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Identification of Restorers and Maintainers in Test Cross Hybrids in Rice (*Oryza sativa* L.)

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ABSTRACT

Two cytoplasmic male sterile (CMS) lines APMS 6A and APMS 8A of rice having wild abortive (WA) cytoplasmic male sterility source were crossed with fifty genotypes to identify their restorer/maintainer nature. A total of 100 test cross hybrids were subjected to pollen fertility and spikelet sterility analysis. Most of the genotypes expressed differential fertility restoration with 2 CMS lines. The genotype, AM 643 was identified as maintainer for the both CMS lines while eight genotypes *viz.,* AM 620, AM 638, BM 382, BM 392, HR 1-1, HR 1-6, HR 1-10 and HR 1-11 were identified as restorers for both the CMS lines. Ten genotypes were identified as a partial restorers for both CMS line and six genotypes were identified as partial maintainers for both the CMS line. The restorers identified in the present investigation could be used to develop good, high yielding and promising rice hybrids and maintainers identified can be repeatedly back crossed with the CMS lines to develop new stable CMS lines.

Key words: *Rice, CMS lines, Restorers, Maintainers, Pollen fertility and Spikelet sterility.*

Rice is the most important cereal crop of the world. Globally, it is cultivated in an area of about 154.31 Mha with an annual production of 472.9 Mt (United States Department of Agriculture, 2015-16). In India, rice is cultivated over an area of 43.39 Mha with a production of 104.32 Mt and productivity of 2404 kg ha^{-1}. In Andhra Pradesh the area, production and productivity of rice are 21.60 lakh ha, 74.9 L t and 3466 kg ha^{-1}, respectively (Agriculture Statistics at a Glance, 2016).

It is grown under diverse eco-geographical conditions in various tropical and subtropical countries including India. To meet the future food demand, anticipated from the projected world population increase, there is an urgent need to take necessary steps for enhancing the productivity of rice. Heterosis breeding is a tool for the exploitation of cross combinations and at commercial level under different environmental conditions. In rice, male sterility system has been exploited and further identification of restorers and maintainers is crucial to decide appropriate parents in producing superior hybrids.

Successful vigour in rice is largely depends on availability of locally adopted cytoplasmic male sterile (CMS) lines and restorer lines. CMS lines introduced from elsewhere may not be well adapted to a given target area. Maintainer lines are used for conversion into new CMS lines and restorer lines are used subsequently as a male parent in hybrid development programme. Test cross programme helps to identify maintainers as well as restorers. The establishment of test cross nursery for identification of restorers and maintainers is the first step in heterosis breeding (Akthar *et al* 2008 and Prasad *et al.* 2017). Keeping this in view the present investigation is carried out with two CMS lines and 50 testers to identify the best suitable contribution for exploitation.

MATERIAL AND METHODS

The experimental material comprised of two CMS lines from Wild Abortive source *viz.,* APMS 6A and APMS 8A and 50 genetically diverse testers - As shown in Table 1 as males. A set of 100 test cross hybrids were generated in Line × Tester fashion (Kempthrone, 1957) in *Rabi*, 2016-17 and evaluated along with their corresponding parents in Randomized Block Design with two replications during *kharif*, 2017. Each genotype was grown in two rows of 5.1 m long with 30 × 15 cm spacing at Regional Agriculture Research Station, Maruteru, West Godavari district, Andhra Pradesh. Single seedling per hill was transplanted and recommended package of practices were adopted for a good crop.

Estimation of pollen fertility

Pollen studies were carried out at flowering time to assess fertility / sterility status of F_1 plants. For this purpose, 15-20 spikelets from the just emerged panicles of five randomly selected plants were collected in a vial containing 70% ethanol. All the anthers from at least three to four spikelets were taken out with the help of forceps and placed on a glass slide with a drop of distill water. The anthers were gently crushed by using a needle to release the pollen grains. Then the pollen grains were stained with one per cent iodine potassium iodide (I-KI) solution. After removing the debris, a cover slip was placed and the slide was observed under the microscope to estimate the pollen fertility (%). The following formula was used to calculate pollen fertility (%).

