RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
48 (2) 145-153, 2016

IDENTIFICATION OF ELITE GRAIN QUALITY RESTORERS AND MAINTAINERS FOR WA CMS LINES OF RICE (*Oryza sativa* L.)

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SUMMARY

Twenty one premium grain quality genotypes of rice were used for identification of restorers and maintainers for 3 WA cytoplasmic male sterile lines. Six genotypes (Sanwal Basmati, Pusa Sugandh-2, Pusa Sugandh-3, Pusa Sugandh-5, Pusa 2517-2-51-1 and HUR-JM-59221) were found to exhibit stable restorer behaviour for all the 3 CMS lines (IR-58025A, IR-68897A and Pusa 6A). Moreover, Pusa-44 was found to exhibit the stable fertility restoring ability for IR-58025A, whereas Pusa Basmati-1121 revealed the stable restoring potential for IR-68897A. Three pollen parents (Pusa Basmati-1, Pusa-1460 and HUR-LP-191123) exhibited stable maintainer behaviour for all the 3 CMS lines. In addition, 3 genotypes *i.e.*, HURPB-1M-98, HURPB-1S-97 and TBD-2-1 were found to show the stable maintainer behaviour for CMS line IR-58025A. The male parents showing stable fertility restoring behaviour along with the female parents and hybrids were evaluated with the help of molecular markers to establish the presence of fertility restoring genes. The primers RM171 and RM6100 linked to *Rf4* gene revealed distinguishing banding pattern between CMS lines and fertility restorers, no such polymorphism was observed for the primers RM315 and RM443 linked to *Rf3* gene. The genotypes HUR-JM-59221 and Pusa-44 revealed slightly different banding pattern for the primer RM171 than rest of the pollen parents.

Key words: CMS lines, maintainers, restorers, *Rf3*, *Rf4*, rice, SSR markers

Key findings: Six genotypes (Sanwal Basmati, Pusa Sugandh-2, Pusa Sugandh-3, Pusa Sugandh-5, Pusa 2517-2-51-1 and HUR-JM-59221) were found to be stable restorers for the CMS lines IR-58025A, IR-68897A and Pusa 6A. Moreover, 3 pollen parents *i.e.*, Pusa Basmati-1, Pusa-1460 and HUR-LP-191123 were found to be stable maintainers for all the 3 CMS lines.

Manuscript received: July 16, 2015; Decision on manuscript: August 15, 2015; Manuscript accepted: October 18, 2015.

Communicating Editor: Akshaya Biswal

INTRODUCTION

About half of the world population depends on rice for their survival. It is cultivated in 114 countries across the globe, but 90 percent of world's rice is grown and consumed in Asia. Global rice production for 2013-14 was estimated as 475.9 million tonnes (on milled basis) over an area of 160.9 million ha (USDA Rice Outlook, 2014). There is an urgent need to increase the rice production to meet the demand of ever growing population. In order to narrow the gap between production and demand, increase in productivity is the only option left since the other alternatives like expanding cultivable land and water are unavailable and

resources are either stagnant or declining (Yashitola *et al.,* 2002). Exploitation of heterosis in the form of hybrid rice technology has been contemplated as a potential strategy for yield enhancement in rice. The average yield of hybrid rice is at least 15-20 percent more than that of inbred rice and it has been anticipated that hybrid rice technology will play a key role in ensuring food security worldwide in the future decades (Sabar and Akhter, 2003).

Although, research on the commercial utilization of heterosis in rice has made tremendous gains during the last 20 years, it is still in its stage of infancy due to the lack of desirable quality in F_1 produce. The grain quality of rice hybrids depends on the grain quality of parents. In order to develop rice hybrids possessing premium grain quality like that of basmati, at least one or both parents must possess basmati quality (Virmani *et al.,* 1998). It is therefore important that only parents that show consumer acceptability are chosen to make hybrids. Thus, identification of restorers among elite quality cultivars can serve as important tool for the development of better quality rice hybrids.

The present study has been attempted to identify restorers and maintainers among the elite quality genotypes of rice. The pollen parents showing stable fertility restorer behaviour (over years) were evaluated with the help of molecular markers to establish the presence of fertility restoring genes.

MATERIALS AND METHODS

This study was carried out over 3 seasons *i.e.* wet seasons of 2011, 2012 and 2013 at the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (UP). The site of study is situated at $25^0$18´N latitude and $83^0$03´E longitude at an elevation of 80.71 m above mean sea level.

