Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 7 [7] June 2018 : 15-19

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

Identification of Restorers and Maintainers in Local Land Races of Rice (*Oryza Sativa* L.)

Shweta Singh, Parmeshwar K. Sahu and *Deepak Sharma
Department of Genetics and Plant Breeding, CoA, Indira Gandhi Krishi Vishwavidyalaya, Raipur-492012 (Chhattisgarh)
*Email: deepakhybridrice@gmail.com

ABSTRACT
The experiment was conducted to identify stable maintainers and restorers for three cytoplasmic male sterile (CMS) lines. Three CMS viz., IR 58025A (WA cytoplasm), CRMS 31A and CRMS 32A (Kalinga cytoplasm) lines with were crossed with 6 local landraces of rice to identify restorers and maintainers during 2016-17. The generated F1's expressed various degrees of fertility reactions. Out of 18 test crosses evaluated two parents viz., Bakramudi and Siyar as restorers, 07 parents as partial restorers and 09 parents were identified as partial maintainers for different CMS lines on the basis of pollen fertility and spikelet sterility. Identified restorers will be utilized to develop hybrids and maintainers will be used to develop locally adapted CMS lines through recurrent back crossing.
Keywords: *Rice, CMS Lines, pollen sterility, spikelet fertility, maintainer, restorer*

Received 11.04.2018 Revised 10.05.2018 Accepted 23.05.2018

INTRODUCTION

Rice is the world's most important food crop and a primary food source for more than one third of world's population. It is a staple food of millions of mankind from the dawn of civilization. More than 90% of the world's rice is grown and consumed in Asia, where 60% of the global populations live [1]. It is widely cultivated throughout the world and occupies about 163.2 million hectare of land with 751.9 million tons production (in form of paddy) worldwide [2]. Asia is the leader in rice production accounting for 678.6 million tons during 2016 which is about 90% of the world's production [3].

Breeding strategies for developing the hybrids with high yield potential and better grain quality require the expected level of heterosis and combining ability. Combining ability analysis is one of the effective approaches available for estimating the combining ability effects that help in selecting desirable parents and crosses for the exploitation of heterosis [4].

The success of future hybrid rice programme depends upon identification of parents having good combining ability with higher magnitude of heterosis and good restorer and maintainer capabilities. The use of male sterility system in developing hybrids in crops is possible only when effective maintainers and restorers are identified [5]. Identification of locally adapted maintainers and restorers which show complete sterility and consistently high degree of restoration of CMS lines would be of great value in commercial hybrid programme, if restoring ability is combined with high combining ability.

The establishment of test cross nursery to identify restorers and maintainers is the first step in three line heterosis breeding [6]. In three line breeding system restorers and maintainers will be identified from test cross nursery. Maintainer lines are used for conversion into new CMS lines and restorer lines are subsequently used as male parent in hybrid development program. CMS lines introduced from elsewhere may not be well adapted to a given target area. Successful use of hybrid vigor in rice largely depends on availability of locally developed CMS and restorer lines [7].

MATERIALS AND METHODS

The experiment was conducted at Research cum Instructional Farm, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) in *kharif* 2016 and *kharif* 2017. The biological materials used in the research work comprises of three CMS lines *viz.*, IR 58025A, CRMS 31A and CRMS 32A and six rice

landraces as testers *viz*., Kadamphool, Bakramudi, Maidubraj, Reladhan, Chandan and Siyar and their eighteen hybrids.

The eighteen corresponding hybrids were attempted by adopting L x T design [8]. The hybrids were evaluated along with their parents in Randomized Complete Block Design with two replications under rainfed conditions. Twenty one days old seedlings of total 21 hybrids and their parents were transplanted in the field. A standard spacing of 20 x 15 cm was adopted for the planting.

The panicles of CMS plant were bagged with butter paper before anthesis period. Pollen from pollen parents were dusted on bagged panicles of CMS line separately. So utmost care was taken while test crossing and crossed seeds from the combinations were collected for their evaluation.

Estimation of pollen fertility

Pollen fertility test of F1 in test cross nursery was carried out for their fertility or sterility responses. The spikelets from the just emerged panicle of 05 randomly selected plants were collected in vial containing 70 percent ethanol. With the help of forceps, the anthers from the spikelets were placed on a glass slide containing 2% Iodine Potassium Iodide (IKI) strain. Then the anthers were gently crushed by using needle to release the pollen grains. After removing the debris, a covers lip was put on the slide and observed under microscope.

$$\text{Pollen fertility (\%)} = \frac{\text{No. of fertile pollen grains}}{\text{Total no. of pollen grains}} \times 100$$

Estimation of spikelet fertility

Estimation of spikelet fertility was done on three panicles per plant (two selected at random and one from the main culm) from five randomly selected plants for each test cross hybrid at maturity and were bagged to asses spikelet fertility/sterility. Spikelet fertility of hybrids was assessed by taking the count of well filled and chaffy spikelet in each panicle.

$$\text{Spikelet fertility (\%)} = \frac{\text{No. of filled spikelets}}{\text{Total no. of spikelets}} \times 100$$

Classification of pollen parents

The criteria for classifying the parental lines as maintainers, partial maintainers, partial restorers and restorers were used as proposed by [9]. The categories of parental lines according to pollen fertility % and spikelet fertility % are given below in Table 1.1.