Pollen fertility (%) =

$$\frac{\text{Number of stained pollen grains}}{\text{Total no.of pollen grains}} \text{ x } 100$$

Estimation of spikelet fertility

Estimation was done on five panicles per plant from randomly selected for each test cross hybrids at maturity. The well filled and chaffy spikelets of each panicle were counted and spikelet fertility (%) was estimated. The following formula was used to calculate spikelet fertility (%).

Spikelet fertility (%) =

$$\frac{\text{Total no.of filled grains per panicle}}{\text{Total no.of grains per panicle}} \text{ x } 100$$

The pollen parents were classified into four categories- Maintainers (M), Partial Maintainers (PM), Partial Restorers (PR) and Restorers (R) based on the pollen and spikelet fertility (Table 1). The criteria proposed by Virmani *et al.* (1997) was used for classifying the pollen parents.

Table 1. Classification of Pollen Parents

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

RESULTS AND DISCUSSION

The parent study revealed that among the 100 test crosses evaluated, 31 test crosses showed restorer reaction, 42 test crosses gave partial restorer reaction, 25 test crosses gave partial maintainer reaction and remaining two test crosses gave maintainer reaction and the result was presented in Table 1. Similar results were also reported by Jayashudha and Sharma (2010), Veeresha *et al.* (2013), Singh *et al.* (2014) and Hasan *et al.* (2015).

Among the test crosses with the CMS line APMS 6A, pollen fertility ranged from 0.12 % (APMS 6A × AM 643) to 90.11 % (APMS 6A × AM 622) and spikelet fertility ranged from 0.0 % (APMS 6A × AM 643) to 92.40 % (APMS 6A × AM 622) and in test crosses with APMS 8A the pollen fertility ranged from 0.6 % (APMS 8A × AM 643) to 90.15 % (APMS 8A × SM 13) and spikelet fertility ranged from 0.0 % (APMS 8A × AM 643) to 92.90 % (APMS 8A × SM 13). Among the 100 crosses highest pollen fertility (90.15 %) and spikelet fertility (92.90 %) was observed in test cross, APMS 8A × SM 13. The lowest pollen fertility (0.12 %) and spikelet fertility (0 %) was observed in APMS 6A × AM 643. Among one hundred test crosses thirty one crosses exhibited more than 80 per cent pollen fertility and more than 75 per cent spikelet fertility indicating that male parent of these test crosses can be used as restorers. Both the CMS lines exhibited 100 % pollen sterility and 0 % spikelet fertility when crossed with the parent AM 643 indicating this test cross parent can be used as maintainer.

Fertility restoration of different CMS lines with different testers as presented in Table 2 and number of testers identified as restorers, partial restorers, partial maintainers and maintainers are presented in the Table 3. Among the 50 genotypes more than 80 % pollen fertility and 75 % spikelet fertility restoration was observed in eight genotypes with both the CMS lines, 9 genotypes with CMS line APMS 8A and 6 genotypes with CMS line APMS 6A. The occurrence of restorers was high with CMS line APMS 8A (34 %) followed by APMS 6A (28 %). Eight genotypes viz., AM 620, AM 638, BM 382, BM 392, HR 1-1, HR 1-6, HR 1-10 and HR 1-11 are identified as common restorers for both the CMS lines. 50 % to 80 % pollen fertility and 50% to 75 % spikelet fertility was observed in ten genotypes viz., AM 651, CM 300, CM 306, CM 308, CM 313, CM 314, HR 1-7, SM 1, SM 8 and SM 10 and were identified as a partial restorers for both CMS lines, ten genotypes with APMS 6A and eleven genotypes with APMS 8A gave partial restorer reaction. The occurrence of partial restorer was higher in CMS line APMS 8A (44 %) followed by APMS 6A (40 %). The pollen fertility per cent ranged from 1.1 % to 50 % and spikelet fertility per cent ranged 0.1 % to 50 % were observed in six genotypes *viz.,* BM 386, BM 394, CM 302, CM 317, SM 4 and SM 9 and these can be exploited as partial maintainers for both CMS lines. Further, nine genotypes for the CMS line, APMS 6A and four genotypes with APMS 8A are also identified as partial maintainer. The occurrence of partial maintainers was higher in CMS line APMS 6A (30 %) compared to APMS 8A (20 %). The study on pollen fertility and spikelet sterility percentages identified maintainer line, AM 643 for both CMS lines as it showed complete pollen and spikelet sterility.

