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Screening of rice (*O. sativa* L.) germplasm accessions for fertility restoration genes *Rf3* and *Rf4*

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Abstract
To study the identification of restorers with different molecular markers which are linked to fertility restorer genes *Rf3* and *Rf4* of WA-CMS system, 43 parental lines were screened for fertility restoration with the help of molecular markers linked to major fertility genes *Rf3* and *Rf4*. The SSR primer RM6100 linked to *Rf4* gene on chromosome 10 and SF 21-5 linked to *Rf3* gene on chromosome 1 are used efficiently for identifying restorer lines in crop improvement programmes. Therefore, these markers are useful tool for evaluating large number of breeding lines to know about their fertility restoration in a short period of time without generating and evaluating large number of test crosses. Ten potential restorers identified (*Rf4* and *Rf3* present) with hundred percentage efficiency based on molecular screening, if candidate genes based markers are developed and validated for both *Rf4* and *Rf3* genes.

Keywords: Fertility restoration, Rf4, Rf3, molecular markers, WA-CMS

Introduction
Rice (*Oryza sativa* L.) is the most important staple food for more than half of the world's population.Its cultivation is of immense importance for food security of Asia, where more than 90% of the global rice is produced and consumed. Heterosis breeding has been successfully utilised to increase rice productivity in China with the development of F1 rice hybrids using cytoplasmic male sterility and fertility restoration system. The hybrids out yielded the best pure line varieties by 20-30 % (Lin and Yuan, 1980) [2]. Hence, a technology with such a potential may prove very relevant to developing countries like India. However, still it is at its infancy because of the non-availability of stable male sterile lines, maintainers and a low degree of fertility restoration by restorer lines. Therefore, it is necessary to identify maintainers and restorers for commercial exploitation of hybrid rice in India. In rice, primarily, three types of CMS systems are deployed for commercial hybrid seed production, namely Wild Abortive (WA), Bao Tai (BT) and Honglian (HL). Among these three, the WA cytoplasm is the most widely used since it is a more stable system and the pollen sterility is almost nearly complete (Shinjyo and Omura 1966) [4]. Pollen abortion in WA-type CMS is sporophytic, forming typical abortive pollen (Huang *et al*. 2003). Earlier studies designated that a major dominant gene controls fertility restoration of WA-CMS (Huang *et al*. 1986; Anandakumar and Subramaniam 1992). Later, it was discovered that fertility restoration is controlled by two independent dominant nuclear genes with one stronger in action than the other (Young and Virmani 1984; Virmani *et al*. 1986). Hybrid rice seed production technology using CGMS system, the combination of a CMS line, maintainer line and restorer line carrying the restorer gene (s) (*Rf)* For fertility restoration it is required for the development of superior hybrids. The most widely used cytoplasmic male sterile (CMS) in rice is based on wild abortive (WA) cytoplasm derived from *Oryza sativa* f. sp. *spontanea*. WA based CMS lines are highly stable and also their pollen sterility is complete. Fertility restoration of WA-CMS is extensively investigated trait. All the studies have consistently demonstrated that two dominant independent loci controlling fertility restoration of WA-CMSsystem. Screening of fertility restoration genes in the elite germplasm will help to develop combination to achieve higher yields among the hybrids. The present study was focused on screening of diverse set of rice germplasm lines for fertility restoration genes.

Materials and Methods

The plant material for the present study included that seventy two genotypes and three CMS lines in that two received from International Rice Research Institute (IRRI), Philippines. IR58025A, IR79156A and one from APMS maruteru research station west Godavari, Andra Pradesh. APMS6A were included in present study.

Experimental site

The present research study conducted during *rabi*, 2014 *karif* at Indian Institute of Rice Research, Research farm at ICRISAT campus. Experimental farm is situated at 17.53ºN latitude, 78.27ºE longitude and altitude of 545m above mean sea level. High yielding Varieties (HYVs), Released cultivars, Exotic germplasm (NERICA, IRHTN) from Africa were collected. A Total of 43 varieties were grown in source nursery at IIRR, ICRISAT campus in *rabi*, 2014-15. Total genomic DNA was isolated from young leaves by c-TAB protocol of Dellaporta *et al*., PCR reactions was carried out using 50 ng/l of template DNA, containing 2.5 mM of each dNTP, 0.5 µl of each forward and reverse primer, 0.2 µl of Taq DNA polymerase, 10X PCR reaction buffer in a total volume of 10µ l in thermal cycler (Eppendorf, USA). The amplified PCR products along with 100 bp molecular marker (Bangalore Genie, India) were separated on a 3.0% Seakem® LE agarose gel stained with ethidium bromide and documented using Gel documentation system (AlphaInnotech).

