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Identification of restorers and maintainers in rice (*Oryza sativa* L.) using SSR markers

SK Singh, S Sonkar, PR Vennela*, and DK Singh

Institute of Agricultural Sciences, Banaras Hindu University, Varanasi - 221005, UP, India

Corresponding author e-mail: vprudhviraj.2@gmail.com

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ABSTRACT

For the ever growing requirements in cereals production, rice occupies the top position. To meet the future food demand, there is an urgent need to break the yield barriers. The utilization of hybrid rice technology has been contemplated as a potential strategy for yield enhancement which has successfully been demonstrated and commercialized. It has been anticipated that hybrid rice technology will play a key role in ensuring food security worldwide in the future decades. In order to deploy CMS system to develop commercial rice hybrid, it is essential to have an effective restorer line. Thus, identification of restorers among elite quality cultivars can serve as important tool for the development of better rice hybrids. In the light of above points, the present investigation was conducted to identify the restorers and maintainers from elite quality cultivars. Thirty six genotypes and their F_1s were used for identification of restorers and maintainers using pollen and spikelet fertility for a cytoplasmic male sterile (CMS) line (Pusa 6A). The pollen parents showing stable fertility restorer behavior were evaluated with the help of SSR markers to establish the presence of fertility restoring genes. The present observations revealed that out of 36 rice genotypes 20 were found as restorer and 16 were maintainers.

Key words: *Hybrid rice technology, CMS, restorers, maintainers and SSR markers*

Rice (*Oryza sativa* L.) belongs to the genus *Oryza* of family Poaceae. 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 in Asia. India has the largest area under rice among the rice growing countries in the world and ranks second in production after China. The present world rice area, production and productivity is 160.9 mha, 476.00 mt and 2.95 t/ha, respectively (FAO, 2014). In India, it is being grown in 44.00 mha area with production of 106.65 mt and productivity of 2.42 t/ha. (Directorate of Economics & Statistics, 2014). In India, among the major rice growing states, Uttar Pradesh ranks first in area (5.86 mha), West Bengal ranks second in area (5.43 mha) and Uttar Pradesh ranks second in production (14.41 mt) after West Bengal (15.02 mt). Rice area, production and productivity in Uttar Pradesh is 5.86 mha, 14.41 mt and 2.45 t/ha, respectively (Directorate of Economics and Statistics, Ministry of Agriculture 2014). To feed the ever growing population, the targeted rice production of the world, China and India for the year 2030 is envisaged as 771.02, 168.90 and 130.02 million tonnes, respectively (United State Department of Agriculture 2014). To get success in achieving the target, the increase in rice productivity is the only option left, since the other alternatives like cultivable land, water and other natural resources are either stagnant or declining (Singh *et al.* 2015). Ever since the report of Jones (1926), utilization of hybrid rice technology has been contemplated as a potential strategy for yield enhancement in rice, which has successfully been demonstrated by China after the

commercial release of hybrids. The average yield of hybrid rice is at least 15-20 per cent 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). Hence, there is an urgent need to boost the rice production through its enhanced productivity which may be done by the breeders through adaptation of hybrid rice technology at larger scale.

As hybrid rice technology is one of the strongest tools to break the yield barriers and if CMS based hybrid rice is developed then it needs restorer lines to make the hybrid rice technology practically feasible. Fertility restorer genes (*Rf* genes) make the male parents, as a restorer line. It is tedious and time taking to study the restorer lines through conventional approach. Now, the molecular markers are available which made it easy to study the restorer lines among the rice genotypes, which is a quicker method of study. As diversity is one of the major criteria to get the higher magnitude of heterosis in the F_1 hybrids, it is necessary to study the genetic diversity among the rice genotypes to make the heterosis breeding programme practically useful.

MATERIALS AND METHODS

The present study was carried out during *kharif* , 2014 (at the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, U.P., India). The molecular analysis was accomplished at the Molecular Biology Laboratory (Niche Area Lab) of the Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (U.P.), India. The experimental material for this investigation comprised of 36 F_1s which were obtained by crossing 36 rice genotypes with the male sterile line Pusa 6A which posses wild abortive cytoplasm.The experiment was carried out in one Season as described below.

