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RESEARCH ARTICLE

Identification of restorers and maintainers for WA based Indica CMS lines of rice

■ PRGYA PARMITA AND ASHISH GOSWAMI

SUMMARY

Among the innovative genetic options available for enhancing the rice production hybrid rice is one of the technologies which is feasible and readily adoptable. In the early stage of the hybrid rice breeding programme, breeders identified restorer lines by testcross screening from rice germplasm pools. The availability of stable cytoplasmic male sterility and fertility restoring system is vital for commercial exploitation of heterosis in rice. In the present experiment, seven cms lines of divergent origin were studied for their pollen fertility and spikelet fertility in first year at DRR, Hyderabad. Out of them top five were reevaluated in next year at Varanasi location. These five stable cytoplasmic male sterile lines (CMS) of rice having wild abortive (WA) cytoplasmic male sterility source were crossed with 30 genotypes to identify their restorer/ maintainer nature. Most of the genotypes expressed differential fertility reactions when cross with cms lines. Among the genotypes tested, no one was found to be common as effective restorer for all the five cms lines. Genotypes found to be effective restorers for four CMS lines, Sarju 52, Malviya 36, HUR 105, Narendra 359, Pusa Sugandh 3, Pusa Sugandh 4 and NDR 118 were the promising effective restorers for most of the CMS lines.

Key Words : Rice, CMS lines, Maintainers, Restorers

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Rice (*Oryza sativa* L.) is the staple food crop of more than half of world's population and the world population particularly that of rice consuming countries increasing at a faster rate. By the year 2025, about 785 million tones of paddy which is 70 per cent more than the current production will be needed to meet the growing demand (Manomani and Fazlullah Khan, 2003). To meet the demand of increasing population there is need to increase production and productivity. Among the different improved technologies to increase the productivity of rice, the exploitation of hybrid technology appears to be promising.

The major mandate of the National Food Security Mission which was presented few days before in the Parliament was to enhance the annual rice production

MEMBERS OF THE RESEARCH FORUM

Author to be contacted :
ASHISH GOSWAMI, Department of Genetics and Plant Breeding, Udai Pratap Autonomous College, VARANASI (U.P.) INDIA
Email : ashishgoswamiupc2013@gmail.com

Address of the Co-authors:
PRGYA PARMITA, Department of Genetics and Plant Breeding, Udai Pratap Autonomous College, VARANASI (U.P.) INDIA

by an additional 10 million tons. Among the innovative genetic options available for enhancing the rice production hybrid rice technology has been widely accepted to be the most promising approach for breaking the yield plateaus (Siddiq, 1997).

This technology has been developed, extensively tested across the country and has been adopted in a limited area of about 1.1 million hectares at present. Of the targeted 10.9 million tons of additional annual rice production envisaged during next five years, hybrid rice may contribute easily upto 2 to 3 million tons of additional production, if adopted in 2-3 million hectares during the next five years. For this to happen and become a reality concerned efforts are needed by all the stakeholders, hybrid rice researchers, seed producers, both in public and private sectors and above all the technology transfer personnel. High yield of F_1 hybrids depends largely upon high pollen or spikelet fertility which is determined by the mode of genes prevalent in the restorer lines of the hybrids (Eckardt, 2006). The three line breeding method adopted in hybrid production technology involves the use of cytoplasmic male sterile line (A line), its maintainer (B line) and restorer (R line). Improvement of parental lines A, B and R are congenial for successful development of hybrid rice. Hence, evaluation of CMS lines for their adaptability and pollen and spikelet fertility was taken for into consideration while conducting the present study. Hundred of CMS lines have been developed, but only a few have been used in hybrid breeding programme due to the reason that many of these do not have effective restorers and only few CMS lines are stable for sterility over environments (Virmani, 1996). Most of the hybrids released throughout the world are based on a single sterile cytoplasmic source *i.e.* WA or wild abortive. Only limited efforts have been made to improve the grain quality of hybrid rice (Kush *et al.*, 1986).

In heterosis breeding programme using cytoplasmic male sterility (CMS) system, identification of maintainers and restorers is fundamental. Restorers for different cytosterile sources will increase the cytoplasmic diversification, which in turn can prevent genetic vulnerability due to the use of single CMS source (Pradhan *et al.*, 1992). A number of restorers also have been identified so far for different CMS lines in different regions (Virmani and Edwards, 1983; Govinda and Virmani, 1988; Prasad *et al.*, 1993; Venugopal, 1997; Kumeri *et al.*, 1998; Rosamma and Vijayakumar, 2005 and Krishnalatha and Sharma, 2012) but these are not sufficient and many restorers did not performed well in other regions due to environmental effect.