Three cytoplasmic male sterile lines (IR-58025A, IR-68897A and Pusa 6A) having Wild abortive (WA) cytoplasm as a source of male sterility were used as female parents in crossing programme. Twenty one pollen parents (Sanwal Basmati, Pusa Sugandh-2, Pusa Sugandh-3, Pusa Sugandh-5, Pusa 2517-2-51-1, HUR-JM-59221, Pusa-44, Pusa Basmati-1121, Pusa Basmati-1, Pusa-1460, HUR-LP-191123, HURPB-1M-98, HURPB-1S-97 TBD-2-1, Taraori Basmati, Ranbir Basmati, Type-3, HUR-ML-1131221, Basmati-370, HUBR-2-1 and Kasturi Basmati) selected on the basis of yield and quality parameters, were used as male parents in the hybridization programme. All the genotypes were obtained from the 'All India Coordinated Rice Improvement Project (AICRIP)' at the Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (U.P.).

During wet season-2011, 3 cytoplasmic male sterile (CMS) lines along with their maintainers and the pollen parents were raised in a staggered manner at 3 dates (10 days apart) to facilitate synchronization of flowering. Single seedling per hill was planted and recommended package of practices were followed to raise a good crop. Crosses were made between 3 CMS lines and 21 pollen parents to generate a set of 63 hybrids in line x tester manner as proposed by Kempthorne (1957). In wet season-2012, the seeds of 63 hybrids (generated during previous season) were raised to estimate their pollen and spikelet fertility. On the basis of pollen and spikelet fertility percentage of F_1s, the pollen parents were classified as maintainers, partial maintainers, partial restorers and restorers based on the criteria proposed by Virmani *et al.* (1997) *i.e.* restorer (pollen fertility >80%; spikelet fertility >75%), partial restorer (pollen fertility: 50.1-80%; spikelet fertility: 50.1-75%), partial maintainer (pollen fertility: 1.1-50%; spikelet fertility: 0.1-50%) and maintainer (pollen fertility: 0-1%; spikelet fertility: 0%). The CMS lines, their isogenic B lines and 21 pollen parents were also raised, and crosses made as in wet season-2011, to confirm the findings of wet season-2012 during next year (wet season-2013).

The fertility restoring ability of the pollen parents identified as restorers were confirmed with the help of microsatellite markers using parental polymorphism survey. The molecular markers, previously reported to be linked to fertility restoring (*Rf*) genes were validated for the pollen parents showing stable restorer behaviour during pollen and spikelet studies. The SSR primers RM171 and RM6100

linked to *Rf4* gene on chromosome 10, and SSR primers RM315 and RM443 linked to *Rf3* gene on chromosome 1 were used to identify the fertility restorer genes.

RESULTS

Six genotypes (Sanwal Basmati, Pusa Sugandh-2, Pusa Sugandh-3, Pusa Sugandh-5, Pusa 2517-2-51-1 and HUR-JM-59221) were found to be stable restorers for all the 3 CMS lines (IR-58025A, IR-68897A and Pusa 6A). Moreover, Pusa-44 and Pusa Basmati-1121were found to behave as stable restorers for IR-58025A and IR-68897A, respectively. Furthermore, 3 pollen parents (Pusa Basmati-1, Pusa-1460 and HUR-LP-191123) exhibited stable maintainer behaviour for all the 3 CMS lines. In addition, 3 genotypes *i.e.*, HURPB-1M-98, HURPB-1S-97 and TBD-2-1 were found to show stable maintainer behaviour for CMS line IR-68897A. Rest of the male genotypes were either stable partial restorers or stable partial maintainers for respective CMS lines, or their fertility restoration behaviour varied over years (Table 1).

The frequency of stable restorers was observed to be highest for CMS lines IR-58025A and IR-68897A (7/21), followed by Pusa 6A (6/21). Similarly, the frequency of stable maintainers was highest for IR-68897A (6/21), while IR-58025A and Pusa 6A revealed the lowest frequency of stable maintainers (3/21). Ten genotypes (Pusa-44, Pusa Basmati-1121, HURPB-1M-98, HURPB-1S-97, TBD-2-1, Taraori Basmati, Ranbir Basmati, Type-3, HUR-ML-1131221 and Basmati-370) showed variable fertility restoration behaviour over the CMS lines.

