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Identification of Potential Maintainer and Restorer Lines from Elite Germplasm of Rice

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ABSTRACT: The present investigation was conducted for the identification of potential restorers and maintainers from the elite germplasm of rice. Cytoplasmic-genetic male sterility (CGMS) system used predominantly in rice for hybrid seed production which requires three lines A (male sterile), B (maintainer line) and R (restorer line). Sometimes it is very difficult to develop hybrids locally by using imported CMS lines from another country. So identification of effective maintainer and restorer lines from local germplasm is a prerequisite before starting hybrid seed production in rice. In the present investigation, four CMS lines were crossed with 23 genotypes as 'testers' to get 92 F_1. The 92 F_1 were subjected to spikelet fertility analysis. The spikelet fertility was ranged from 0 per cent (Pant CMS 3A × UPR 3807-1-6 and Pant CMS 3A × UPR 3807-1-1) to 83.61 per cent (IR 58025 A × UPR 3506-1-2). Among the 92 F_1, 7 F_1 were expressed as fully fertile, 2 complete sterile, and 83 intermediate types. Eight testers UPR 3405-1-2, UPR 3506-1-1, UPR 3507-1-3, UPR 3607-1-1, UPR 3607-1-2, UPR 3707-1-2, UPR 3807-1-4 and UPR 3807-1-7 identified as partial maintainers for all the four CMS lines. Tester UPR 3507-1-1 was identified as a partial restorer for all four CMS lines. None of the testers was found to be a complete restorer or complete maintainer for all the four CMS lines. For male sterile line Pant CMS-3A two testers (UPR 3807-1-1 and UPR 3807-1-6) were found to be fully maintainers. Four testers (UPR 3405-1-3, UPR 3507-1-2, UPR 3607-1-3 and UPR 3707-1-4) exhibited full restoration after crossing with male sterile line Pant CMS-3A. Out of 92 crosses, only 9 crosses contributed unambiguously to the identification of fully restorer and maintainer parents. Other 83 crosses were found intermediate types which showed neither complete sterility nor fertility. All the effective maintainers identified in the current study can be used for the development of component CMS lines and restorers might be used directly for hybrid development. So, the identified complete maintainer and restorer lines in the present study can be exploited in a heterosis breeding programme in coming years.

Keywords: Cytoplasmic-genetic male sterility, fully restorer, fully maintainer, heterosis.

INTRODUCTION

Rice is a self-pollinated, diploid crop (2n = 24) belonging to the genus *Oryza* having 22 wild and 2 cultivated species (Hashim *et al*., 2021). Among all the breeding techniques to break the yield barrier in rice utilization of heterosis in rice is atmost important because of the dependency of half of the world population on rice (Islam *et al*., 2019; Majid *et al*., 2020). The utilization of male sterility and fertility restoration system in rice for exploitation of heterosis has already paved milestones throughout the world (Mian and Bashar, 2015). In the three lines system of hybrid seed production A line in the male sterile line, B is the maintainer line and R is the restorer line. B and R line crossing with A-line maintain and restore the sterility and fertility of A-line respectively. If any unidentified line after crossing with A-line gives no functional pollen and spikelet fertility in F_1 it can be considered as an effective maintainer. A New CMS line can be developed by 5-6 times backcrossing of F_1 with the recurrent parent. Those genotypes which give higher fertility (>75% spikelet fertility) after crossing with CMS lines are called potential restorers and can be used as male parents in hybrid rice breeding programmes. So depending upon the ability of a genotype to maintain complete sterility or restore normal fertility, recognized as a potential maintainer or restorer (Hariprasanna *et al*., 2006). In the R line presence of any dominant fertility restoration gene (*Rf*) is responsible for restoration which interacts with male-sterile cytoplasm and confers male fertility. Restorer lines have already been identified for different male sterile cytoplasmic sources like CMS-WA, CMS-HL, CMS-BT, CMS-DA and CMS-D1 in China and other countries. Identification of different restorer lines with versatile fertile restoration capacity will help in the development of more heterotic combinations.

