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Research Article

Identification and evaluation of aromatic short grain and coarse grain potential restorers and maintainers in rice hybrids

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Abstract
Three CMS lines IR58025A (Wild abortive (WA) cytoplasm of *Oryza perennis*), CRMS31A (WA- cytoplasm) and CRMS32A ("Kalinga"cytoplasm from 'Dunghansali') and 20 diverse testers were crossed in line × tester fashion to generate 60 hybrids. The experiment was laid out in a complete randomized block design with two replications. Observations of pollen fertility and spikelet fertility were carried out for the identification of maintainers and restorers. Out of 60 test crosses 9 restorers exhibited more than 80 % pollen/spikelet fertility with heterotic morphological attributes. Out of 9 restorers two are aromatic short grain type and others 7 are coarse grain type. One maintainer was also identified. Frequency of partial restorer (37) and partial maintainer (11) were high. All the F1 are semi tall except CRMS31A/Jaldubi and CRMS31A/Adhamchini. F_1's *viz*. IR58025A/ IR72164-352-2-5-5 and IR58025A/ TOX 981-11-2-3 show high spikelet fertility. No effective maintainer was identified from aromatic short grain rices.

Key words: Rice, CMS, aromatic short grain, restorer, maintainer.

Introduction

Cytoplasmic male sterility-fertility restoration (CMS) system based three line hybrid rice breeding is popular in rice production worldwide. Experience in China (Ma and Yuan 2003) IRRI (Virmani 2003, 2006), India (Mishra *et al.*, 2003), Vietnam (Hoan and Naghia 2003), the Philippines (Redona *et al.*, 2003), Bangladesh (Julfiquar and Virmani 2003) clearly shown that hybrid rice varieties have a 1-1.5 t ha^{-1} yield advantage over semi dwarf inbred high yielding varieties (HYVs) in farmer's fields. Cytoplasmic male sterility (CMS) is a common phenomenon that has been extensively used for production of hybrid seeds in various crops. *Rf* genes are needed for restoring fertility to CMS lines. The basic step in hybrid development program is identification of restorers and maintainers through test cross evaluation. Potential restorers will be utilized to develop hybrids whereas potential maintainers will be converted into new CMS lines through recurrent back cross. With this objective, a set of local and exotic germplasm were evaluated to identify restorers and maintainers with CMS lines in the background of locally adapted varieties/elite breeding lines to develop aromatic short grain and coarse grain rice hybrids.

Material and methods

Local and exotic germplasm was evaluated to identify the commercially usable restorers and maintainers in aromatic short grain and coarse rices at Research-cum-Instructional farm of Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh during *Kharif* 2011 to *Kharif* 2012. Three CMS lines viz., IR58025A, CRMS31A, and CRMS32A and 20 testers as varieties including accessions viz., Swarna selection-1, Swarna selection-2, R1557-1317-1-580-1, Nagina-22, Tarun Bhog, R1659-3629-1-462-1, Mancha, R-1240-927-3-1056-1, Jaldubi, Adhamchini, MTU-1001, TOX 981-11-2-3, Chinikapoor, R1949-1196-2-1, IR73007-44-1-2-3, IR72164-352-2-5-5, IR72910-177-1-1-3, R1213-3, Mahsuri Sel-1 and HR-NPT-6-3 were used in this study. The 3 CMS lines have cytoplasm derived from a WA and Kalinga cytoplasmic sources. Lines (CMS) were crossed with 20 testers to generate 60 hybrid combinations in line x tester mating design. The hybrids were evaluated along with parents in a randomized complete block design with 2 replications.

Observation on pollen and spikelet fertility (%):
Pollen fertility and seed-setting rate were used as the main criteria for the evaluation of fertile and sterile plants.
Estimation of pollen fertility: Pollen fertility test of test cross F1 was carried out for their fertility or sterility responses. The spikelets (5 to10) from the just emerged panicle of 3 randomly selected plants were collected in vial containing 70 percent ethanol (Tan *et al.,* 2008). With the help of forceps, the anthers from the spikelet were placed on a glass slide containing 1% Iodine Potassium Iodide (IKI) stain (Sarial and Singh, 2000). Then the anthers were gently crushed by using needle to release the pollen grains. After removing the debris, a cover slip was put on the slide and observed under microscope.