Three CMS lines along with the 8 pollen parents showing stable fertility restoring ability and their 20 hybrids were evaluated with the help of microsatellite markers to confirm the presence of fertility restorer genes. The SSR primers RM171, RM315, RM443 and RM6100 were used to identify the presence of fertility restoring genes. Although, the primers for *Rf4* gene (RM171 and RM6100) revealed distinguishing banding pattern between maintainer lines and fertility restorers, no such polymorphism was observed for the primers of *Rf3* locus (RM315 and RM443) (Figure 1). The genotypes HUR-JM-59221 and Pusa-44 revealed slightly different banding pattern than rest of the pollen parents.

DISCUSSION

Identification of restorers and maintainers

Identification of restorers and maintainers from elite breeding lines or landraces through test crossing and their use in further breeding programmes are the initial and foremost steps in three-line heterosis breeding. The restorers can be used to develop hybrids and the maintainers to be used for development of new CMS lines.

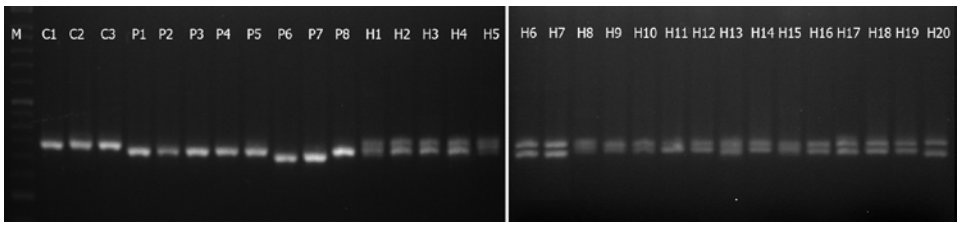
In this study, 3 cytoplasmic male sterile (CMS) lines were tested for their stability with respect to sterility and subsequently crossed with 21 pollen parents for isolation of restorers and maintainers. The frequency of stable restorers was higher than the frequency of stable maintainers for all the CMS lines. High frequency of restorers than the maintainers were also reported during their respective studies by Virmani and Edwards (1983), Jaiswal and Parveen (2009), Upadhyay and Jaiswal (2012), Das *et al.* (2013), Veeresha *et al.* (2013) and Reddy *et al.* (2014). Contrary to the present finding, Ali and Khan (1996) reported nearly 4 times higher frequency of maintainers than restorers in the indica genotypes they studied. Similarly, significantly higher frequency of maintainers as compared to restorers was also reported by Sabar and Akhtar (2003), Singh *et al.* (2010) and Seesang *et al.* (2014). Sabar *et al.* (2007) also reported higher frequency of maintainers than restorers among the traditional basmati genotypes (Basmati 370, Super Basmati and Shaheen Basmati) for certain WA CMS lines. On the other hand Rosamma and Vijayakumar (2005), and Ingale *et al.* (2005) reported nearly equal proportions of restorer and maintainer in their study materials.

Table 1. Restoration ability of various rice genotypes for the three WA CMS lines.

