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IDENTIFICATION OF FERTILITY RESTORER LINES AND VALIDATION OF MARKER RM 6100 FOR DEVELOPMENT OF NEW RICE HYBRIDS

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ABSTRACT

An experiment was conducted at Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist-Ahmednagar during 2014. Cytoplasmic male sterile (CMS) line IR58025A having wild abortive (WA) was crossed with 25 genotypes to assess their maintainer and restorer behaviour. Among the genotypes tested, IR55838, IR48715, IR54742 and SYE 2001 were considered as potential restorers for the CMS line IR58025A as their respective hybrids have shown more than 80% pollen fertility and more than 75% spikelet fertility. Rice varieties, Phule Radha and RTN 24 produced sterile hybrids. Simultaneously the accuracy of the marker RM6100 in predicting fertility restoration was validated in 7 restorers and 10 maintainers. RM 6100 amplified the *Rf4* linked allele in the restorers and was found to be perfectly co-segregating with phenotype.

(Key words: Fertility restorer, male sterility, Rice hybrids, RF locus, SSR markers,validation)

INTRODUCTION

It is necessary to identify maintainers and restorers from the germplasm for development of superior rice hybrids. Test crosses are used to identify restorers and maintainers in the three line heterosis breeding (Akhter *et al.,* 2008). Identification of maintainers or restorers by test cross method is time consuming and laborious. Validation of molecular markers tightly linked to *Rf* genes for fertility restoration so that marker-aided selection (MAS) which can ease out the identification more efficiently. Present investigation was therefore undertaken to identify new maintainers/restorers and validation of highly efficient SSR marker RM6100 linked to *Rf* locus for identification of fertility restorers or maintainer trait.

MATERIALS AND METHODS

The male sterile line IR58025A based on wild abortive (WA) cytoplasmic source and 25 genetically diverse cultivated genotypes were used for crossing and 25 F_1's were evaluated during *kharif* 2014 for pollen and spikelet fertility.

Estimation of Pollen fertility/sterility

The pollen sterility was scored by using 1% iodine–potassium iodide stain. Unstained, half stained, irregular, shrivelled and empty pollen grains were classified as sterile while, well filled, dark stained and round pollen grains were recorded as fertile. Plants with both the types were classified as partial fertile (Chaudhary *et al.,* 1981) (Fig. 1).

Estimation of Spikelet fertility

Emerging panicles of F_1 hybrid were bagged before flowering with glassine paper bags prior to anthesis to prevent cross-pollination. Bagged panicles were harvested 25-30 days after flowering and number of filled and unfilled spikelets were counted and proportion to total number of spikelets was worked out to determine spikelet fertility percentage.

$$\text{Spikelet fertility (\%)} = \frac{\text{Number of filled spikelets in a panicle}}{\text{Total number of filled and unfilled spikelets in the panicle}} \times 100$$

Based on the scores recorded for pollen fertility and spikelet fertility, the genotypes were classified as maintainers and restorers (Virmani *et al.,* 1997).

Marker Aided Selection

The SSR marker RM6100 has been reported to be linked to *Rf-4* (Singh *et al.,* 2005). Seven known restorers, eight known and two newly identified maintainer lines for wild abortive (WA) type of cytoplasm were analyzed. The genomic DNA was isolated using leaf sample of thirty days old seedlings. DNA was prepared as per the modified Cetyl trimethyl ammonium bromide (CTAB) method (Cao and Oard, 1997). PCR amplication was carried out following the standard procedures. The bands generated by microsatellite primer were assigned and scored as M for maintainer type allele and R for restorer type alleles. The allelic pattern of polymorphic marker RM6100 on each genotype was compared with pollen fertility and spikelet fertility to know the efficiency of this SSR marker.

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Table 1. Categorization of genotypes based on pollen fertility and spikelet fertility (%) in F_1