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

Estimation of spikelet fertility: Estimation was done on three panicles per plant (two selected at random and one from the main culm) from five randomly selected plants for each testcross hybrid at maturity. Spikelet fertility of hybrids was assessed by taking the count of well filled and chaffy spikelets in each panicle.

$$\text{Spikelet fertility \%} = \frac{\text{No. of filled spikelets per panicle x 100}}{\text{Total no. of spikelets per panicle}}$$

The panicles that emerged from the primary tiller were bagged before anthesis and the number of filled grains and chaffs in the panicle were counted at the time of maturity. The ratio of filled grains to the total number of spikelets was expressed as seed setting rate (He *et al*., 2006). The goodness of fit to Mendelian segregation pattern of fertile, partially fertile, partially sterile was assessed and completely sterile plants were classified in hybrids. Classification of pollen parents : The pollen parents were classified into four categories (Fig.1) *viz.* Maintainers (M), Partial Maintainers (PM), Partial Restorer (PR) and Restorer (R) according to Virmani *et al.* (1997) and Sabar *et al.* (2007) (Table 1).

Results and discussion

Out of 60 tests crosses 9 restorers, 1 maintainer and 11 partial maintainers were found on the basis of pollen fertility, while 37 partial restorers were found on the basis of spikelet/pollen fertility studies under binocular microscope. The frequency of restorers, partial restorers, partial maintainers and maintainers were 17%, 62%, 18% and 3% respectively (Fig.2). Das *et al.* (2013) were also reported higher frequency of restorers (51.3%) than that of maintainer (6.3%) from evaluation of 224 test crosses and Khan *et al*. (2012) reported same higher frequency of restorers (27%) than that of maintainers (4%) from 100 test crosses. Raju *et al*. (2006) also reported the high frequency of restorers as compare to maintainers. But Akhter *et. al*. (2008) reported higher frequency of maintainers (17%) than that of restorers (11%) from 65 test crosses. Sri Krishnalatha and Sharma (2012) reported the equal frequency of restorers and maintainers out of 18 hybrids *viz.* two genotypes having restorer reaction, nine genotypes exhibited partial restorer reaction, five genotypes were partial maintainers and two genotypes were maintainers. Soni *el al.* (2012) stated the high frequency of potential restorers. They identified 3 partial maintainer, 6 partial restorers and 6 potential restorers and no effective maintainer could be identified in the material in their based on the pollen fertility and spikelet fertility percentage. Jayasudha and Sharma (2010) also concluded that the frequency of potential restorers was much higher in number and no effective maintainer could be identified.

Two aromatic short grain rice (ASG) and seven coarse lines were identified as effective restorers from the tested genotypes (Table 2). The maximum pollen fertility (97.60%) was observed in the cross of IR72164-352-2-5-5 / IR58025A, while the complete sterility (0.00) was observed in the cross of R1557-1317-1-580-1 / CRMS32A. Among these 9 restorers, two aromatic short slender rices *viz.* Chinikapoor and Tarun Bhog are the popular aromatic short grain varieties of Chhattisgarh state having good cooking quality traits.

Out of these 60 testcrosses only one was identified as effective maintainers) and others 9 were classified as partial maintainers (Table 3). No effective maintainer/partial maintainer was identified for aromatic short grain type. The morphological characters of identified maintainers are suitable for development of CMS lines and can be converted into new CMS lines for the development of local rice hybrids. Morphological attributes of hybrids having good restoration are presented in Table 4. In the present study it was revealed that nine combinations had more than 80% spikelet fertility with acceptable flowering days. Plant heights of all these hybrids were also less except CRMS31A/Jaldubi and CRMS31A/Adhamchini than check variety.