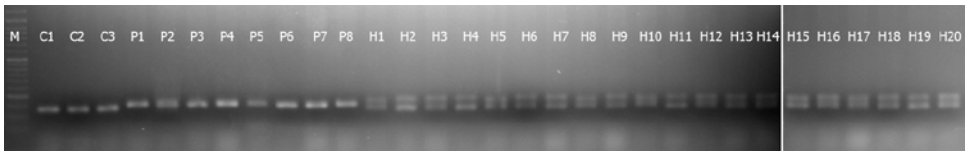
No.	Cross combinations	IR-58025A						IR-68897A						Pusa 6A					
		2012			2013			2012			2013			2012			2013		
		PF (%)	SF (%)	Class	PF (%)	SF (%)	Class	PF (%)	SF (%)	Class	PF (%)	SF (%)	Class	PF (%)	SF (%)	Class	PF (%)	SF (%)	Class
1.	Sanwal Basmati	84.2	82.0	R	84.6	82.1	R	85.5	81.1	R	89.0	84.4	R	90.5	87.6	R	88.9	81.3	R
2.	Pusa Sugandh-2	87.5	83.7	R	82.0	77.0	R	92.8	81.0	R	91.6	82.0	R	98.8	86.3	R	84.5	78.7	R
3.	Pusa Sugandh-3	84.5	83.8	R	81.0	77.8	R	82.0	81.8	R	83.6	77.6	R	96.0	84.5	R	88.3	77.8	R
4.	Pusa Sugandh-5	89.5	88.0	R	81.2	76.2	R	92.5	85.5	R	81.0	76.2	R	95.5	91.1	R	81.6	76.2	R
5.	Pusa 2517-2-51-1	90.5	81.1	R	81.5	77.2	R	80.1	76.1	R	81.5	77.1	R	91.0	89.3	R	91.4	81.1	R
6.	HUR-JM-59221	92.0	86.6	R	84.3	77.2	R	88.5	85.0	R	82.5	78.3	R	95.0	88.2	R	81.4	77.3	R
7.	Pusa-44	83.5	78.2	R	82.7	78.5	R	85.5	77.1	R	75.8	70.1	PR	75.5	71.5	PR	77.5	74.0	PR
8.	Pusa Basmati-1121	73.2	71.4	PR	77.2	73.2	PR	82.8	81.5	R	80.6	75.3	R	68.5	65.0	PR	61.4	52.4	PR
9.	Pusa Basmati-1	0.9	0.0	M	0.8	0.0	M	0.5	0.0	M	0.6	0.0	M	0.7	0.0	M	0.6	0.0	M
10.	Pusa-1460	0.7	0.0	M	0.9	0.0	M	0.8	0.0	M	0.9	0.0	M	0.8	0.0	M	0.5	0.0	M
11.	HUR-LP-191123	0.3	0.0	M	0.5	0.0	M	0.2	0.0	M	0.8	0.0	M	0.5	0.0	M	0.2	0.0	M
12.	HURPB-1M-98	23.0	4.0	PM	11.4	1.2	PM	0.5	0.0	M	0.2	0.0	M	12.8	11.0	PM	4.6	1.0	PM
13.	HURPB-1S-97	13.0	2.5	PM	3.5	0.5	PM	0.8	0.0	M	0.7	0.0	M	9.5	3.0	PM	5.5	0.5	PM
14.	TBD-2-1	4.2	0.2	PM	3.5	1.0	PM	0.3	0.0	M	0.8	0.0	M	6.5	2.0	PM	2.5	0.5	PM
15.	Taraori Basmati	78.2	73.1	PR	81.0	76.2	R	78.1	73.8	PR	77.5	72.5	PR	76.8	72.6	PR	81.5	76.8	R
16.	Ranbir Basmati	85.2	77.3	R	74.6	68.2	PR	58.5	52.5	PR	72.8	66.1	PR	73.5	71.0	PR	77.5	72.2	PR
17.	Type-3	72.0	69.0	PR	73.8	69.1	PR	65.0	62.2	PR	69.3	63.5	PR	55.5	52.1	PR	88.4	76.2	R
18.	HUR-ML-1131221	78.2	58.2	PR	71.5	66.3	PR	78.5	64.3	PR	61.4	51.9	PR	88.5	83.3	R	73.6	69.5	PR
19.	Basmati-370	78.5	73.8	PR	73.5	67.5	PR	49.5	48.8	PM	47.1	44.8	PM	77.5	72.5	PR	72.4	68.3	PR
20.	HUBR-2-1	7.5	4.4	PM	3.5	0.1	PM	12.5	10.5	PM	6.5	0.1	PM	11.1	7.3	PM	3.9	0.1	PM
21.	Kasturi Basmati	77.2	62.3	PR	48.5	44.2	PM	72.4	57.7	PR	49.0	44.5	PM	75.5	72.1	PR	48.3	42.2	PM

PF = Pollen fertility, SF = Spikelet fertility, R = Restorer (PF >80%; SF >75%), PR = Partial restorer (PF: 50.1-80%; SF: 50.1-75%), PM = Partial maintainer (PF: 1.1-50%; SF: 0.1-50%), M = Maintainer (PF: 0-1%; SF: 0%). [According to Virmani *et al.*, 1997]

