



Research Note

Fertility restoration analysis in different CMS sources in rice (*Oryza sativa* L.)

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Abstract

Amongst 275 test crosses, 57.45 %, 7.27 %, 35.27 % and 21.09 % were observed as restorers, maintainers, partial restorer and partial maintainers respectively for all the CMS lines included in the study. The pollen and spikelet fertility in the test cross progenies ranged from 0.0 to 94.66 and 0.0 to 81.10 per cent, respectively. The highest pollen and spikelet fertility was observed in test cross; RTN 17A/Pusa-44 (94.66 and 89.10 %) followed by RTN 3 x PR-115 (94.43 and 87.29 %). Among 55 high yielding varieties/ promising lines, 47 genotypes exhibited fertility restoration for any one of the CMS line. Out of 47 genotypes, 14 varieties (25.45 %) were identified as common restorers for all the five CMS lines of five different sources. The frequency of the male parents behaving as effective restorers was found to be maximum (65.45 %) for RTN 2A of ARC source followed by RTN 17A (60.0 %) of Dissi source, KJTCMS-6A (56.36 %) of WA sources, RTN 3A (54.55 %) of Mutant of IR 62829B source and RTN 13A (50.91 %) of Gambiaca source indicating that the fertility in these CMS lines were easy to restore. The genotypes, viz., IR-8866, BL-184AR, IET-13840-RP-66-67, RDN-97-3-2-37-14, PR-115, PKV-Makarand, GR-7, IR-54742-22-19-3, Pusa Sugandha-3, Pusa Sugandh-5, Super basmati, Phule Radha, RP-BIO-226 and GR-11, NVSR-20, IR-63879-195-195-2-2-3-2, IR-22273 and PR-118 were identified as effective restorers may be exploited to develop commercial hybrids and the genotypes, viz., VDN-9-10-1, SYE-4, GR-4, Gurjari, SKL-8, IR-34, SYE-6, SYE-5 and GR-5 were identified as maintainers may be exploited for conversion of CMS sources in above said locally adaptable genotypes.

Key words

Restores, maintainers, partial restorers / partial maintainers, pollen fertility, spikelet fertility, CMS sources

Rice, the traditional, self-pollinated crop has witnessed all round success of hybrid cultivars in China, where hybrid rice technology has enable to increase in both biomass and harvest index (Yuan, 1992). Hybrid rice technology has been successfully developed and is one of the potent options for increasing rice production in irrigated areas. India and Vietnam are other important countries, which adopted hybrid rice technology on large scale. The availability of stable cytoplasmic male sterility and fertility restorer system is vital for commercial exploitation of heterosis in any crop. With the discovery of the wild abortive (WA) male sterility inducing cytoplasm from *Oryza sativa spontanea* and subsequent development of three lines hybrids make a breakthrough in exploitation of heterosis in rice. The low frequency of restores and maintainers in rice cultivar is serious handicap in exploitation of rice hybrids of various groups with quality traits. So far sixty-eight CMS sources have been reported of which the wild abortive (WA) cytoplasm is used extensively for the production of hybrids. Yuan and Virmani reported CMS sources used in China to develop commercial F₁ rice hybrids, those include WA, Gambiaca (GAM), CMS-Boro (BT), Dissi (Di), HL and dwarf wild rice with abortive pollen (DA). About 95% of the area under cytoplasmic male sterile (CMS) derived hybrids are occupied from wild abortive (WA) cytosterility system. Outside China, WA cytosterility system is mostly used to develop suitable CMS lines. In India, 46 hybrids in rice have been developed by

public and private sectors are based on WA cytosterility (Ramesha *et al.*, 2007).

Present investigation was undertaken to identify effective restorers and maintainers among different high yielding, locally adaptable varieties/ promising lines for different male sterile lines having different cytoplasmic sources (WA, ARC, Gambiaca, Dissi and mutant cytoplasm).

Two hundred and seventy five crosses were made in line x tester mating design by using five CMS lines from different CMS sources (KJTCMS-6A - WA sources, RTN 2A -ARC source, RTN 3A - Mutant of IR 62829B source, RTN 13A - Gambiaca source and RTN 17A - Dissi source) and fifty five males by hand pollination at National Agricultural Research Project Farm, Navsari. Before pollination, sterility of female plants was checked and insured to have 100 per cent sterility. The 275 F₁s, were evaluated in Randomized Block Design (RBD), replicated thrice in the three different locations viz., Navsari (Loc-I), Bardoli (Loc-II) and Vyara (loc-III) by planting a single row plot of 30 plants, placed at 20 x 15 cm. All the agronomical practices and plant protection measures were followed as per recommendations. Observations were recorded on pollen fertility and spikelet fertility.