MATERIAL AND METHODS

Present study was conducted consecutively for two years at different locations in different seasons *viz.*, from July, 2012 to Oct., 2012 (On season trial) at Research Farm, Faculty of Agriculture, Udai Pratap Autonomous College, Varanasi and from Nov., 2012 to March, 2013 (Off season trial) at Research Farm, Directorate of Rice Research (DRR), Rajendra Nagar, Hyderabad. The experimental material for this study consisted of seven ‘WA’ (Wild Abortive) cyto-sterile lines of rice *viz.*, IR 58025A, PMS 8A, PMS 10A, DDR 9A, DRR 10A, IR 68897A and IR 79156A and a pool of varieties/cultures *viz.*, Sarju 52, Narendra 80, BPT 5204, MTU 7209, Malviya 36, HUBR 2-1, HUR 105, JR 32, Pusa Basmati 1, Kala Namak, Narendra 6093, Narendra 359, Pusa Sugandh 3, Pusa Sugandh 4, Badshah Bhog, Taraori Basmati, Type 3, Narendra 97, NPT 80-1, NPT 9, Jaya, NDR 118, Pant Dhan 6, Heera, Anjali, Rupali, Pusa 44, BVD 109, BAU 170-90 and Pant Dhan 10. The objective of this experiment was to isolate effective restorer and maintainers. Twenty one days old seedlings were transplanted with spacing of 20x15 cm and all recommended agronomical practices were followed. The stability for sterility was estimated on the basis of pollen and spikelet sterility. At flowering stage, spikelets were randomly collected and fixed in aceto-alcohol (1:3). Mature anthers from five randomly selected spikelets were removed and then smeared and stained in freshly prepared 1 per cent IKI solution and were examined under compound microscope. About 100-200 pollen grains were scored at three different spots. Unstained shrivelled and empty pollen grains were counted as sterile, while stained well filled and round pollen grains as fertile. Pollen sterility was counted in percentage. For estimation of spikelet sterility, four to five panicles were randomly bagged with butter paper bags before initiation of flower and were harvested at maturity. The empty spikelets were counted as sterile and spikelets filled with grains as fertile. Spikelet sterility was also computed in percentages. CMS lines with more than 95 per cent pollen and spikelet sterility were considered as stable. For test cross study, staggered sowing of the CMS lines was done to ensure synchronous flowering. 22 days old seedlings were transplanted on 28 July, 2012. Each CMS line was planted in twin rows, with 15 plants alternating pollen parents (in single rows at 20x15 cm spacing).

Recommended package of practices was followed during crop growth period. Panicles of CMS lines were bagged prior to anthesis. Pollen from male parents were collected at the time of anthesis and dusted separately on the bagged panicles. All possible cross combinations were attempted and mature seeds were collected from these. In second year, 150 test crosses attempted during on-season trial were evaluated for their fertility restoration or sterility maintaining ability on the basis of pollen sterility and spikelet fertility. Test crosses involving PMS 8A and PMS 10A CMS lines were discarded as instability of those lines. The F_1 seeds were grown in the field in single rows having 15 plants alternated by male parents with spacing of 20x15 cm. Recommended package of practices was followed during crop growth period. Before anthesis three panicles of each F_1 plant were bagged so as to avoid contamination. Pollen fertility and spikelet fertility were recorded from bagged panicles and the test entries were classified as below;

Criteria of classification of restorers and maintainers:

If > 80% pollen and spikelet fertility → Potential restorer

If 21-80% pollen and spikelet fertility → Partial restorer

If 1-20% pollen and spikelet fertility → Partial maintainer

If < 1% pollen and spikelet fertility → Effective maintainer

Pollen fertility of hybrids was assessed at flowering time. For pollen fertility assessment, about two or three spikelets were collected from freshly emerged panicles and examined under microscope with one per cent iodine-potassium iodide (IKI).

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

Spikelet fertility of hybrids were assessed at maturity time by taking the count of well filled and chaffy spikelet in each panicle.

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

Also, Pollen sterility (%) =100 - pollen fertility (%)
Spikelet sterility (%) = 100 - Spikelet fertility(%)

RESULTS AND DISCUSSION

Success of hybrid breeding programme in self pollinated crop like rice would primarily depend on stable and effective cytoplasmic-nuclear male sterility-fertility restoration system. Data collected for pollen and spikelet sterility in 1st season for different CMS lines are given in Table 1.

In general, pollen sterility percentage was higher than the spikelet sterility percentage in all the seven CMS lines. IR 58025A and IR 68897A highly stable were very important lines for Indian condition, since they have been especially bred at IRRI to develop commercial hybrids for Indian sub-continent. However, PMS 10A and PMS 9A showed low sterility percentage and considered as unstable so they were not taken in next year. In 2nd year only 5 CMS lines trial *viz*., IR 58025A, IR 68897A, IR 79156A, DRR 8A, and DRR 10A were re-evaluated for pollen and spikelet sterility at Hyderabad location. Data collected for pollen and spikelet sterility for five CMS lines are given in Table 2.