Thirty six F_1s were raised in nursery bed and 25 days old seedling were transplanted in the main field in RCBD design at the standard spacing of 20 x 15 cm in 4 m row length. Single seedling hill was planted and recommended package of practices were followed to raise a good crop.

Observations recorded

The following observations were recorded for fertility restoration related traits, yield parameters and quality characteristics.

Fertility restoration related traits

Pollen fertility

Atm flowering stage panicles were collected randomly from each genotype and fixed in aceto-alcohol (1:3) for 24 hours, and then preserved in 70 percent ethanol. Mature anthers from five randomly selected spikelets each from top, middle and bottom of the panicle were taken, crushed, smeared and stained in freshly prepared 1 per cent I2-KI solution separately, and examined under a light microscope. About 5000 pollen grains were scored for each genotype. Deeply stained, fully developed and round pollen grains were counted as fertile, whereas weakly stained/unstained and irregular shaped pollens were grouped as sterile. Pollen fertility was computed in percentage according to the formula given by Choudhary *et al.* (1981).

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

Spikelet fertility

Five randomly selected panicles from each line/F1 were covered with butter paper bags before anthesis. At maturity the bagged panicles were harvested 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).

$$\text{Spiklet fertility (\%)} = \frac{\text{Number of filled spikelet}}{\text{Total number of spikelet}} \times 100$$

On the basis of pollen fertility 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) (Table 1).

Table 1. Classification of fertility restoration (Virmani *et al.* 1997)

Pollen fertility (%)	Category	Spikelet fertility (%)
0-1	Maintainer	0
1.1-50	Partial maintainer	0.1-50
50.1-80	Partial Restorer	50.1-75
>80	Restorer	>75

Molecular analysis

A total of four simple sequence repeat (SSR) markers, *i.e.*, RM315, RM443, RM171 and RM6100, reported to be linked with fertility restorer genes were used for parental polymorphism survey. RM315 and RM443 have been reported to be linked with *Rf3* gene on chromosome 1, whereas RM171 and RM6100 reported to be linked with *Rf4* gene on chromosome 10 (Ahmadikhah *et al.* 2007; Bazrkar *et al.* 2008; Sattari *et al.* 2008). The details of SSR primers used are presented in Table 2.

RESULTS AND DISCUSSIONS

Identification of restorers and maintainers

Thirty six rice genotypes and their F_1 hybrids obtained by crossing with Pusa 6A (CMS line) were raised in RCBD design and used for identification of restorer and maintainers during *kharif* season, 2014. The pollen and spikelet fertility per cent of 36 F_1 hybrids were used to assess fertility restoring ability. On the basis of pollen and spikelet fertility, genotypes were classified as restorers, and maintainers.

The male parents were classified (Virmani et al. 1997) as fertility restorers (>80 % pollen and >75% spikelet fertility), partial restorers (pollen fertility: 50.1-80 % and spikelet fertility: 50.1-75 %), partial maintainers (pollen fertility: 1.1-50 % and spikelet fertility: 0.1-50 %) and maintainers (pollen fertility: 0-1 % and spikelet fertility 0 %) for the CMS line (Table 3).

Out of 36 rice genotypes, 20 were restorer and 16 were maintainer. Genotypes; IET 22202, Vardhan, Akshaya Dhan, BPT 5204, B.G.-102, MTU-1010, NDR-97, Pant Dhan-12, Pusa6 B, Shusk Samrat, IR-64, Baranideep, URG-1, URG-3, URG-5, URG-19, URG-22, URG-30, HUR-10-9, Dantaswari are found to be effective restorers and HUR-3022, HUR-105,