In some cases, the same testers behaved as a restorer for one CMS line and as partial maintainer or partial restorer for the other CMS line. Similar results were also reported by Bisne and Motiramani (2005) and Upendi *et al.* (2017).

Thus the above results it can be concluded that the variations in behavior of fertility restoration

Table 2. Fertility restoration of different CMS lines with different genotypes.

Sl. No	Test crosses	Wild abortive			Sl. No	Wild abortive		
	Females	APMS 6A				APMS 8A		
	Male	SF (%)	PF (%)	Based on		SF (%)	PF (%)	Based on
1	AM 619	79.21	81.23	R	51	61.44	67.27	PR
2	AM 620	77.46	80.85	R	52	75.07	80.30	R
3	AM 621	74.41	78.26	PR	53	81.12	85.34	R
4	AM 622	92.40	90.11	R	54	68.46	73.61	PR
5	AM 623	76.08	80.97	R	55	74.66	78.23	PR
6	AM 638	82.74	83.03	R	56	79.25	81.72	R
7	AM 641	71.20	77.96	PR	57	92.82	88.27	R
8	AM 643	0.00	0.12	M	58	0.00	0.60	M
9	AM 644	65.09	69.28	PR	59	75.29	82.23	R
10	AM 645	78.86	81.31	R	60	72.36	76.39	PR
11	AM 651	53.96	60.75	PR	61	59.98	62.74	PR
12	BM 377	59.80	52.26	PR	62	38.35	30.66	PM
13	BM 378	52.19	55.73	PR	63	36.52	39.32	PM
14	BM 381	39.31	42.94	PM	64	54.85	59.98	PR
15	BM 382	87.41	89.17	R	65	78.15	81.13	R
16	BM 384	66.87	72.33	PR	66	87.31	86.17	R
17	BM 386	16.65	18.66	PM	67	18.62	21.75	PM
18	BM 387	28.13	21.78	PM	68	57.51	61.69	PR
19	BM 388	43.38	49.05	PM	69	80.74	82.42	R
20	BM 389	46.46	41.32	PM	70	83.57	85.04	R
21	BM 390	41.81	44.78	PM	71	59.67	64.13	PR
22	BM 392	82.45	84.13	R	72	83.26	85.29	R
23	BM 394	42.20	45.66	PM	73	47.71	49.23	PM
24	BM 397	37.11	40.67	PM	74	53.63	57.26	PR
25	CM 300	62.63	57.08	PR	75	68.86	74.88	PR
26	CM 302	12.08	16.62	PM	76	22.93	29.52	PM
27	CM 306	55.75	60.45	PR	77	71.81	75.27	PR
28	CM 307	53.87	57.43	PR	78	81.87	83.19	R
29	CM 308	57.42	62.89	PR	79	66.89	71.95	PR
30	CM 313	57.26	59.61	PR	80	64.80	67.57	PR
31	CM 314	60.51	65.53	PR	81	65.26	70.49	PR
32	CM 315	21.84	26.38	PM	82	72.11	75.16	PR
33	CM 317	15.93	16.73	PM	83	30.63	35.63	PM
34	L 550	24.59	31.22	PM	84	87.22	81.47	R
35	L 564	49.52	47.63	PM	85	50.99	58.98	PR
36	L 565	75.85	80.77	R	86	73.75	77.83	PR
37	2615-28-2-2	83.83	82.23	R	87	72.63	77.29	PR
38	HR 1-1	84.03	85.53	R	88	83.33	83.24	R
39	HR 1-6	83.97	87.26	R	89	88.64	89.18	R
40	HR 1-7	74.78	78.97	PR	90	74.13	78.90	PR
41	HR 1-10	81.69	82.01	R	91	80.23	82.16	R
42	HR 1-11	83.75	85.10	R	92	89.37	89.88	R
43	SM 1	73.73	78.23	PR	93	72.93	78.55	PR
44	SM 3-1	59.35	64.02	PR	94	30.47	23.29	PM
45	SM 4	22.89	28.37	PM	95	16.16	17.93	PM
46	SM 8	54.40	63.35	PR	96	74.26	79.58	PR
47	SM 9	12.65	15.78	PM	97	15.28	16.09	PM
48	SM 10	66.41	71.93	PR	98	70.79	75.45	PR
49	SM 12	58.14	62.37	PR	99	40.87	42.98	PM
50	SM 13	54.98	58.29	PR	100	92.90	90.15	R
	Maximum	92.40	90.11			92.90	90.15	
	Minimum	0.00	0.12			0.00	0.60	