Table 1.1 Criteria for classifying the parental lines

Pollen fertility (%)	Spikelet fertility (%)	Category
0-1	0	Maintainers
1.1-50	0.1-50	Partial maintainers
50.1-80	50.1-75	Partial restorers
>80	>75	Restorers

RESULTS AND DISCUSSION

Identification of restorers and maintainers was carried out by considering observations on pollen fertility and spikelet fertility percentage as per the classification is given by (9).

The restorers and maintainers identified in the present investigation are presented in the Table 1.2 and their frequencies are illustrated in Table 1.3 and Figure 1.

The studies on pollen and spikelet fertility percentages indicated that none of the testers could be identified as potential maintainer. The frequency of potential maintainers is very low as compare to restorers. Results were confirmed by [10] as they were also not found any potential maintainers from their study.

Tester Kadamphool has been identified as partial maintainer for the CMS line CRMS 31A. The Kadamphool is very popular aromatic short grain variety. Therefore for converting this variety as potential maintainer its crosses were attempted with other CMS lines. Its partial maintainer will be further crossed with other potential maintainer lines (*i.e.* B x B crosses). The parents Reladhan and Chandan have been identified as partial maintainers for all the three lines. The genotype Siyar have been identified as partial maintainer in relation to lines CRMS 31A and CRMS 32A. Similar work plan was reported by [10-12].

Table 1.2 List of Identified Restorers and Maintainers

CMS Line	Potential Maintainer (Pollen fertility 0-1% and spikelet fertility 0-0.1%)	Partial Maintainer (Pollen fertility 1.1- 50% and spikelet fertility 0.1-50%)	Partial Restorer (Pollen fertility 50.1-80% and spikelet fertility 50-75%)	Potential Restorer (Pollen fertility > 80% and spikelet fertility > 75%)
IR 58025A	-	Reladhan, Chandan	Kadamphool, Bakramudi, Maidubraj	Siyar
CRMS 31A	-	Kadamphool, Reladhan, Chandan, Siyar	Maidubraj	Bakramudi
CRMS 32A	-	Reladhan, Chandan, Siyar	Kadamphool, Bakramudi, Maidubraj	

Table 1.3 Frequency of Restorers and Maintainers

CMS Lines	Classification based on															
	Pollen Fertility								Spikelet Fertility							
	R	%	PR	%	PM	%	M	%	R	%	PR	%	PM	%	M	%
IR 58025A	1	5.55	3	16.66	2	11.11	0	0	1	5.55	3	16.66	2	11.11	0	0
CRMS 31A	1	5.55	1	5.55	4	22.22	0	0	1	5.55	1	5.55	4	22.22	0	0
CRMS 32A	0	0	3	16.66	3	16.66	0	0	0	0	3	16.66	3	16.66	0	0
Total	**2**	**11.12**	**7**	**38.89**	**9**	**49.99**	**0**	**0**	**2**	**11.12**	**7**	**38.88**	**9**	**50**	**0**	**0**

