spikelet fertility		Based on both
	Per cent	Class	Per cent	Class	
L_1 x T_1	85.79	R	77.45	R	R
L_1 x T_2	80.10	R	82.74	R	R
L_1 x T_3	85.95	R	94.70	R	R
L_1 x T_4	57.57	PR	8.66	PR	PR
L_1 x T_5	80.25	PR	75.95	R	R
L_1 x T_6	99.11	R	54.19	PR	R/PR
L_1 x T_7	56.28	PR	14.48	PR	PR
L_1 x T_8	39.48	PR	46.29	PR	PR
L_1 x T_9	18.06	PR	15.35	PR	PR
L_1 x T_{10}	72.24	PR	28.21	PR	PR
L_1 x T_{11}	100.00	R	77.79	R	R
L_2 x T_1	81.84	R	72.97	R	PR/R
L_2 x T_2	100.00	R	63.40	PR	R/PR
L_2 x T_3	80.21	R	72.82	R	R
L_2 x T_4	32.14	PR	7.39	PR	PR
L_2 x T_5	82.57	R	70.68	R	PR/R
L_2 x T_6	73.61	PR	48.08	PR	PR
L_2 x T_7	28.33	PR	30.49	PR	PR
L_2 x T_8	80.21	R	87.73	R	R
L_2 x T_9	18.94	PR	26.28	PR	PR
L_2 x T_{10}	78.26	PR	39.17	PR	PR
L_2 x T_{11}	88.22	R	80.28	R	R
L_3 x T_1	97.34	R	85.67	R	R
L_3 x T_2	58.20	PR	52.58	PR	PR
L_3 x T_3	91.38	R	75.31	R	R
L_3 x T_4	31.15	PR	65.42	PR	PR
L_3 x T_5	80.12	R	70.63	R	R
L_3 x T_6	80.25	R	70.86	R	R
L_3 x T_7	55.17	PR	58.97	PR	PR
L_3 x T_8	67.44	PR	53.93	PR	PR
L_3 x T_9	77.45	PR	54.72	PR	PR
L_3 x T_{10}	79.59	PR	38.37	PR	PR
L_3 x T_{11}	98.34	R	88.95	R	R
L_4 x T_1	92.08	R	86.42	R	R
L_4 x T_2	55.57	PR	65.04	PR	PR
L_4 x T_3	86.08	R	91.34	R	R
L_4 x T_4	58.11	PR	44.12	PR	PR
L_4 x T_5	83.11	R	88.46	R	R
L_4 x T_6	86.68	R	96.98	R	R
L_4 x T_7	89.87	R	86.71	R	R
L_4 x T_8	86.46	R	72.91	R	R
L_4 x T_9	45.43	PR	14.21	PR	PR
L_4 x T_{10}	89.65	R	33.45	PR	R/PR
L_4 x T_{11}	81.02	R	77.77	R	R
L_5 x T_1	80.29	R	80.18	R	R
L_5 x T_2	80.77	R	77.39	R	R
L_5 x T_3	93.45	R	82.88	R	R
L_5 x T_4	96.77	R	71.66	R	R
L_5 x T_5	89.45	R	70.10	R	R
L_5 x T_6	94.52	R	86.42	R	R
L_5 x T_7	80.69	R	84.26	R	R
L_5 x T_8	80.52	R	70.20	R	R
L_5 x T_9	91.67	R	59.32	PR	R/PR
L_5 x T_{10}	78.70	PR	64.63	PR	PR
L_5 x T_{11}	88.22	R	82.45	R	R
L_6 x T_1	11.82	PR	63.90	PR	PR
L_6 x T_2	29.04	PR	42.07	PR	PR
L_6 x T_3	93.33	R	88.20	R	R