Results and Discussion

Based on the banding pattern gels were scored for presence and absence of bands as restorers and non- restorers. The 43 varieties without any information about fertility restoration status along with one known restorer (KMR-3) were screened with two SSR primers namely RM6100 linked to fertility restorer gene *Rf4* located on chromosome 10 and SF 21-5 linked to fertility restorer gene *Rf3* located on chromosome 1. The lines were scored as restorers based on the presence of restorer specific allele band. The amplification pattern of SSR markers linked to *Rf4* and *Rf3* genes were shown in the Figs. 1 & 2. Among the forty three parental lines screened for the presence of the fertility restorer gene *Rf4* with RM 6100 primer 23 lines were identified *Rf4* present, these lines may be restorers and 20 lines were *Rf4* absent. In same way for *Rf3* gene with SF 21-5 primer, twenty two accessions were identified with Rf3 present allele and hence these lines are restorers and twenty one accessions were non restorer lines. 10 parental lines identified to possess both the *Rf4* and *Rf3* genes. Based on spikelet fertility out of forty three one line maintainer line (0%), six partial maintainer lines, (0.1 -50 ± 5%), ten partial restorer lines (50 – 75 ± 5%), and 26 Restorer lines (>75.) (Table1). Identification of effective restorers and maintainers is the primary step in three line heterosis breeding. The identified lines from the present study can be immediately utilized in hybrid rice breeding programme. Further the primers are also useful for identification of restorer lines for marker assisted selection. However, the SSR primers RM6100 and RM SF 21-5 can be utilized to screen the germplasm accessions to identify restorers with 80 to 85% efficiency.

The SSR primer RM6100 linked to *Rf4* gene amplified restorer specific allele in forty three lines based on spikelet fertility and showed 84% efficiency in identifying restorer lines. With respect SF 21-5 linked to *Rf3* gene showed 81% efficiency in comparison with spikelet fertility, RM6100 and RM SF 21-5 can be utilized to screen the accessions to identify restorers.

Table 1: Primer sequences of Rf4 & Rf3 linked markers

Molecular marker/ primer	Sequence (5' to 3')	Gene linked	Chromosomal location	Amplification product size in restorer (base pair)	Amplification product size in non-restorer (base pair)
RM6100	TTCCCTGCAAGATTCTAGCTACACC TGTTCGTCGACCAAGAACTCAGG	*Rf4*	10	185	175
RM SF 21-5	ACTTACACAAGGCCGGGAAAGG TGGTAGTGGTAACTCTACCGATGG	*Rf3*	1	188	175

Table 2: Screening results of *Rf4* (RM6100) and *Rf3* (SF 21-5)

S. No.	Genotype	Gel Code	Spikelet fertility	Remarks	RM6100 (*Rf4*)	SF 21-5 (*Rf3*)
1	2012-131	29	90.5	R	*Rf4*	*Rf3*
2	2010-112	5	55.5	PR	NO	NO
3	2015-110	6	75.5	R	NO	NO
4	2012-110	9	52.2	PR	NO	NO
5	2012-128	10	75.1	R	*Rf4*	*Rf3*
6	EC460	12	79	R	*Rf4*	NO
7	MANDYA VIJAYA	17	77	R	*Rf4*	*Rf3*
8	2012-130	18	83	R	NO	*Rf3*
9	SUGANDAMATI	19	82	R	NO	*Rf3*
10	2014-130	20	40.5	PM	*Rf4*	NO
11	STYASRI	21	88.6	R	*Rf4*	NO
12	DHANARASI	23	72.8	PR	NO	NO
13	DRRDHAN46	24	82.9	R	*Rf4*	NO
14	DRRDHAN43	25	44.5	PM	NO	NO
15	DRRDHAN42	26	43.5	PM	NO	NO
16	BPT5204	27	76.5	R	*Rf4*	*Rf3*
17	2010-131	28	82	R	NO	*Rf3*
18	RNR15048	4	42.5	PM	NO	NO
19	2012-126	30	75.5	R	*Rf4*	NO
20	2012-113	31	85	R	*Rf4*	NO