The CMS lines are mostly caused by abnormal mitochondrial genes encoding cytotoxic proteins responsible for anther abortion. The maintainer line is the isogenic line of A-line with normal anther production. Restorer lines contain fertility restorer (*Rf*) genes which inhibit the effect of cytotoxic mitochondrial genes responsible for male sterility (Liao *et al*., 2021). Identification of these lines is a prerequisite in rice for hybrid development from diverse germplasm lines and it is a backbone of hybrid rice breeding programme (Mian and Bashar, 2015). They are identified either by phenotypic evaluation (pollen fertility and spikelet fertility) or marker-based selection from testcross hybrids. Abebrese *et al*., (2018); Majid *et al*., (2020) used marker-assisted selection for the identification of those restorer lines that possess *Rf3* and *Rf4* fertility restorer genes in rice. The maintainer and restorer line will show complete spikelet sterility and fertility respectively after crossing with the male sterile line (Abebrese *et al*., 2018). Therefore, this study was undertaken for the identification of restorer (partial and full) and maintainer (partial and full) lines which can be used subsequently for hybrid development and maintenance of the male-sterile line.

MATERIAL AND METHODS

Experimental materials in the present study for the identification of effective maintainers and restorers comprised of 92 tests cross progenies derived from combinations involving 4 CMS lines (Wild abortive) and 23 pollen parents (Table 1). Those materials were grown in the experimental field of Norman Borlaug crop research Centre, GBPUA&T, Pantnagar during *Kharif* 2016 and 2017. All the parents were planted in the plot with a spacing of 15 cm (plant to plant) × 20 cm (row to row). Pollen from all the twenty-three pollen parents was dusted on bagged panicles of CMS lines. Along with this, for facilitating hybridization plants of four CMS lines at the flowering stage were transferred to pots that were filled with soil. Clipping of CMS panicles was done in the evening and hand pollination was carried out the following morning by dusting pollen from 23 selected elite germplasm. So all possible 92 cross combinations were attempted and crossed seeds were collected for evaluation. Identification of maintainers and restorers in the present investigation was carried out by categorizing all F_1 based on spikelet fertility percentage. As per the classification given by Virmani *et al*., (1997**)** dividing all crosses into four classes namely, fully restorer, partial restorer, fully maintainer, and partial maintainer (Table 2).

Table 1: Genotypes used in the study.

Sr. No.	Source	Source	Sr. No.	Source	Source
Pollen Parents					
1.	UPR 3405-1-1	GBPUA&T, Pantnagar	13.	UPR 3707-1-1	GBPUA&T, Pantnagar
2.	UPR 3405-1-2	GBPUA&T, Pantnagar	14.	UPR 3707-1-2	GBPUA&T, Pantnagar
3.	UPR 3405-1-3	GBPUA&T, Pantnagar	15.	UPR 3707-1-3	GBPUA&T, Pantnagar
4.	UPR 3506-1-1	GBPUA&T, Pantnagar	16.	UPR 3707-1-4	GBPUA&T, Pantnagar
5.	UPR 3506-1-2	GBPUA&T, Pantnagar	17.	UPR 3807-1-1	GBPUA&T, Pantnagar
6.	UPR 3506-1-3	GBPUA&T, Pantnagar	18.	UPR 3807-1-2	GBPUA&T, Pantnagar
7.	UPR 3507-1-1	GBPUA&T,Pantnagar	19.	UPR 3807-1-3	GBPUA&T, Pantnagar
8.	UPR 3507-1-2	GBPUA&T, Pantnagar	20.	UPR 3807-1-4	GBPUA&T, Pantnagar
9.	UPR 3507-1-3	GBPUA&T,Pantnagar	21.	UPR 3807-1-5	GBPUA&T, Pantnagar
10.	UPR 3607-1-1	GBPUA&T, Pantnagar	22.	UPR 3807-1-6	GBPUA&T, Pantnagar
11.	UPR 3607-1-2	GBPUA&T, Pantnagar	23.	UPR 3807-1-7	GBPUA&T, Pantnagar
12.	UPR 3607-1-3	GBPUA&T, Pantnagar			
Male Sterile Line					
1.	IR 79165A	International Rice Research Institute (IRRI), Philippines			
2.	IR 58025A	International Rice Research Institute (IRRI), Philippines			
3.	Pant CMS 2A	GBPUA&T, Pantnagar			
4.	Pant CMS 3A	GBPUA&T, Pantnagar			