Table 3. Fertility classification of 36 genotypes for restorer and maintainer

Sl. No.	Genotypes	BHU 2014	
		Restorer	Maintainer
1.	Akshaya Dhan	P	A
2.	Danteswari	P	A
3.	Vandana	A	P
4.	Anjali	A	P
5.	Sahabhagi	A	P
6.	B.G.-102	P	A
7.	NDR - 97	P	A
8.	Nagina - 22	A	P
9.	Shusk Samrat	P	A
10.	IR-64	P	A
11.	IR-36	A	P
12.	NDR - 359	A	P
13.	MTU-1010	P	A
14.	Pant Dhan -12	P	A
15.	Baranideep	P	A
16.	Lalat	A	P
17.	Birsa- 105	A	P
18.	URG-1	P	A
19.	URG-3	P	A
20.	URG-5	P	A
21.	URG-8	A	P
22.	URG-19	P	A
23.	URG-22	P	A
24.	URG-24	A	P
25.	URG-28	A	P
26.	URG-30	P	A
27.	URG-42	A	P
28.	IET - 22202	P	A
29.	BPT - 5204	P	A
30.	HUR - 105	A	P
31.	HUR - 3022	A	P
32.	HUR-10-9	P	A
33.	Vardhan	P	A
34.	Pusa-6-B	P	A
35.	IR-68897-B	A	P
36.	IR - 79156-B	A	P

Total Restorer =20 Total Maintainer =16

P=Present, A=Absent

Table 2. Details of the microsatellite primers used in present study

Microsatellite locus	Location/ Chromosome	Nearby *Rf* gene	Forward/ Reverse	Sequence 5'----------> 3'
RM315	1	*Rf3*	Forward	GAGGTACTTCCTCCGTTTCAC
			Reverse	AGTCAGCTCACTGTGCAGTG
RM443	1	*Rf3*	Forward	GATGGTTTTCATCGGCTACG
			Reverse	AGTCCCAGAATGTCGTTTCG
RM171	10	*Rf4*	Forward	AACGCGAGGACACGTACTTAC
			Reverse	ACGAGATACGTACGCCTTTG
RM6100	10	*Rf4*	Forward	TCCTCTACCAGTACCGCACC
			Reverse	GCTGGATCACAGATCATTGC

Nagina-22, Anjali, Vandana, Sahabhagi, IR-36, NDR-359, Lalat, Birsa, URG-8, URG-24, URG-28, URG-42, IR-68897-B, IR-79156-B, are found to be effective maintainers.Out of thirty six pollen parents, 9 genotypes were classified as restorers, 11 per cent as partial restorer, 12 as maintainers and 4 as partial maintainers for the CMS line Pusa 6A. The pollen and spikelet fertility % is given in the table 4.

Molecular marker based identification of Rstorers and maintainers

Identification of restorers and maintainers forms the first step for commercial exploitation of heterosis in rice. Commercial exploitation of heterosis involves the use of cytoplasmic male sterility (CMS) which is a maternally inherited trait and results in an inability of the plant to produce fertile pollen. Pollen fertility is restored by nuclear-encoded genes called fertility restorer (*Rf*) genes. The lack of morphological markers that help early detection of the potential plants possessing the fertility restorer gene makes the breeding process inefficient and equally intense only at anthesis stage. The process of screening for the trait of fertility restoration is laborious and time consuming as it involves test crossing with a set of CMS lines and evaluation of genotype for pollen and spikelet fertility. 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.

Table 4. Per cent pollen and spikelet fertility of 36 F_1 involving cytoplasmic male sterile line (Pusa 6A)

Sl.No.	F1	PF	SF
1.	Pusa 6A x Akshaya Dhan	86.89	84.65
2.	Pusa 6A x Danteswari	85.33	84.08
3.	Pusa 6A x Vandana	0.00	0.00
4.	Pusa 6A x Anjali	0.00	0.00
5.	Pusa 6A x Sahabhagi	63.92	57.21
6.	Pusa 6A x B.G.-102	23.11	12.95
7.	Pusa 6A x NDR - 97	59.49	55.48
8.	Pusa 6A x Nagina - 22	0.00	0.00
9.	Pusa 6A x Shusk Samrat	81.25	78.35
10.	Pusa 6A x IR-64	65.65	58.54
11.	Pusa 6A x IR-36	0.00	0.00
12.	Pusa 6A x NDR - 359	66.47	54.57
13.	Pusa 6A x MTU - 1010	89.83	88.08
14.	Pusa 6A x Pant Dhan -12	45.93	37.76
15.	Pusa 6A x Baranideep	86.05	84.00
16.	Pusa 6A x Lalat	0.00	0.00
17.	Pusa 6A x Birsa Dhan-105	87.58	79.79
18.	Pusa 6A x URG -1	39.69	32.43
19.	Pusa 6A x URG -3	86.63	84.77
20.	Pusa 6A x URG -5	85.05	83.32
21.	Pusa 6A x URG -8	66.47	54.57
22.	Pusa 6A x URG -19	59.49	55.48
23.	Pusa 6A x URG -22	70.67	59.24
24.	Pusa 6A x URG -24	28.41	31.44
25.	Pusa 6A x URG -28	25.07	19.62
26.	Pusa 6A x URG -30	0.00	0.00
27.	Pusa 6A x URG -42	0.00	0.00
28.	Pusa 6A x IET - 22202	82.94	81.18
29.	Pusa 6A x BPT - 5204	81.91	79.79
30.	Pusa 6A x HUR - 105	0.00	0.00
31.	Pusa 6A x HUR - 3022	0.00	0.00
32.	Pusa 6A x HUR -10-9	83.34	81.34
33.	Pusa 6A x Vardhan	87.84	86.01
34.	Pusa 6A x Pusa-6-B	0.00	0.00
35.	Pusa 6A x IR-68897-B	0.00	0.00
36.	Pusa 6A x IR-79156 -B	0.00	0.00