The fertility restoration performance of the genotypes varies with genetic background of CMS lines. More emphasis should be given to utilize proper rice cultivar in rice breeding as parental lines to achieve the goal of superior hybrid with better grain quality. The identified restorers and maintainers from this study are locally adopted. The identified restorer lines *viz.*IR72164-352-2-5-5, TOX 981-11-2-3, IR72164-352-2-5-5, R1949-1196-2-1, Mahsuri-Sel-1, MTU-1001, and HR-NPT-6-3 will be utilized to develop coarse grain hybrids whereas Chinikapoor, Tarun Bhog will be utilized to develop aromatic short grain hybrids. New restorer may be also developed through crossing programme which can expand the genetic base of restorer by pyramiding complementary traits from diverse sources according to breeding objectives. It was also noticed that local germplasm had more frequency of restorer lines as compared to maintainers. New restorer lines should be developed using the local germplasm / aromatic short grain varieties for the development of good aromatic and non aromatic rice hybrids. From this study, it is evident that the frequency of partial restorers is quite higher than the restorers amongst the tested genotypes. Identified promising maintainer may be backcrossed to develop a cms lines with agronomic superiority and also for other desirable floral traits.

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Table 1. Classification of parental lines into restorers and maintainers (Virmani *et al*., 1997)

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

Table 2. Genotypes identified as effective restorers and their pollen and spikelet fertility.

CMS used	Effective Restorers	Groups	Pollen Fertility %	Spikelet Fertility %
IR58025A	IR72164-352-2-5-5	Coarse	97.60	95.52
IR58025A	TOX 981-11-2-3	Coarse	94.75	93.71
CRMS31A	IR72164-352-2-5-5	Coarse	93.78	90.89
IR58025A	R1949-1196-2-1	Coarse	92.50	90.42
CRMS31A	Chinikapoor	ASG	89.50	90.14
CRMS32A	Tarun Bhog	ASG	87.25	89.53
CRMS31A	Mahsuri Sel.-1	Coarse	86.40	87.74
CRMS31A	MTU-1001	Coarse	86.14	86.67
IR58025A	HR-NPT-6-3	Coarse	86.00	83.04
	S.E.		1.45	1.23
	C.D.(P=0.05)		3.54	2.85

ASG: Aromatic Slender Grain

Table 3. Genotypes identified as maintainer and partial maintainers

CMS used	Effective Maintainers	Groups	Spikelet Fertility%	Pollen Fertility %
CRMS32A	R1557-1317-1-580-1	Coarse	0.00	0.00
CRMS32A	R-1240-927-3-1056-1	Coarse	1.00	1.93
CRMS32A	Swarna Selection-1	Coarse	2.63	2.40
IR58025A	Jaldubi	Coarse	3.65	4.65
IR58025A	R-1240-927-3-1056-1	Coarse	4.54	3.44
IR58025A	R1557-1317-1-580-1	Coarse	4.67	5.90
CRMS31A	Nagina-22	Coarse	4.71	3.75
CRMS31A	Jaldubi	Coarse	5.16	7.65
CRMS31A	IR73007-44-1-2-3	Coarse	5.30	9.00
CRMS31A	Mancha	Coarse	6.83	5.55
	S.E.		0.66	0.86
	C.D.(P=0.05)		1.49	2.79

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Table 4. Morphological attributes and spikelet fertility of cross combination with good spikelet's fertility