Sr.no.	Genotypes	Country of origin	Pollen fertility in hybrid (%)	Spikelet fertility in hybrid (%)	Inference on test line
1	RDN-2000-2-16-20	India	55.12	54.65	**PR**
2	IR58025B	India	00.00	00.00	**M**
3	Phule Samrudhi	India	35.35	38.45	**PM**
4	RTN 24	India	01.00	00.00	**M**
5	SYE-35-4-16-63	India	32.15	47.25	**PM**
6	VDN-10-18	India	60.35	62.65	**PR**
7	Indrayani	India	01.00	00.00	**PR**
8	Phule Radha	India	00.50	00.00	**M**
9	DRR2B	India	01.00	00.00	**M**
10	DRR3B	India	01.00	00.00	**M**
11	IR 65483	India	83.15	84.25	**R**
12	IR 62829B	India	01.00	00.00	**M**
13	Kundalika	India	66.12	68.71	**PR**
14	IR 68886B	India	01.00	00.00	**M**
15	IR 48715	India	86.72	87.10	**R**
16	IR 55838	India	88.15	86.35	**R**
17	IR-72	IRRI	83.12	84.65	**R**
18	IR 69715	India	84.85	86.35	**R**
19	IR 68888B	India	01.00	00.00	**M**
20	VDN-1-3-5-1-4	India	84.85	86.43	**R**
21	PKV HMT	India	46.12	47.65	**PM**
22	SYE 2001	India	86.30	87.56	**R**
23	IR 54742	India	85.60	88.40	**R**
24	IR 69628B	India	01.00	00.00	**M**
25	IR 68897B	India	01.00	00.00	**M**

R- Restorer, PR-partial restorer, PM-partial maintainer, M-maintainer

RESULTS AND DISCUSSION

Twenty five F_1 hybrids produced on CMS line IR58025A behaved differently with regard to pollen and spikelet fertility (Table 1).The pollen fertility ranged from 0.0 to 88.15% and spikelet fertility varied from 0.0 to 88.40%. Out of the 25 F_1 hybrids tested, 8 were fertile with pollen fertility 78% and spikelet fertility >75 % (more than 75 %)and 10 were sterile (pollen fertility less than 1 % and spikelet fertility zero per cent). The remaining 7 hybrids, 4 were partial restorer (pollen and spikelet fertility less than 50 per cent) and 3 partial maintainers. IR55838, IR48715, IR54742 and SYE 2001 can be considered as potential restorers for the CMS. The identified restorer lines can be used as pollen parent in developing new commercial hybrid variety.

Cross IR58025A X IR55838 showed the highest pollen fertility (88.15%) and spikelet fertility (86.35%) followed by crosses IR58025A X IR48715, IR58025A X SYE-2001 and IR58025A X IR54712. Therefore, these crosses may be tested for getting high yielding rice hybrids. All F_1 plants of these pollen parents with CMS line IR58025A showed a rate of 0% spikelet fertility and 0.5 to 1% pollen fertility.

Emphasis should be given to utilize popular rice cultivars in hybrid rice breeding as parental lines to achieve superior hybrid with better grain quality. The identified maintainers and restorers are locally adopted. Rice varieties, Phule Radha and RTN24 produced sterile hybrids. They could be utilized in backcross breeding programmes for the development of male sterile versions which may help