The present study was carried out to establish the presence of fertility restoring genes for WA CMS lines of rice with the help of molecular markers. The molecular markers, which have been previously reported to be linked to fertility restorer (*Rf*) gene (s) were validated for the genotypes revealing stable fertility restoring behaviour. The existence of polymorphism between CMS lines and their restorers were used for detection of *Rf* gene (s). Thirty six rice genotype were evaluated with the help of four SSR markers. The SSR markers RM171 and RM6100 linked to Rf4 gene on chromosome 10, and SSR markers RM315 and RM443 linked to *Rf3* gene on chromosome 1 were used to identify the presence of fertility restoring genes. The female parent (Pusa 6A) din't show any band for all the four markers used for the study. Although, the primers RM171 and RM6100 revealed distinguishing banding pattern between fertility restorers (Fig. 2), but such polymorphism was not observed for the primers RM315 and RM443 (Fig. 1)

The absence of band in the female and presence of band in the restoring lines helps in identification of restorer lines. The absence of bands for marker RM 315 and RM 443 confirms the phenotypic data.

The present observations revealed that F_1 hybrids produced by crossing different rice genotypes with CMS line behaved differently with regard to fertility restoration. Out of 36 rice genotype 20 were found as restorer, and 16 were maintainers. Genotypes; IET 22202, Vardhan, Akshaya Dhan, BPT 5204, Shusk

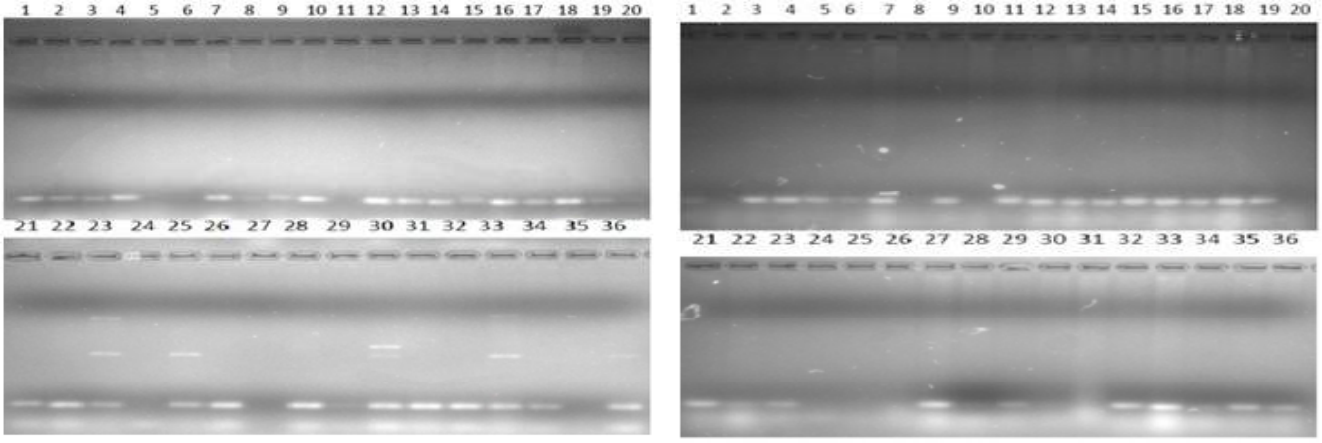

Fig. 1. Showing SSR banding profile obtained by marker RM 315and RM 443 . Lane 1-36 represents rice cultivar