Ten to fifteen spikelets having full-matured anthers (about to dehisce) were collected in a vial

containing 70 % alcohol. Those were from middle to top portion of the every two panicles of each randomly selected five plants in each of the 275 test cross hybrids. Two to three anthers from each spikelet were placed together on a glass slide, squashed and pollen grains were stained by 1 % iodine - potassium iodide stain (the stain was prepared by dissolving of 1 g iodine and 2 g of potassium iodide in 100 ml distilled water) and observation for fertile pollen grains were made under light microscope in three microscopic fields. Unstained, half stained, shriveled and empty pollen grains were classified as sterile while well filled, stained and round pollen grains were recorded as fertile and others were mixed of the above both the types.

The pollen fertility was calculated as Number of fertile pollen grains/ Total number of pollen grains examined $\times 100$. Based on the score of pollen fertility, the cultivars were classified as effective restorers (> 80 pollen fertility), partial restorers (50.1 to 80 pollen fertility), partial maintainers (1.1 to 50 % pollen fertility) and maintainers (0 to 1 pollen fertility) on the basis of their pollen fertility (Virmani *et al.*, 1997).

Five randomly selected emerging panicles from each F_1 hybrid were bagged (to avoid out crossing) after heading and before flowering. The spikelet fertility and sterility were calculated on the basis of five randomly selected panicles from each F_1 at the time of maturity. Spikelet fertility was calculated as a percentage of filled grains. The percentage of spikelet fertility was calculated as given below: Spikelet fertility (%) = Number of fertile spikelets in a panicle (filled) / Total number of spikelets in a panicle (filled and unfilled) $\times 100$. Based on the score of spikelet fertility, cultivars were classified as effective restorers (>75 % spikelet fertility), partial restorers (50.1 to 75 spikelet fertility), partial maintainers (0.1 to 50 % spikelet fertility) and maintainers (0 % spikelet fertility) on the basis of their spikelet fertility (Virmani *et al.*, 1997).

The 55 male parents of test cross hybrids were categorized as restorers, partial restorer, partial maintainers and maintainers. The information on fertility reaction of 275 testcross hybrids and classification of genotypes in four categories is presented in table 1. It was revealed that, amongst 275 test crosses, 57.45 and 7.27 per cent were observed as restorers and maintainers, respectively. The bulk of the male parents (35.27 %) were categorized as partial restorer (21.09 %) and partial maintainers (14.18 %) for all the CMS lines included in the study. The pollen and spikelet fertility in the test cross progenies ranged from 0.0 to 94.66 and 0.0 to 81.10 per cent, respectively. The highest pollen and spikelet fertility were observed in test cross; RTN 17A/Pusa-44 (94.66 and 89.10 %) followed by RTN 3 $\times$ PR-115 (94.43

and 87.29 %). One hundred and sixty test crosses exhibited more than 80 per cent pollen fertility and 75 per cent spikelet fertility indicating fertile category *i.e.* effective fertile hybrids.

Among 55 high yielding varieties/ promising lines 47 genotypes exhibited fertility restoration for any one of the CMS line. Out of 47 genotypes, 14 varieties (25.45 %) were identified as common restorers for all the five CMS lines of five different sources (Table 1 & 2). The frequency of the male parents behaving as effective restorers was found to be maximum (65.45 %) for RTN 2A of ARC source followed by RTN 17A (60.0 %) of Dissi source, KJTCMS-6A (56.36 %) of WA sources, RTN 3A (54.55 %) of Mutant of IR 62829B source and RTN 13A (50.91 %) of Gambiaca source indicating that the fertility in these CMS lines were easy to restore. The genotype, VDN-9-10-1 identified effective maintainers for all the five CMS lines showing that absence of restoring gene (s) for restorations of fertility for all five cytoplasmic sources (Table 2). Cytoplasmic male sterility in rice is controlled by the interaction of cytoplasmic and nuclear genes. Presence or absence of dominant fertility restorer nuclear gene(s) is known to confirm fertility restoring or sterility maintaining ability of a genotype for a specific male fertility inducing cytoplasm.