21	AJAYA	32	89.3	R	*Rf4*	*Rf3*
22	2012-105	33	54.8	PR	NO	*Rf3*
23	2012-106	34	59	PR	NO	*Rf3*
24	2014-315	39	87.7	R	NO	*Rf3*
25	RPBIO226	36	89.5	R	NO	*Rf3*
26	PRATAP SINGH-1	37	81	R	NO	*Rf3*
27	PR116	38	64.3	PR	*Rf4*	NO
28	2012-112	35	76.5	R	*Rf4*	NO
29	NDR359	40	1.3	M	NO	NO
30	MTU1010	41	72.5	PR	*Rf4*	*Rf3*
31	KNM118	42	69	PR	*Rf4*	NO
32	IR64	43	22.5	PM	NO	NO
33	EC444	44	79	R	NO	*Rf3*
34	SAMPADA	68	74.5	R	*Rf4*	*Rf3*
35	CPAU-23	70	41.5	PM	*Rf4*	NO
36	2014-116	71	84.5	R	*Rf4*	NO
37	2009-110	72	83.2	R	*Rf4*	*Rf3*
38	2011-115	79	62	PR	*Rf4*	NO
39	2012-133	80	75.3	R	*Rf4*	*Rf3*
40	CR DHAN 201	81	67.5	PR	NO	*Rf3*
41	NAVEEN	84	84	R	*Rf4*	*Rf3*
42	JAYA	92	74.7	R	NO	*Rf3*
43	N22	93	90.5	R	*Rf4*	*Rf3*

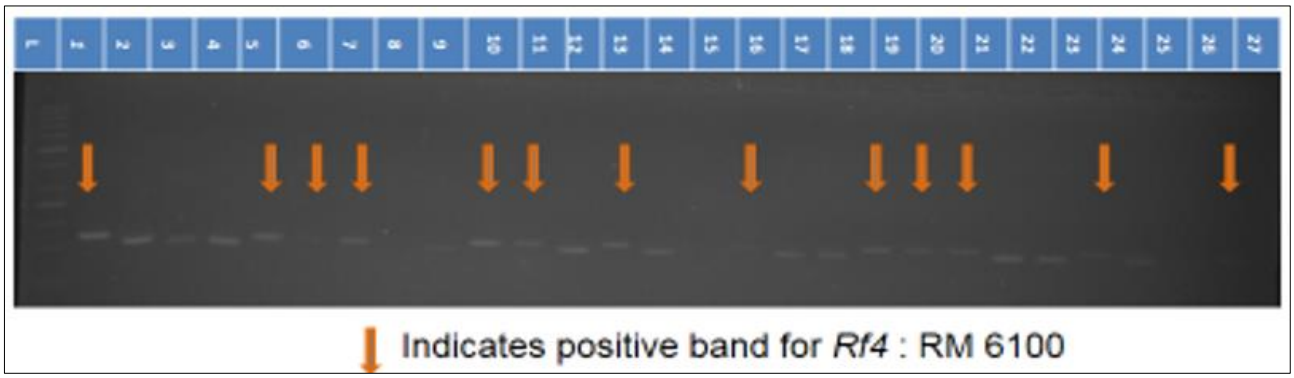

Fig 1: Parental lines conformed with *Rf4* RM 6100 (Arrow marks indicating positive Acc.)

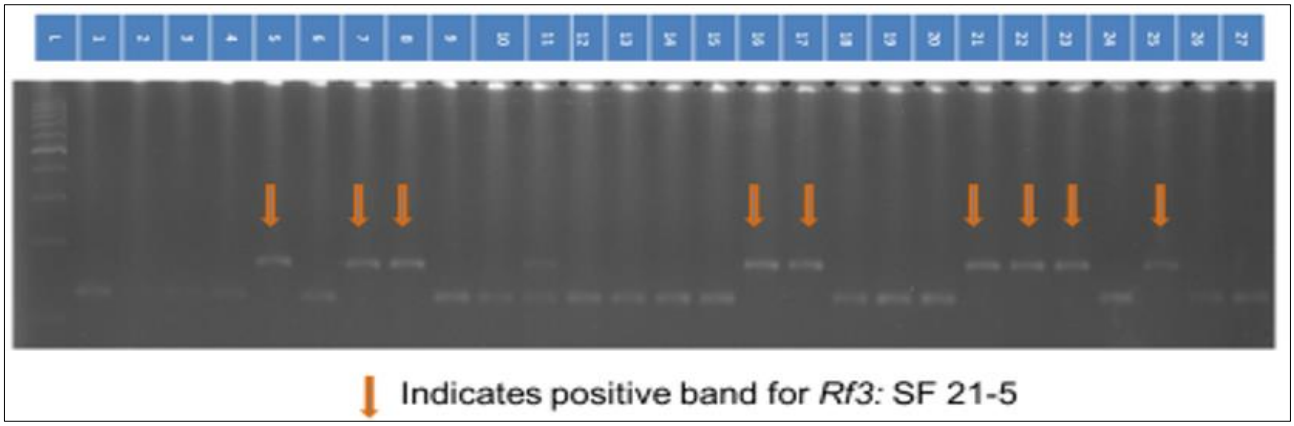

Fig 2: Parental lines conformed with *Rf3* marker SF 21-5 (Arrow showing *Rf3* positive Acc.)

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