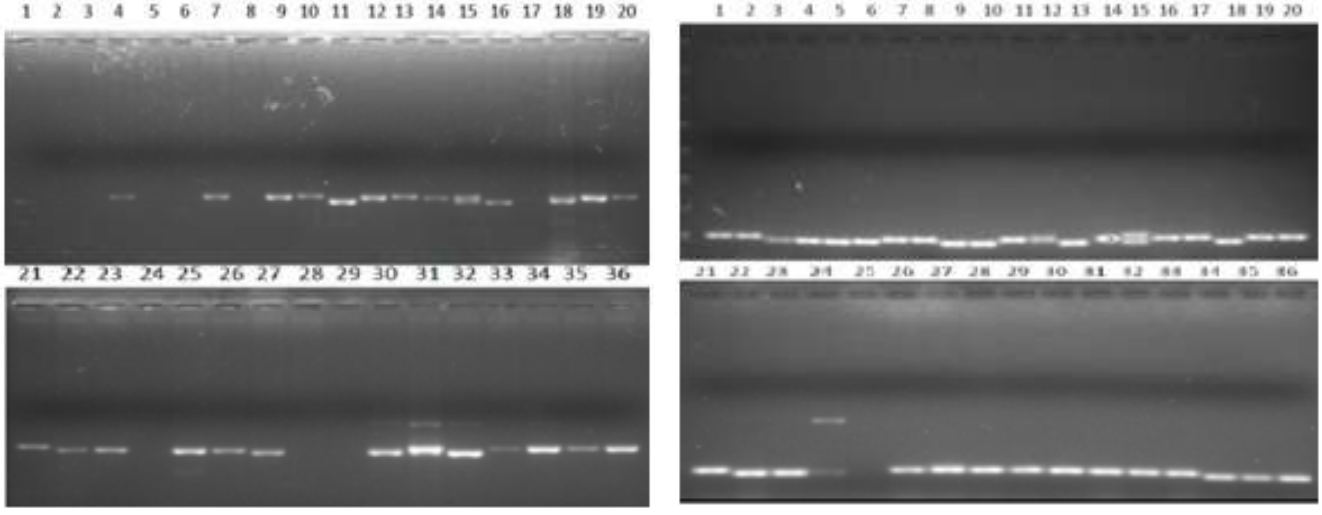

Fig. 2. Showing SSR banding profile obtained by marker RM 171and RM 6100. Lane 1-36 represents rice cultivar

1=Akshaya Dhan, 2= Danteswari, 3=Vandana, 4= Anjali, 5= Sahabhagi, 6= B.G.-102, 7=NDR - 97, 8=Nagina - 22, 9=Shusk Samrat , 10= IR-64, 11= IR-36, 12= NDR- 359, 13= MTU - 1010, 14=Pant Dhan -12, 15= Baranideep, 16= Lalat, 17=Birsa Dhan-105, 18=URG -1, 19=URG -3, 20=URG -5, 21=URG -8, 22=URG -19, 23=URG -22, 24=URG -24, 25=URG -28, 26=URG -30, 27=URG -42, 28=IET - 22202, 29=BPT - 5204, 30=HUR - 10, 31=HUR - 3022, 32=HUR -10-9, 33=Vardhan, 34=Pusa-6-B, 35=IR-68897-B, 36=IR-79156 -B

Samrat,IR-64, Baranideep, MTU-1010, URG-3, URG-5, HUR-10-9, Dantaswari Sahabhagi, NDR 97, Pant dhan -12, NDR-359, URG-8, URG-19, URG-22, URG 30 were found to be restorers. Genotypes Vandana, Anjali, Nagina 22, IR 36, Lalat, Birsa 105, URG-42, HUR-105, HUR-3022, Pusa-6B, IR-68897B, IR-79156B, BG-102, URG-1, URG-24 and URG-28 as maintainers.

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