Out of 55 genotypes crossed, fourteen genotypes *viz.*, IR-8866, BL-184AR, IET-13840-RP-66-67, RDN-97-3-2-37-14, PR-115, PKV-Makarand, GR-7, IR-54742-22-19-3, Pusa Sugandha-3, Pusa Sugandh-5, Super basmati, Phule Radha, RP-BIO-226 and GR-11 were identified as common effective restorers for all the five CMS lines having different cytoplasmic source *viz.*, WA, ARC, Mutant, Gambiaca and Dissi. The genotypes *viz.*, NVSR-20, IR-63879-195-195-2-2-3-2, IR-22273 and PR-118 were identified as effective restorers for KJTCMS-6A, RTN-2A, RTN-3A and RTN-17A and behaved as partial restorer for RTN-13A. GR-3 and KJT-11-1-26-5-11 were identified as potential restorer for all CMS lines except KJTCMS-6A. Rice genotypes, Dandi and PKV-Ganesh were identified as potential restorers for all CMS lines except RTN-17A and RTN-3A respectively. The genotype, KJT-3-2-861-25-15-5 was identified as potential restorer for KJTCMS-6A, RTN-3A and RTN-17A and behaved as partial restorer for RTN-2A and RTN-13A.

The genotypes, SYE-4, GR-4 Gurjari and SKL-8 identified as effective maintainer for RTN-17 CMS line of Dissi cytoplasmic source. Rice cultivars, SKL-8, IR-34, SYE-6, SYE-5 and GR-5 identified as maintainer for RTN-13A CMS line of Gambiaca source and IR-34, SYE-5 and GR-5 as maintainer for RTN-3A CMS line of Mutant of IR 62829B source. This showed that there was differential behaviour of different genotypes with different

CMS sources for their fertility restoration. The variation in the fertility restoration ability of genotypes for the same source as well as different sources of cytoplasm have also been confirmed by the studies of several earlier workers (Jayamani *et al.*, 1995).

The effect of minor or modifier genes present in the pollinator could also result in differential fertility restoration (Yadav *et al.*, 1997). These presumptions also hold good for the differential behaviour of CMS lines of WA, Dissi, Mutant, Gambiaca and ARC cytoplasm for fertility restoration in the present study. The results of present study suggest that fertility restoration is CMS lines specific. Therefore, a pollen parent identified as a restorer cannot eventually be taken as a restorer for other CMS lines even of the same source. The genotypes, viz., IR-8866, BL-184AR, IET-13840-RP-66-67, RDN-97-3-2-37-14, PR-115, PKV-Makarand, GR-7, IR-54742-22-19-3, Pusa Sugandha-3, Pusa Sugandh-5, Super basmati, Phule Radha, RP-BIO-226 and GR-11, NVSR-20, IR-63879-195-195-2-2-3-2, IR-22273 and PR-118 were identified as effective restorers may be exploited to develop commercial hybrids and the genotypes, viz., VDN-9-10-1, SYE-4, GR-4, Gurjari, SKL-8, IR-34, SYE-6, SYE-5 and GR-5 were identified as maintainer may be exploited for conversion CMS sources in locally adaptable above said genotypes.

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Table 1. Fertility reaction of test crosses involving five CMS lines of five different cytoplasmic sources