Parentage	Days to Flowering	Plant Height (cm)	Tillers / Plant	Filled spikelets/ Panicle	Spikelet Fertility %
IR58025A / IR72164-352-2-5-5	97.00	113.95	13.75	254.85	95.52
IR58025A / TOX 981-11-2-3	95.25	116.40	6.50	304.80	93.71
CRMS31A / IR72164-352-2-5-5	105.90	94.20	7.55	195.25	90.89
IR58025A / R1949-1196-2-1	84.75	118	7.45	273.25	90.42
CRMS31A / Chinikapoor	92.00	108.80	8.50	325.60	90.14
CRMS32A / Tarun Bhog	107.75	75.93	7.68	371.25	89.53
CRMS31A / Mahsuri Sel.1	108.75	137.20	9.35	186.35	87.74
CRMS31A / MTU-1001	104.00	69.84	12.95	450.50	86.67
IR58025A / HR-NPT-6-3	96.00	124.40	4.70	195.50	83.04
KRH-2 (check)	104.77	133.03	11.40	156.20	79.90
S.E.	2.48	7.16	0.91	29.52	1.48
C.D.(P=0.05)	5.60	16.19	2.07	66.72	3.34

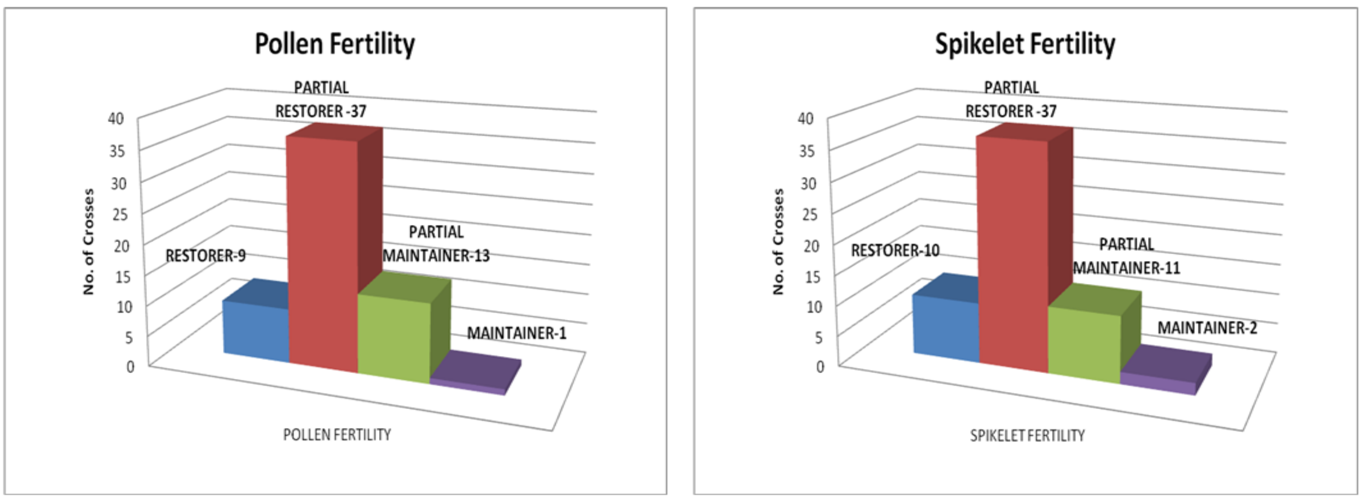

Fig. 1 Frequency of pollen and spikelet fertility of the genotypes under study

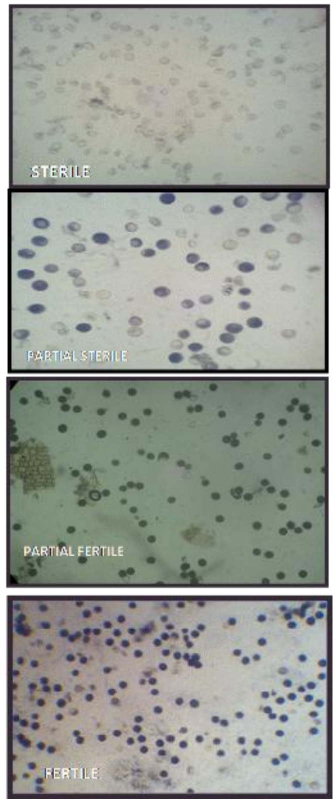

Fig. 2 Pollen and spikelet fertility of the genotypes under study