In off season trial, IR 58025 showed again highest pollen sterility (100%) while DRR 10A showed highest spikelet sterility (99.80%). Other CMS lines also showed considerable pollen and spikelet sterility. Data of both trials were somehow different may be due to climatic differences. Less spikelet sterility than pollen sterility was may be some seed set due to cross pollination. Singh and Singh (2000) and Sidharthan *et al*. (2007) also reported IR 58025 as stable CMS line.

Test cross data collected from F_1 for their pollen fertility (%) and spikelet fertility (%) and classification of pollen parents on the basis of pollen fertility (%) and spikelet fertility (%) are presented in Table 3.

Table 1 : Spikelet and pollen sterility of seven CMS lines having WA cytoplasm in on-season trial

Sr. No.	CMS lines	Sterility %		Remarks
		Spikelet sterility	Pollen sterility	
1.	IR 58025A	96.88	100	Stable
2.	IR 68897A	97.80	100	Stable
3.	IR 79156A	97.20	98.88	Stable
4.	DRR 10A	98.00	99.25	Stable
5.	DRR 9A	95.60	98.00	Stable
6.	PMS 10A	92.30	94.30	Unstable
7.	PMS 8A	90.45	92.78	Unstable

The present observations revealed that out of 30 genotypes no one was found to be common effective restorer for all the five CMS lines. However, Malviya 36, HUR 105, narendra 359, Pusa sugandh 3 and pusa sugandh 4 were found to be effective restorers for four CMS lines. Highest effective restorers (18) were observed for CMS lines IR 68897A, followed by IR58025A (16), IR 79156A (14) and DRR 10A (8) while only two genotypes BPT 5204 and Pusa sugandh 3 were found to be restorers for DRR 9A. Malviya 36, HUR 105, narendra 359, Pusa sugandh 3,Pusa sugandh 4, Sarju 52, Badshah Bhog, Type 3 and NDR 118 produced higher fertile hybrids and behaved as effective restorers for all the three IR series CMS lines for which more restorers

Table 2 : Spikelet and pollen sterility of five CMS lines having WA cytoplasm in 2nd season

Sr. No.	CMS lines	Sterility %		Remarks
		Spikelet sterility	Pollen sterility	
1.	IR 58025A	96.28	100	Stable
2.	IR 68897A	97.40	99.25	Stable
3.	IR 79156A	96.25	98.48	Stable
4.	DRR 10A	99.80	99.25	Stable
5.	DRR 9A	95.25	98.20	Stable

Table 3 : Classification of pollen parents into effective restorers/maintainers and partial restorers/maintainers on the basis of pollen sterility (%) and spikelet sterility (%)