A. RM171

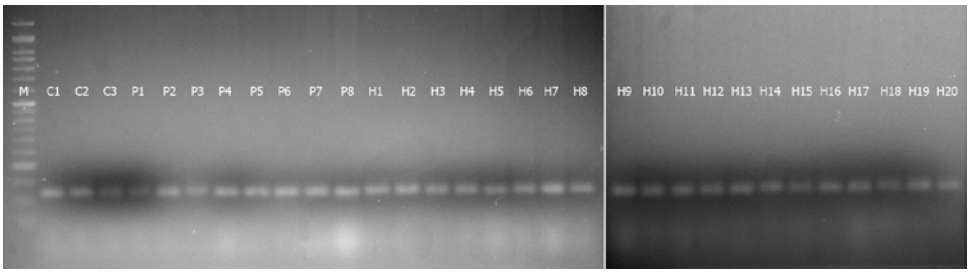


B. RM6100



C. RM315

D. RM443



M = Marker (50 bp DNA Ladder), C1 = IR-58025B, C2 = IR-68897B, C3 = Pusa 6B, P1 = Sanwal Basmati, P2 = Pusa Sugandh-2, P3 = Pusa Sugandh-3, P4 = Pusa Sugandh-5, P5 = Pusa 2517-2-51-1, P6 = HUR-JM-59221, P7 = Pusa-44, P8 = Pusa Basmati-1121, H1 = IR-58025A x Sanwal Basmati, H2 = IR-58025A x Pusa Sugandh-2, H3 = IR-58025A x Pusa Sugandh-3, H4 = IR-58025A x Pusa Sugandh-5, H5 = IR-58025A x Pusa-2517-2-51-1, H6 = IR-58025A x HUR-JM-59221, H7 = IR-58025A x Pusa-44, H8 = IR-68897A x Sanwal Basmati, H9 = IR-68897A x Pusa Sugandh-2, H10 = IR-68897A x Pusa Sugandh-3, H11 = IR-68897A x Pusa Sugandh-5, H12 = IR-68897A x Pusa 2517-2-51-1, H13 = IR-68897A x HUR-JM-59221, H14 = IR-68897A x Pusa Basmati-1121, H15 = Pusa 6A x Sanwal Basmati, H16 = Pusa 6A x Pusa Sugandh-2, H17 = Pusa 6A x Pusa Sugandh-3, H18 = Pusa 6A x Pusa Sugandh-5, H19 = Pusa 6A x Pusa 2517-2-51-1, H20 = Pusa 6A x HUR-JM-59221.

Figure 1. Banding pattern obtained using various SSR primers.

The differential restoration reaction of rice genotypes with different CMS lines of same cytoplasmic source was observed in the present investigation. Similar findings have also reported by Kumar *et al.* (2002), Hariprasanna *et al.* (2005), and Upadhyay and Jaiswal (2012). They reported that the genotypes acting as restorers may also behave as partial restorer against a similar source of CMS lines. Rosamma and Vijaykumar (2005) reported that rice genotypes expressed differential fertility reactions when crossed with different CMS lines having WA cytoplasm. Similar results were also reported by Virmani *et al.* (1997),

Bisne and Motiramani (2005), and Durai and Nadrajan (2007). The change in fertility restoration behaviour of restorers with CMS lines of same source or of different source could either be due to fertility restoring genes or may be affected by modifier genes (Waghmode and Mehta, 2011). Ganeshan *et al.* (1998) also suggested that minor and modifier gene(s) present in the pollinators may be responsible for such type of variation. The differential reaction of pollen parents with different CMS lines may also be due to the influence of female genetic background on the restoration behaviour of genotypes tested. Thus, the variation may be attributed to the difference in pollen fertility restoring genes, or their penetrance or expressivity differed with genotypes (Umadevi *et al.*, 2010), or due to existence of modifier genes (Pande *et al.,* 1990). These probable explanations given by different workers also hold good for the differential behaviour of CMS lines towards fertility restoration in this study.

Some of the genotypes in present study were observed to show unstable fertility restoration behaviour (over years) for the respective CMS lines. Sarial and Singh (2000) observed that performance of restorers (Chandan and SAF khalsa 7) and maintainer (Pusa Basmati-1) varied with location and season of testing, and suggested that the differential ability to restore fertility in WA cyto-sterile lines could result from different nuclear backgrounds of the CMS lines. Restoration reaction has also been reported to be influenced by environmental factors (Govindraj *et al.*, 1984). Virmani and Edwards (1983) reported that the restoring ability of some genotypes was found to be site specific. Some partial restorers or partial maintainers (either stable or unstable) for the respective CMS lines were also observed in this study, but they have no utility in hybrid rice breeding (Gautam and Singh, 2004).