Fig 1. Differential staining of rice pollen grains in hybrids

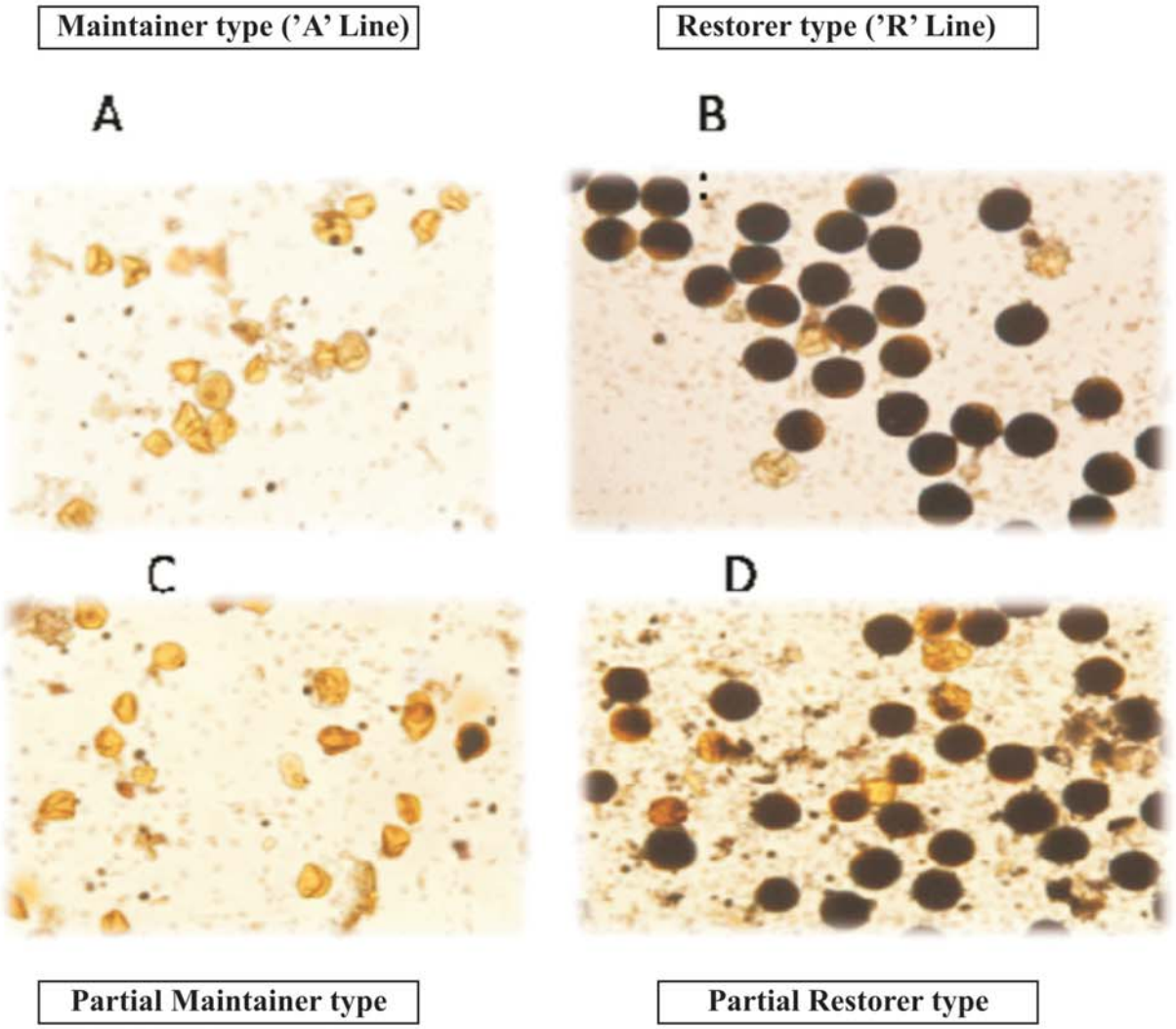
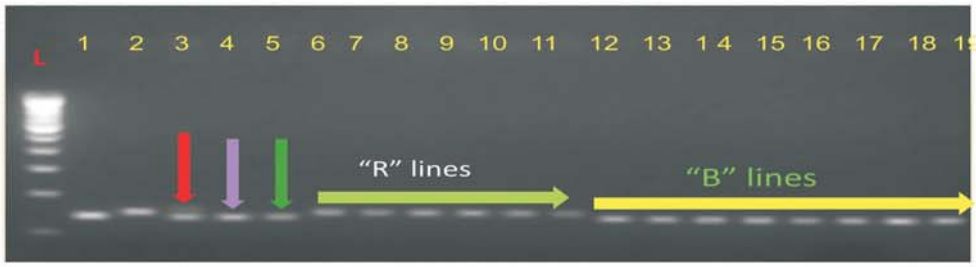


Fig. 2. Screening of R and B lines using polymorphic *Rf* locus specific SSR marker Rm6100



1	IR 58025 B	(129 bp)	7	IR 54742	12	DRR 3B (129 bp)
2	IR 72	(143 bp)	8	IR 69715	13	IR 62829B
3	F1		9	IR 55838	14	IR 68886B
4	RTN24	(129 bp)	10	IR 48715	15	IR 69628B
5	Phule Radha	(129 bp)	11	IR 65483	16	IR 68897B
6	IR 65653	(143 bp)			17	IR 68888B
					18	DRR2B

in the development of rice hybrids for Maharashtra. The variety RTN24 is early duration, short slender rice variety may be useful for development of early duration, fine grain rice hybrids. Oka (1974) suggested that the genetic background of a female parent could influence pollen and spikelet fertility of F_1 hybrids in inter-varietal rice hybrids.

The eight male parents were scored against SSR marker RM6100. Based on banding pattern of standard checks IR58025 and IR72 (Fig.2). There was highly positive association with molecular classification of fertility restoration based on pollen fertility and spikelet fertility.

The marker RM 6100 is linked to *Rf4* locus which is located on chromosome 10 (Sheeba *et al*., 2009). However, in present study marker RM6100 showed 100 per cent accuracy for selecting genotypes for fertility restoration as suggested by Mallikarjun, 2011; Veeresha *et al.,* 2013; Naresh Babu, 2010 and Naik, 2015. RM6100 is co-dominant and is capable of discriminating three kinds of genotypes; Rf/Rf, Rf/rf, rf/rf. The marker may be used for evaluation of seed purity of hybrid seed and can become an alternative to the time consuming and laborious grow out test (GOT).

The present investigation could thus be concluded that RM6100 is a good marker for identification of restorers from germplasm as well as it can be used in hybridity test of F_1 seeds.

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