Sr. No.	Test crosses Female → Male ↓	WA			ARC			Mutant			Gambiaca			Dissi		
		KJTCMS-6A			RTN-2A			RTN-3A			RTN-13A			RTN-17A		
		PF%	SF%	FR	PF	SF	FR	PF	SF	FR	PF	SF	FR	PF	SF	FR
1	IR-8866	86.50	79.96	R	90.53	84.77	R	86.70	80.96	R	84.38	76.65	R	87.49	81.07	R
2	IR-22273	83.49	77.82	R	86.91	81.33	R	87.67	82.27	R	70.56	67.19	PR	86.22	81.21	R
3	KJT-10-3-39-7-42	6.07	0.48	PM	86.86	81.30	R	92.41	86.86	R	70.21	64.65	PR	80.66	75.11	R
4	IR-28	36.70	31.14	PM	83.77	78.22	R	73.29	67.74	PR	80.57	75.01	R	83.34	77.79	R
5	RTN-22-3-1-1-1	85.80	80.24	R	72.85	67.29	PR	57.07	51.51	PR	84.27	78.71	R	81.65	76.09	R
6	KJT-3-2-861-25-15-5	89.22	85.25	R	85.01	78.68	PR	87.64	82.84	R	77.04	72.04	PR	90.42	84.96	R
7	Parag	83.94	78.38	R	92.58	87.02	R	2.42	0.04	PM	83.18	77.62	R	18.48	12.93	PM
8	GR-4	83.54	77.98	R	61.11	55.55	PR	8.34	2.01	PM	81.62	76.07	R	0.00	0.00	M
9	IR-68	87.88	82.32	R	90.07	84.52	R	29.49	23.94	PM	32.73	27.17	PM	83.75	78.20	R
10	BL-184AR	82.65	75.83	R	82.57	76.99	R	83.40	77.94	R	80.57	75.23	R	87.50	81.90	R
11	IET-13840-RP-66-67	89.74	84.04	R	84.70	79.33	R	82.85	77.61	R	85.49	81.62	R	83.32	77.33	R
12	KJT-11-1-26-5-11	76.23	70.92	PR	86.64	81.45	R	81.16	75.25	R	79.70	78.95	R	85.92	80.32	R
13	VDN-9-10-1	0.83	0.00	M	0.00	0.00	M	0.64	0.00	M	0.00	0.00	M	0.00	0.00	M
14	RDN-97-3-2-37-14	90.09	85.01	R	88.28	82.87	R	88.85	83.74	R	82.34	77.66	R	89.13	83.21	R
15	SYE-4	27.71	22.15	PM	61.63	56.08	PR	2.49	0.00	PM	74.99	69.43	PR	0.00	0.00	M
16	PR-115	82.40	77.63	R	88.12	82.99	R	94.43	87.29	R	85.94	79.67	R	85.37	82.18	R
17	PKV-Makarand	88.47	83.43	R	83.93	77.74	R	85.89	80.50	R	86.07	80.24	R	82.22	79.71	R
18	GR-3	77.20	71.48	PR	87.17	81.14	R	87.88	81.55	R	86.78	81.54	R	80.34	76.79	R
19	IR-44	60.39	54.83	PR	72.50	66.95	PR	68.67	63.12	PR	44.17	38.62	PM	58.87	53.31	PR
20	SKL-4-43-28-46-48	81.50	75.95	R	83.98	78.42	R	61.82	56.26	PR	66.17	60.62	PR	80.71	75.16	R
21	PR-116	85.44	79.46	R	87.15	82.07	R	90.25	85.32	R	85.74	82.71	R	79.05	73.30	PR
22	PKV-Ganesh	89.79	84.26	R	85.78	80.20	R	82.54	72.42	PR	92.65	86.67	R	84.59	79.26	R
23	GR-7	91.43	87.01	R	82.92	77.53	R	89.28	86.68	R	87.57	81.57	R	88.84	83.88	R
24	IR-26	27.44	21.88	PM	92.68	87.12	R	62.09	56.54	PR	69.15	63.60	PR	4.40	0.00	PM



Table 1. Contd.,

Sr. No.	Test crosses		WA			ARC			Mutant			Gambiaca			Dissi		
	Female	→	KJTCMS-6A			RTN-2A			RTN-3A			RTN-13A			RTN-17A		
	Male ↓		PF%	SF%	FR	PF	SF	FR	PF	SF	FR	PF	SF	FR	PF	SF	FR
25	IR-66		80.15	74.60	PR	84.10	78.55	R	55.24	49.69	PM	62.30	56.74	PR	62.52	56.96	PR
26	IR-54742-22-19-3		85.21	80.47	R	87.35	82.36	R	88.65	85.30	R	85.11	81.17	R	88.17	83.46	R
27	SKL-22-63-21-48		59.48	53.92	PR	42.15	36.59	PM	84.59	79.03	R	81.28	75.72	R	78.06	72.51	PR
28	Pusa Sugandha-3		91.30	85.75	R	89.49	83.93	R	84.14	78.59	R	82.79	77.24	R	84.63	79.08	R
29	Pusa Sugandh-5		82.93	77.35	R	87.13	81.50	R	84.71	80.31	R	82.03	77.04	R	85.81	80.37	R
30	CR-57-MR-1523		77.71	72.75	PR	75.49	70.32	PR	88.95	83.51	R	83.04	78.15	R	81.59	76.78	R
31	Gurjari		31.45	25.89	PM	75.38	69.83	PR	87.72	82.16	R	4.84	0.26	PM	1.25	0.00	M
32	Super basmati		84.51	79.27	R	90.91	86.00	R	86.54	82.11	R	79.68	76.20	R	87.02	82.04	R
33	PR-114		77.94	72.56	PR	80.81	75.22	R	76.90	71.14	PR	76.70	70.84	PR	85.73	79.10	R
34	PR-118		90.97	85.41	R	86.55	80.99	R	82.81	77.26	R	72.60	67.04	PR	90.77	85.22	R
35	Phule Radha		87.10	81.36	R	86.52	81.12	R	83.04	80.18	R	84.20	80.29	R	91.11	85.86	R
36	SKL-8		8.64	2.57	PM	78.95	73.39	PR	9.98	4.46	PM	0.00	0.00	M	0.00	0.00	M
37	NPT-5.1		3.98	0.24	PM	12.33	6.77	PM	87.82	82.27	R	63.60	58.04	PR	78.20	72.65	PR
38	NVSR-20		90.03	84.56	R	84.12	78.72	R	88.10	84.11	R	79.42	74.79	PR	85.35	79.50	R
39	IR-34		78.94	73.38	PR	27.64	22.08	PM	1.11	0.00	M	1.10	0.00	M	3.99	0.16	PM
40	IR-63879-195-195-2-2-3-2		85.80	80.23	R	87.15	82.41	R	89.29	84.37	R	78.98	73.61	PR	88.04	83.51	R
41	Dandi		83.98	78.81	R	87.29	81.72	R	82.81	77.20	R	87.26	82.12	R	77.73	72.51	PR
42	Pusa-44		89.09	83.53	R	51.74	46.18	PM	73.20	67.65	PR	45.16	39.60	PM	94.66	89.10	R
43	RP-BIO-226		91.04	85.64	R	85.84	80.03	R	83.55	79.06	R	84.93	79.69	R	76.65	75.73	R
44	INP-19		91.00	85.42	R	82.05	76.15	R	74.90	70.14	PR	91.84	85.65	R	83.42	78.05	R
45	GR-11		89.21	84.34	R	80.32	75.51	R	83.51	77.98	R	82.34	77.52	R	88.08	82.94	R
46	IR-48		29.91	24.35	PM	87.68	82.12	R	12.07	5.12	PM	6.43	0.20	PM	10.81	4.42	PM
47	KJT-6		37.57	32.01	PM	16.07	10.51	PM	33.13	27.57	PM	0.00	0.00	M	79.01	73.46	PR
48	IR-32809-26-3-3		53.67	48.12	PM	28.37	22.81	PM	59.24	53.68	PR	56.81	51.26	PR	74.94	69.39	PR