Estimation of spikelet fertility (F_1 crosses). At the time of harvesting, five panicles were harvested from randomly chosen five different plants in each of the F_1 crosses. After threshing the panicles the total number of grains per panicle and filled grains per panicle were counted, and the spikelet fertility was expressed (in percentage) as the number of filled grains per panicle to the total number of spikelets (filled and unfilled spikelet). Based on spikelet fertility of experimental hybrids, pollen parents were classified into four classes (Virmani *et al*., 1997) as given in Table 2.

Spikelet fertility percentage was calculated as under:

Spikelet fertility (%) = filled grains per panicle/ total number of spikelets × 100.

Table 2: Classification of pollen parent based on spikelet fertility percent of the corresponding hybrids.

Class	Spikelet fertility (%)
Fully maintainer (FM)	0
Partial maintainer (PM)	0.10 - 50.00
Partial restorer (PR)	50.10 - 75.00
Fully restorer (FR)	>75

RESULTS AND DISCUSSION

Results showed that F_1 hybrids produced by crossing CMS lines with selected 23 rice genotypes behaved differently about spikelet fertility and are presented in Table 3. Among 92 F_1 hybrids, different levels of spikelet sterility/fertility were observed. The spikelet fertility was ranged from 0 per cent to 83.61 per cent. The highest and lowest percentages of spikelet fertility were found in Pant CMS 3A × UPR 3807-1-1 and IR 58025 A × UPR 3506-1-2 respectively. Out of 92 F_1 hybrids having CMS lines with WA cytoplasm, 2 lines (Pant CMS 3A × UPR 3807-1-6 and Pant CMS 3A × UPR 3807-1-1) were completely sterile. The remaining 90 hybrids expressed varying degrees of spikelet fertility. Twenty-five of them showed fertility between 50–75%, fifty-eight hybrids were in the range of 10-50% fertility, 7 were more than 75% fertility and the remaining two hybrids were fully sterile (based on spikelet fertility).

The frequencies of fully sterile, 10-50% fertile, 50–75% fertile, and >75% fertile hybrids were 2.17, 61.94, 28.24, 7.58 per cent respectively based on spikelet fertility (Table 4). In the present experiment, among 92 F_1 raised through crossing 23 pollen parents with 4 CMS lines, the frequency of maintainers parents was found to be 64.11 per cent (2.17 % for complete maintainers and 61.94 % for partial maintainers) was higher than the frequency of restorers which accounts for 35.82 per cent (28.24 % for the partial restorer and 7.58 for the complete restorer. Akhter *et al*., 2008; Hossain *et al*., 2018 reported similar results where the frequency of maintainers was higher than that of the restorers.