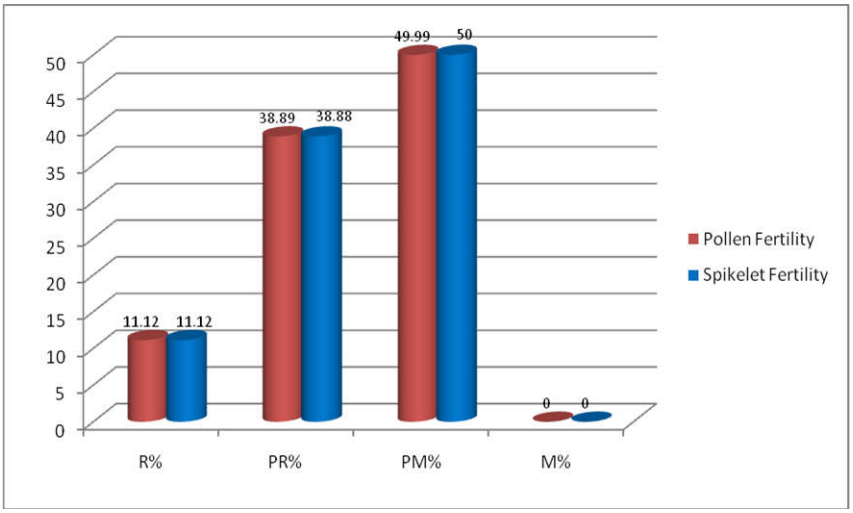

Fig 1: Frequency of Restorers and Maintainers

Two genotypes *viz*., Bakramudi and Siyar were identified as potential restores for CMS lines CRMS 31A and IR58025A respectively. The hybrid CRMS 31A/ Bakramudi showed highest pollen fertility (85.77%) followed by IR 58025A/ Siyar (83.64%) whereas spikelet fertility percentage is higher in IR 58025A/ Siyar (81.58%) followed by CRMS 31A/ Bakramudi (77.18%). Therefore these crosses can be effectively utilized as good restorer lines to develop high yielding rice hybrids. These kinds of reports were also found by other scientists. 07 hybrids were found potential restorer out of 21 hybrids by [10] and 18 hybrids performed as effective restorer out of 38 genotypes by [7].

In some cases, the same genotype behaved as a restorer for one CMS line and as partial maintainer or partial restorer for the other CMS line. Similar type of results was reported by [13].

Tester Kadamphool behaved as partial maintainer for CMS line CRMS 31A and partial restorer for both CMS line IR 58025A and CRMS 32A based on pollen and spikelet fertility respectively. Genotype Bakramudi behaved as potential restorer for CRMS 31A and partial restorer for IR 58025A and CRMS 32A.Genotype Maidubraj behaved as partial restorer for all the CMS line IR 58025A, CRMS 31A and CRMS 32A. Genotype Reladhan behaved as partial maintainer for all lines *i.e.* IR 58025A, CRMS 31A and CRMS32A. Tester Chandan behaved as partial maintainer for all lines. Tester Siyar behaved as effective restorer for line IR 58025A and partial maintainer for both lines CRMS 31A and CRMS 32A. The reason behind these variations in behavior of fertility restoration indicates that the fertility-restoring genes are different or their penetrance and expressivity varied according to genotypes of the parents or the modifiers of female background. This could be due to differential nuclear cytoplasmic interactions between the testers and CMS lines. This kind of the differential reaction of the same genotype in restoring the fertility of different CMS lines of same cytoplasmic source was reported by [5, 14 and 15].

The parents Kadamphool and Bakramudi performed as partial restorer for lines IR 58025A and CRMS 32A whereas, Maidubraj acted as partial restorer for all the three lines.

Table 1.3 Fertility restoration study for identification of restorers and maintainers

Crosses	Days to50% Flowering	Filled spikelets per panicle	Pollen fertility (%)	Spikelet fertility percent (%)	Fertility reaction
IR 58025A x Kadamphool	92.75	147.25	75.72	73.37	PR
IR 58025A x Bakramudi	93.25	161.75	79.85	67.62	PR
IR 58025A x Maidubraj	99.50	167.25	73.59	63.43	PR
IR 58025A x Reladhan	104.75	69.00	25.63	32.54	PM
IR 58025A x Chandan	95.25	43.25	15.59	25.47	PM
IR 58025A x Siyar	100.50	177.75	83.64	81.58	R
CRMS 31A x Kadamphool	94.75	114.50	35.69	42.72	PM
CRMS 31A x Bakramudi	100.00	213.25	85.77	77.18	R
CRMS 31A x Maidubraj	99.75	164.00	77.67	69.64	PR
CRMS 31A x Reladhan	101.25	11.25	14.82	6.65	PM
CRMS 31A x Chandan	100.50	7.00	13.75	6.04	PM
CRMS 31A x Siyar	105.00	71.00	20.84	28.57	PM
CRMS 32A x Kadamphool	95.25	178.25	71.40	69.27	PR
CRMS 32A x Bakramudi	92.25	164.00	78.51	71.66	PR
CRMS 32A x Maidubraj	107.50	147.75	73.96	64.40	PR
CRMS 32A x Reladhan	105.00	82.75	33.42	40.76	PM
CRMS 32A x Chandan	101.50	4.75	10.02	2.82	PM
CRMS 32A x Siyar	101.00	92.50	27.85	39.13	PM

*R= Restorer, PR= Partial restorer, PM= Partial Maintainer

Conclusion

In the present investigation two genotypes *viz.,* Bakramudi and Siyar were identified as potential restorers for CMS lines CRMS 31A and IR 58025A respectively. Potential restorers identified in the present study can be further used for developing good, high yielding and quality rice hybrids. Whereas short grain aromatic rice Kadamphool identified as partial maintainer will be further improved by attempting B x B crosses and converted into locally adapted similar grain type CMS line.

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CITATION OF THE ARTICLE

Shweta Singh, Parmeshwar K. Sahu and Deepak Sharma. Identification of Restorers and Maintainers in Local Land Races of Rice (*Oryza Sativa* L.). Bull. Env. Pharmacol. Life Sci., Vol 7 [7] June 2018 : 15-19