L_6 x T_4	86.84	R	72.60	R	R
L_6 x T_5	88.36	R	73.68	R	R
L_6 x T_6	80.61	R	90.26	R	R
L_6 x T_7	84.62	R	79.05	R	R
L_6 x T_8	86.93	R	70.13	R	R
L_6 x T_9	91.37	R	73.67	R	R
L_6 x T_{10}	90.65	R	76.37	R	R
L_6 x T_{11}	81.79	R	74.89	R	R
L_7 x T_1	84.38	R	73.22	R	R
L_7 x T_2	97.37	R	87.35	R	R
L_7 x T_3	94.92	R	90.11	R	R
L_7 x T_4	88.14	R	70.19	R	R
L_7 x T_5	37.50	PR	64.82	PR	PR
L_7 x T_6	87.12	R	81.45	R	R
L_7 x T_7	47.34	PR	54.03	PR	PR
L_7 x T_8	83.21	R	91.37	R	R
L_7 x T_9	77.63	PR	48.05	PR	PR
L_7 x T_{10}	87.36	R	58.20	PR	R/PR
L_7 x T_{11}	87.86	R	81.66	R	R
L_8 x T_1	80.35	R	70.98	R	R
L_8 x T_2	65.40	PR	65.51	PR	PR
L_8 x T_3	83.50	R	90.24	R	R
L_8 x T_4	81.76	R	73.46	R	R
L_8 x T_5	34.52	PR	62.59	PR	PR
L_8 x T_6	97.13	R	57.03	PR	R/PR
L_8 x T_7	87.52	R	79.30	R	R
L_8 x T_8	84.38	R	85.66	R	R
L_8 x T_9	80.01	R	79.32	R	R
L_8 x T_{10}	93.45	R	34.77	PR	PR
L_8 x T_{11}	88.13	R	72.78	R	R
L_9 x T_1	81.43	R	76.62	R	R
L_9 x T_2	71.54	PR	53.63	PR	PR
L_9 x T_3	89.74	R	94.00	R	R
L_9 x T_4	96.94	R	78.78	R	R
L_9 x T_5	93.75	R	61.07	PR	R/PR
L_9 x T_6	84.48	R	92.82	R	R
L_9 x T_7	77.58	PR	50.03	PR	PR
L_9 x T_8	83.09	R	83.03	R	R
L_9 x T_9	92.39	R	64.64	PR	R/PR
L_9 x T_{10}	84.76	R	65.03	PR	R/PR
L_9 x T_{11}	80.50	R	87.04	R	R

Table 7: Classification of pollen parent into Restorer (R) and Partial Restorers (PR) across nine CMS lines

Lines/Restorers	L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8	L_9
T_1	R	R	R	R	R	PR	R	R	R
T_2	R	R/PR	PR	PR	R	PR	R	PR	PR
T_3	R	R	R	R	R	R	R	R	R
T_4	PR	PR	PR	PR	R	R	R	R	R
T_5	R	R	R	R	R	R	PR	PR	R/PR
T_6	R/PR	PR	R	R	R	R	R	R/PR	R
T_7	PR	PR	PR	R	R	R	PR	R	PR
T_8	PR	R	PR	R	R	R	R	R	R
T_9	PR	PR	PR	PR	R/PR	R	PR	R	R/PR
T_{10}	PR	PR	PR	R/PR	PR	R	R/PR	PR	R/PR
T_{11}	R	R	R	R	R	R	R	R	R

R-Restorer, PR-Partial Restorer

3.4 Differential fertility restoration ability of restorers

Although the CMS lines included in the present study belonged to the same cytoplasmic source, the restorer lines showed differential pollen and spikelet fertility in combination with different CMS lines. The differential reaction of CMS lines with same set of restorer lines reflected that the nuclear background of the female parent also had a profound influence on the expression of fertility in test cross hybrid progenies. Katara *et al.* (2017) reported that differential fertility restoration efficiency observed in F_1s due to variable allelic combinations of *Rf*3 and *Rf*4 genes and also due to the nuclear and cytoplasmic genome interaction provides new leads for deeper insight into the molecular mechanism of fertility restoration.

4. Conclusion

Red kernel rice genotypes ME-19, MSN-10-3, MSN-15-15, IET-14757, MSN-98, and NSN-1-298 were identified as

better restorers based on both phenotypic and molecular data analysis, with only two genotypes, MSN-10-3 and MSN-15-15, classified as effective restorers for all nine CMS lines used, and these lines can be used as potential restorers for developing high yielding and nutrition rich red rice hybrids in the future. Since, markers utilized under study could not able to distinguish the partial restorers from complete restorers, there is need to develop more candidate gene specific markers for Rf locus.

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