Table 1. Contd.,

Sr. No.	Test crosses		WA			ARC			Mutant			Gambiaca			Dissi		
	Female	Male	KJTCMS-6A			RTN-2A			RTN-3A			RTN-13A			RTN-17A		
	→	↓	PF%	SF%	FR	PF	SF	FR	PF	SF	FR	PF	SF	FR	PF	SF	FR
49	MTU-1001		65.08	59.53	PR	61.70	56.14	PR	80.36	74.81	PR	87.05	81.49	R	70.93	65.37	PR
50	BPT-5204		84.41	78.85	PR	40.38	34.82	PM	86.75	81.19	R	83.35	77.79	R	87.83	82.27	R
51	SYE-6		68.77	63.21	PR	88.81	83.26	R	9.88	8.43	PM	3.86	0.04	M	46.30	40.74	PM
52	SYE-5		92.25	86.69	R	89.86	84.31	R	0.00	0.00	M	0.00	0.00	M	85.99	80.44	R
53	GR-5		49.68	44.12	PM	23.93	18.37	PM	0.00	0.00	M	0.00	0.00	M	7.41	1.58	PM
54	GR-9		82.34	76.79	R	82.44	76.89	R	0.00	0.00	M	0.00	0.00	M	7.69	1.70	PM
55	Sathi-34-36		64.01	58.46	PR	63.29	57.73	PR	90.89	85.34	R	77.40	71.85	PR	63.98	58.42	PR

R- Restorer, PR- Partial restorer, PM- Partial maintainer and M- Maintainer; PF- pollen fertility (%), SF- spikelet fertility (%) and FR-fertility reaction

Table 2. Fertility restoration (%) of different CMS lines with different pollinator

CMS line	Source	Restorer	Partial restorer	Partial maintainer	Maintainer	Total
KJTCMS-6A	Wild rice with abortive pollen	31.00 (56.36)	13.00 (23.64)	10.00 (18.18)	1.00 (1.82)	55.00 (100.00)
RTN 2A	Assam Rice Collection	36.00 (65.45)	10.00 (18.18)	8.00 (14.55)	1.00 (1.82)	55.00 (100.00)
RTN 3A	Mutant of IR-62829B	30.00 (54.55)	11.00 (20.00)	9.00 (16.36)	5.00 (9.09)	55.00 (100.00)
RTN 13A	Gambiaca	28.00 (50.91)	14.00 (25.45)	5.00 (9.09)	8.00 (14.55)	55.00 (100.00)
RTN 17A	Dissi	33.00 (60.00)	10.00 (18.18)	7.00 (12.73)	5.00 (9.09)	55.00 (100.00)
Grand total		158.00 (57.45)	58.00 (21.09)	39.00 (14.18)	20.00 (7.27)	275.00 (100.00)
Common restorers for all CMS lines		14.00 (25.45 %)				

Figures in parentheses are percentages