SF - Spikelet fertility, PF - Pollen fertility, R - Restorer, PR - Partial restorer,
PM -Partial maintainer and M- Maintainer

NOTE: Bold figures indicate Maximum and Minimum value of respective CMS lines.

Table 3. Genotypes identified as restorers, partial restorers, partial maintainers and maintainers.

Sl. No	Category	Spikelet fertility (%)	Pollen fertility (%)	Lines	Number of genotypes	Testers
1	Restorer	>75	>80	Both CMS lines	8	AM 620, AM 638, BM 382, BM 392, HR 1-1, HR 1-6, HR 1-10 and HR 1-11
				APMS 6A	6	AM 619, AM 622, AM 623, AM 645, L 565 and 2615-28-2-2
				APMS 8A	9	AM 621, AM 641, AM 644, BM 384, BM 388, BM 389, CM 307, L 550 and SM 13
2	Partial restorer	50.1-75	50.1-80	Both CMS Lines	10	AM 651, CM 300, CM 306, CM 308, CM 313, CM 314, HR 1-7, SM 1, SM 8 and SM 10
				APMS 6A	10	AM 621, AM 641, AM 644, BM 377, BM 378, BM 384, CM 307, SM 3-1, SM 12 and SM 13
				APMS 8A	12	AM 619, AM 622, AM 623, AM 645, BM 381, BM 387, BM 390, BM 397, CM 315, L 564, L 565 and 2615-28-2-2
3	Partial maintainer	0.1-50	1.1-50	Both CMS Lines	6	BM 386, BM 394, CM 302, CM 317, SM 4 and SM 9
				APMS 6A	9	BM 381, BM 387, BM 388, BM 389, BM 390, BM 397, CM 315, L 550 and L 564
				APMS 8A	4	BM 377, BM 378, SM 3-1 and SM 12
4	Maintainer lines	0	0-1	Both CMS lines	1	AM 643

Table 4. Fertility restoration of genotypes with CMS lines (%)

CMS and source	RESTORER		PARTIAL RESTORERS		PARTIAL MAINTAINERS		MAINTAINERS		TOTAL
	No.	%	No.	%	No.	%	No.	%	
APMS 6A (WA)	14	28	20	40	15	30	1	2	50
APMS 8A (WA)	17	34	22	44	10	20	1	2	50
GRAND TOTAL	31	31	42	42	25	25	2	2	100

may be due to different fertility-restoring genes or their penetrance and expressivity varied with the genotypes of the parents or the modifiers present in female background. This could also be due to differential nuclear-cytoplasmic interactions between the genotype and CMS line. This kind of the differential reaction of the same genotype in restoring the fertility of different CMS lines of same cytoplasmic source was also reported by Jayasudha and Sharma (2010) and Upendi *et al*. (2017).

CONCLUSION

From the present study eight genotypes AM 620, AM 638, BM 382, BM 392, HR 1-1, HR 1-6, HR 1-10 and HR 1-11 were identified as restorers for both CMS lines (APMS 6A and APMS 8A). The restorers identified in the present study can be used for developing good, high yielding rice hybrids. The maintainer line identified, AM 643, can be used for developing stable CMS line by repeated back crossings.

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