Sr. No.	Genotypes	IR 79156A	IR 58025A	IR 68897A	DRR 9A	DRR 10A
1.	Sarju 52	R (88.42, 87.45)	R (97.30, 95.82)	R (97.50, 96.30)	PR (79.73, 78.90)	PR (74.12, 72.66)
2.	Narendra 80	PR (74.73, 71.90)	PR (74.73, 72.90)	R (97.50, 95.30)	PR (67.73, 65.90)	R (90.50, 88.30)
3.	BPT 5204	R (92.50, 90.30)	R (90.50, 88.10)	R (97.20, 96.30)	R (86.80, 84.30)	PM (18.55, 16.80)
4.	MTU 7209	PR (74.73, 71.90)	R (86.50, 82.30)	R (87.33, 86.80)	PR (74.73, 71.90)	R (96.50, 92.30)
5.	Malviya 36	R (89.90, 85.80)	R (86.10, 82.45)	R (87.55, 84.00)	PR (57.90, 60.90)	R (82.50, 80.20)
6.	HUBR 2-1	PR (56.45, 50.50)	R (86.20, 82.00)	R (84.80, 81.90)	PM (18.50, 16.20)	PR (56.60, 52.90)
7.	HUR 105	R (88.00, 85.00)	R (90.50, 94.00)	R (86.80, 85.00)	PR (45.45, 48.70)	R (82.00, 80.50)
8.	JR 32	PR (68.80, 72.00)	R (88.80, 90.00)	R (90.20, 86.70)	PM (16.50, 18.80)	PR (74.70, 71.00)
9.	Pusa Basmati 1	R (92.00, 87.80)	R (85.80, 84.00)	PR (58.90, 55.50)	PR (62.00, 60.00)	PR (45.80, 42.80)
10.	Kala Namak	PR (64.90, 61.35)	PR (78.80, 77.00)	R (85.55, 82.20)	PR (71.10, 67.00)	PR (75.00, 71.80)
11.	Narendra 6093	PM (10.60, 9.50)	PM (12.40, 10.00)	M (0.82, 0.36)	M (0.80, 0.30)	PM (18.55, 16.80)
12.	Narendra 359	R (95.20, 90,80)	R (94.80, 92.10)	R (89.60, 84.80)	PR (65.80, 64.00)	R (87.60, 84.80)
13.	Pusa Sugandh 3	R (88.00, 85.00)	R (84.70, 82.50)	R (88.80, 85.20)	R (82.75, 80.50)	PR (69.80, 65.80)
14.	Pusa Sugandh 4	R (88.00, 85.00)	R (90.60, 86.40)	R (85.40, 84.00)	PR (68.00, 65.50)	R (85.50, 82.60)
15.	Badshah Bhog	R (95.80, 93.00)	R (92.90, 88.60)	R (87.80, 85.20)	PR (75.80, 70.80)	PR (72.00, 68.80)
16.	Taraori Basmati	PR (55.60, 48.90)	R (83.00, 81.50)	R (85.00, 82.80)	PM (8.60, 7.68)	PR (48.00, 45.50)
17.	Type 3	R (92.00, 87.80)	R (88.00, 84.60)	R (85.00, 84.00)	PR (68.00, 65.00)	PR (62.00, 60.80)
18.	Narendra 97	PR (78.00, 74.80)	PR (75.80, 72.55)	R (85.60, 83.40)	PR (68.50, 61.00)	R (82.00, 80.50)
19.	NPT 80-1	R (87.00, 84.40)	PR (72.00, 65.00)	PR (72.00, 67.00)	PR (58.00, 53.20)	PR (55.00, 51.80)
20.	NPT 9	R (84.00, 82.20)	R (82.00, 80.80)	PR (72.00, 68.80)	PR (68.00, 62.45)	PR (56.90, 51.55)
21.	Jaya	PM (12.80, 9.80)	PR (35.78, 28.90)	PM (14.00, 10.50)	M (0.89, 0.55)	PR (48.00, 42.60)
22.	NDR 118	R (92.00, 86.90)	R (88.00, 84.60)	R (85.90, 84.00)	PR (70.00, 65.50)	PR (73.00, 68.20)
23.	Pant Dhan 6	PM (15.50, 12.80)	PR (37.90, 32.50)	PR (48.00, 45.50)	M (0.82, 0.36)	PM (18.80, 16.80)
24.	Heera	M (0.80, 0.56)	PM (18.80,16.80)	M (0.80, 0.33)	M (0.92, 0.66)	M (0.90, 0.50)
25.	Anjali	PM (18.55, 16.80)	PM (12.80,10.60)	M (0.85, 0.45)	PM (15.00, 12.80)	PM (13.50, 9.80)
26.	Rupali	PR (46.08, 42.00)	PR (56.00, 48.80)	R (82.00, 80.50)	PM (18.60, 15.25)	R (85.95, 80.60)
27.	Pusa 44	R (81.10, 80.60)	PR (54.20, 50.80)	PR (66.35, 61.99)	PR (65.53, 60.86)	PM (11.19, 9.25)
28.	BVD 109	PM (12.00, 10.00)	PM (15.50, 14.10)	PR (48.26, 46.51)	M (0.85, 0.45)	PM (14.80, 11.20)
29.	BAU 170-90	PM (18.80, 16.40)	PR (58.38, 53.50)	PR (46.30, 43.94)	PR (67.30, 64.57)	PM (18.80, 16.89)
30.	Pant Dhan 10	PR (72.65, 70.55)	PR (74.05, 73.05)	PR (62.00, 58.20)	PM (18.75, 12.80)	PM (16.35, 14.80)

were found than DRR series. It may be due to better adaptability of IR series CMS lines. CMS lines DRR 9A and DRR 10A showed more partial restorers/maintainer reaction with these genotypes. Among the maintainers, Heera was promising one which gave more sterile hybrids and behaved as maintainer for four CMS lines except IR 58025A for which no prospective maintainer was observed. For CMS lines DRR 9A, highest (5) maintainers, 17 partial restorers and 6 partial maintainers were found. As observed in the present study, the differential reaction to fertility restoration by different genotypes, earlier workers (Mohanty and Sharma, 1983; Singh and Sinha, 1988 and Verma, 1998) have also reported the similar results. Sharma *et al.* (2012) also reported similar restoration behaviour of Type 3, HUBR 2-1, Narendra 359,BPT 5204, Malviya 36, Pusa sugandh 3, Pasa sugandh 4, Badshah bhog, Sarju 52, Pusa Basmati 1, Narendra 80, Taraori Basmati and MTU 7209 with IR 58025 and IR 68897 and also maintainer reaction of Heera with IR 58025A. Present restoration behaviour of cultivars Sarju 52 and Narendra 118 is in agreement with the findings of Singh (1989) and Singh and Singh (2000). NPT 80-1 was also reported as potential restorers for IR 79156 by Ghosh *et al.* (2013).

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