Among the 92 F_1, there was a differential sterility/fertility reaction of the same pollen parents when crossed with different CMS lines. The same genotype/pollen parent behaved as a fully maintainer for one CMS background, whereas on crossing with another CMS lines it behaved as a partial maintainer. On another side, the same pollen parent was found to be a partial maintainer after crossing with one CMS line while it was fully/partially restorer for another CMS background as depicted in Table 3. For example, UPR 3506-1-2 behaved as a partial maintainer when crossed with Pant CMS 2A, Pant CMS 3A and IR 79156 A CMS lines, while it behaved as fully restorer when crossed with IR 58025 A female lines. UPR 3807-1-5 behaved as partially restorer when crossed with Pant CMS 2A whereas, it behaved as partial maintainer when crossed with Pant CMS 3A, IR 58025 A, IR 79156 A. UPR 3807-1-3, when crossed with Pant CMS 2A, behaved as fully restorer, with Pant CMS 3A behaved as partially restorer, while, on crossing with IR 58025 A and IR 79156 A behaved as partially maintainer. Such differential fertility restoring ability of the same genotype on crossing with different CMS backgrounds is also reported by many other researchers like, Akhter *et al*., 2008; Ali *et al*., 2014; Itha *et al*., 2020; Jayasudha and Sharma, 2010; Mian and Bashar, 2015; Sahu *et al*., 2014; Umadevi *et al*., 2005; Waza and Jaiswal, (2016).

The pollen parent, UPR 3807-1-1 and UPR 3807-1-6 was found to be effective restorer for male sterile line Pant CMS 3A. These identified maintainers can be recurrently backcrossed with the same CMS line for the development of new CMS lines. But these maintainers should have desirable features for height, and other characters also. Seven pollen parents which showed full restoration with different CMS lines can be used further for location-specific hybrid development. From the above results, it is clear that the fertility restoration behaviour of different restorers depended largely on nucleo-cytoplasmic interactions. It could be due to the influence of the genetic background of the CMS line with that of the pollen parent or the presence of modifier genes present in the male parent. In our study, there was a differential sterility/fertility reaction of the same pollen parents when crossed with different CMS lines. Such variability regarding spikelet fertility indicates the existing variation in reproductive traits among the genotypes. Sometimes the number of sterility genes present in female parents act as inhibitors of fertility restoration. So even well-established restorers may show partial fertility restoration with different male sterile lines due to the presence of these inhibitory genes. Partial maintenance or restoration in some cases is due to the presence of the heterozygous gene in the restoration line.

Table 3: Differential fertility/sterility reaction of the same genotype (male) when crossed with different female parents.

Sr. No.	Male parent	Female parent	Differential fertility /sterility reaction
1.	UPR 3405-1-1	Pant CMS 2A, Pant CMS 3A, IR 58025 A	PR
		IR 79156 A	PM
2.	UPR 3405-1-2	Pant CMS 2A, Pant CMS 3A, IR 58025 A, IR 79156 A	PM
3.	UPR 3405-1-3	Pant CMS 2A, IR 58025 A, IR 79156 A	PR
		Pant CMS 3A	FR
4.	UPR 3506-1-1	Pant CMS 2A, Pant CMS 3A, IR 58025 A, IR 79156 A	PM
5.	UPR 3506-1-2	Pant CMS 2A, Pant CMS 3A, IR 79156 A	PM
		IR 58025 A	FR
6.	UPR 3506-1-3	Pant CMS 2A, Pant CMS 3A, IR 79156 A	PR
		IR 58025 A	PM
7.	UPR 3507-1-1	Pant CMS 2A, Pant CMS 3A, IR 58025 A, IR 79156 A	PR
8.	UPR 3507-1-2	Pant CMS 2A, IR 58025 A, IR 79156 A	PR
		Pant CMS 3A	FR
9.	UPR 3507-1-3	Pant CMS 2A, Pant CMS 3A, IR 58025 A, IR 79156 A	PM
10.	UPR 3607-1-1	Pant CMS 2A, Pant CMS 3A, IR 58025 A, IR 79156 A	PM
11.	UPR 3607-1-2	Pant CMS 2A, Pant CMS 3A, IR 58025 A, IR 79156 A	PM
12.	UPR 3607-1-3	Pant CMS 2A	PR
		Pant CMS 3A, IR 79156 A	FR
		IR 58025 A	PM
13.	UPR 3707-1-1	Pant CMS 2A, Pant CMS 3A, IR 58025 A	PR
		IR 79156 A	PM
14.	UPR 3707-1-2	Pant CMS 2A, Pant CMS 3A, IR 58025 A, IR 79156 A	PM
15.	UPR 3707-1-3	Pant CMS 2A, Pant CMS 3A, IR 79156 A	PM
		IR 58025 A	PR
16.	UPR 3707-1-4	Pant CMS 2A	PR
		Pant CMS 3A	FR
		IR 58025 A, IR 79156 A	PM
17.	UPR 3807-1-1	Pant CMS 2A, IR 58025 A, IR 79156 A	PM
		Pant CMS 3A	FM
18.	UPR 3807-1-2	Pant CMS 2A, Pant CMS 3A, IR 79156 A	PM
		IR 58025 A	PR
19.	UPR 3807-1-3	Pant CMS 2A	FR
		Pant CMS 3A	PR
		IR 58025 A, IR 79156 A	PM
20.	UPR 3807-1-4	Pant CMS 2A, Pant CMS 3A, IR 58025 A, IR 79156 A	PM
21.	UPR 3807-1-5	Pant CMS 2A	PR
		Pant CMS 3A, IR 58025 A, IR 79156 A	PM
22.	UPR 3807-1-6	Pant CMS 2A, IR 58025 A, IR 79156 A	PM
		Pant CMS 3A	FM
23.	UPR 3807-1-7	Pant CMS 2A, Pant CMS 3A, IR 58025 A, IR 79156 A	PM