Molecular marker based identification of fertility restoring genes

The male parents showing stable fertility restoring behaviour along with the female parents and hybrids were evaluated with the help of molecular markers to establish the presence of fertility restoring (*Rf*) genes. The primers RM171 and RM6100 revealed distinguishing banding pattern between CMS lines and fertility restorers, no such polymorphism was observed for the primers RM315 and RM443. Reported genetic analyses have revealed that the number, position and effects of the *Rf* genes for WA CMS system are variable depending upon the materials and methods used. Studies on genetic inheritance of *Rf* genes for WA CMS indicated control of single gene (Shen *et al.*, 1996; Yao *et al.*, 1997), digenic (Bharaj *et al.,* 1991), digenic with different types of interactions (Govindaraj and Virmani, 1988; Waghmode and Mehta, 2011), trigenic (Sarkar *et al.,* 2002), trigenic with interactions (Huang, 1987; Hossain *et al.,* 2010) and tetragenic (Zhu *et al.,* 1996). Only a few researchers observed the fertility restorer gene to be polygenic in nature (Pei, 1980). A majority of studies have reported digenic inheritance governed by 2 independent genes (Li and Yuan, 1986; Virmani *et al.,* 1986; Govindaraj and Virmani, 1988). The chromosomal locations of the two *Rf* genes (*Rf3* and *Rf4*) have also been determined. DNA marker based linkage mapping analysis revealed the location of *Rf3* on Chromosome 1 (Yao *et al.*, 1997; Ahmadikhah *et al.,* 2007; Sattari *et al.*, 2007; Suresh *et al.,* 2012; Cai and Zhang, 2014) and *Rf4* on Chromosome 10 (Yao *et al.,* 1997; Ahmadikhah *et al.,* 2007; Sattari *et al.,* 2007; Sheeba *et al.*, 2009; Cai and Zhang, 2014). SSR marker RM171 has been reported to flank the *Rf4* gene at a genetic distance of 3.2 cM (Ahmadikhah and Karlov, 2006). RM315 and RM443 have been reported to be linked with the *Rf3* gene at a genetic distance of 20.7 and 4.4 cM, respectively (Bazrkar *et al.,* 2008). Sheeba *et al.* (2009) have mapped the *Rf4* gene at 1.2 cM from an SSR marker RM6100.

It appears from the present study that *Rf4* acts as the major fertility restoring gene. This is in accordance with the studies of Suresh *et al.* (2012) who reported that *Rf4* is a major locus for fertility restoring of WA CMS in most of the cases and *Rf3* in some cases. Some studies have reported that the 2 fertility restorer genes are additive in their inheritance and the effect of *Rf4* appeared to be larger than that of *Rf3* (Yao *et al.*, 1997; Sattari *et al.*, 2008; Cai *et al.*, 2013). Some minor genes and modifiers are also reported to be involved in the expression of the

trait for fertility restoration (Govindaraj and Virmani, 1988; Sohu and Phul, 1995).

The markers RM315 and RM443 were unable to distinguish the restorers from the CMS lines. This may also be because these restorer lines may have a different set of fertility restoring gene(s). Mishra *et al*. (2003) also identified *Rf-(u1)*, a new fertility restoring gene for WA CMS system. Another probable reason for monomorphic banding pattern for RM315 and RM443 primers is that a recombination event could have occurred between the marker and the gene during the development of these restorer lines, which could have resulted in non-amplification of the *Rf*3 gene linked allele in these restorers. This can be explained on the basis of linkage distances between *Rf*3 locus and its markers (RM315 and RM443) which are relatively greater.

Moreover, the genotypes HUR-JM-59221 and Pusa-44 revealed slightly different banding pattern for the primer RM171 than rest of the pollen parents, which may be most probably due to the simple change in microsatellite sequence. Furthermore, existence of similar or nearly similar banding patterns within the CMS lines and male parents as revealed in this study indicate of the common origin of these genotypes with respect to fertility restoration.

To summarise, the potential restorers identified in the present investigation can be used for developing rice hybrids with enhanced levels of heterosis for yield and quality traits. The effective maintainers can be used to develop new CMS lines through recurrent back crossing. Pusa Basmati-1 and Pusa-1460 have better grain quality and are effective maintainers for all the 3 CMS lines. Thus, both these maintainers can be used for the production of quality CMS lines. The SSR markers RM171 and RM6100 validated in this study would be useful in identification of restorers while screening rice germplasm for their fertility restoring ability. Moreover, the markers linked to the *Rf* genes could be of significant help in understanding the inheritance of the trait, and targeted identification and introgression of *Rf* genes in breeding programmes.

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