FM- Fully maintainer, **PM-** Partial maintainer, **PR-** Partial restorer, **FR-** Fully restorer

Table 4: Frequency of restorers and maintainers based on spikelet fertility CMS.

CMS lines	Classification based on spikelet fertility							
	FM	%	PM	%	PR	%	FR	%
Pant CMS 2A	0	0	13	14.13	9	9.78	1	1.08
Pant CMS 3A	2	2.17	12	13.04	5	5.43	4	4.34
IR 58025 A	0	0	14	15.21	8	8.69	1	1.08
IR 79156 A	0	0	18	19.56	4	4.34	1	1.08
Total	2	2.17	57	61.94	26	28.24	7	7.58

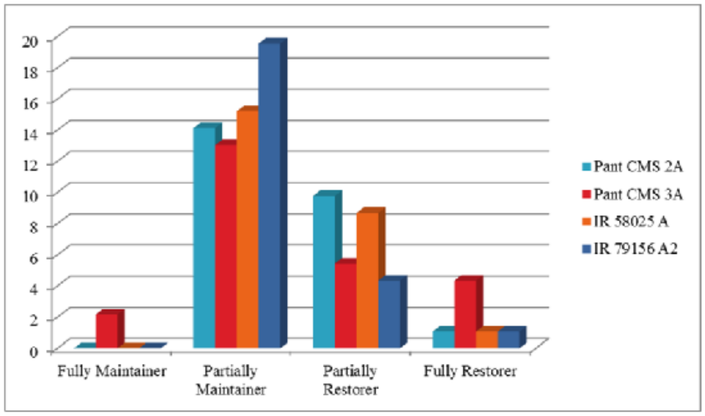

Fig. 1. Frequency of different fertility classes based on spikelet fertility.

CONCLUSION

Identification of potential maintainers and restorers is a prerequisite before starting hybrid seed production in rice. Hence, characterization of elite germplasm of rice based on pollen and spikelet fertility and exploitation of available variation is crucial for the hybrid rice improvement programme. Partial maintainers and restorers found in the experiment can't be used in hybrid rice breeding programmes. The CMS line in which very high variation was found for fertility restoration was Pant CMS 3 A. The fully maintainer lines identified in our experiment can be used for the development of new CMS lines by backcrossing with that F_1. These newly developed CMS lines may be well adapted to local and given target areas to develop adaptable, heterotic hybrids. Newly identified restorers can be used for the development of hybrids with the high level of heterosis and wide adaptation which will also reduce the problem of genetic uniformity. The fully restorer lines may be used for hybrid development after testing their yield performance and combining ability.

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Conflict of Interest. None.

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