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Research Note

Identification of restorers and maintainers among the locally adapted genotypes for hybrid development in rice (*Oryza sativa* L.)

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Abstract
Two cytoplasmic genic male sterile lines of rice *viz.,* CO MS 24A and TNAU CMS 2A having wild abortive source were crossed with 20 testers to identify their restorer/maintainer nature. Total of 40 hybrids were subjected to pollen and spikelet fertility analysis along with their parents. Six effective restorers and eight maintainers were identified among the 20 genotypes. TKM 9 was found to be a common maintainer for CGMS lines TNAU CMS 2A and COMS 24A and the genotype ADT 37 was identified as restorer for both the CGMS lines COMS 24A and TNAU CMS 2A. The identified restorers and maintainers could be utilized for development of new rice hybrids and CMS lines in future.

Keywords
Rice, Restorers, Maintainers

Rice is the staple food for more than half of the world's population. About 3.5 billion people depend on rice for more than 20% of their daily calories. In India, rice is grown in an area of 44 million hectares with a production of 100 million tonnes (Sree Rangasamy *et al.,* 2012). In order to keep pace with the growing population, the estimated rice requirement by 2025 is about 130 million tonnes. Among the several genetic options available to increase the yield, hybrid rice technology is the strongest tool. Most of the rice hybrids in the country and elsewhere in the world are developed by using the CGMS or the three line system. The CGMS system involves three lines, namely a cytoplasmic male sterile line (A line), a maintainer line (B line) and a restorer line, where restorer line (R line) possesses dominant fertility restoring genes. The basic step in hybrid development programme is identification of restorers and maintainers through test cross evaluation. The method for identification of restorers and maintainers is by observation of pollen fertility and spikelet fertility in test crosses involving CGMS lines. Potential restorers will be utilized to develop hybrids, whereas, potential maintainers will be converted into new CGMS lines through back cross breeding.

The experimental material consists of two CGMS lines *viz.,* COMS 24A and TNAU CMS 2A and 20 testers. A set of 40 hybrids were generated in line x tester mating design for the purpose and evaluated along with their corresponding parents in randomized block design with two replications at Vanavarayar Institute of Agriculture, Pollachi during 2014 – 2015. Twenty days old seedlings were transplanted in the main field. A spacing of 20 x 20 cm was maintained in a single row of 1.8m length consisting of 10 plants. Single seedling per hill was transplanted. Recommended package of practices were adopted.

Pollen studies were carried out to assess fertility/ sterility status of F_1 hybrids. For this purpose, 15-20 spikelets from the just emerged panicles of three randomly selected plants were collected in a vial containing 70% ethanol. All the anthers from atleast six spikelets were taken out with forceps and placed on a glass slide with a drop of 1 per cent iodine potassium iodide (I-KI) stain.

The anthers were gently crushed by using a needle to release the pollen grains. After removing the debris, a cover slip was placed and the slide was observed under the microscope. Round well filled and deeply stained pollen grains were counted as fertile and shrivelled and lightly stained were counted as sterile.

$$\text{Pollen fertility} = \frac{\text{Number of pollen grains}}{\text{Total number of pollen grains}} \times 100$$

With regard to pollen fertility of population, plants were classified into the following classes: Fertile (F): Plants showing more than 60% pollen fertility; partially fertile (PF): plants showing 30 to 60% pollen fertility, partially sterile (PS): plants showing 1 to 30% pollen fertility and completely sterile (CS): plants showing no pollen fertility (Gyan *et al.,* 2003).

Spikelet fertility (%) was recorded on five randomly selected and bagged panicles in both replications. Total number of filled grains and ill filled grains on main panicle of each plant was counted separately. The proportion of number of fully developed grains

to the total number of spikelets was calculated as spikelet fertility.

$$\text{Spikelet fertility} = \frac{\text{Number of filled grains}}{\text{Total number of spikelets}} \times 100$$

Based on the spikelet fertility percentage, plants were classified into the following classes according to Virmani *et al.* (1997) as maintainer (0%), partial maintainer (0.1-50%), partial restorer (50.1 – 75%) and restorer (>75%).

Out of 40 F_1s, eight were designated as maintainers, 19 as partial maintainers, seven as partial restores and six as restorers (Table 1.). The restorers and maintainers for WA cytoplasm were reported earlier by Ali *et al.* (2014) and Pankaj Kumar *et al.* (2015). The average proportion of maintainers, partial maintainers/ partial restores and restorers were 20.00%, 40.00 %, 20.00% and 20.0% for COMS 24A and 20.0%, 55.0%, 15.0% and 10.0% for TNAU CMS 2A, respectively (Fig. 1 and 2).

Anna (R) 4, ADT 37, ADT 43 and AS 10070 were identified as restorers for COMS 24A. Similarly, the pollen parents ASD 16 and ADT 37 classified as restorers for the CGMS line TNAU CMS 2A (Table 2.). Restorers for WA-CMS line in rice were reported by many earlier workers namely, Umadevi *et al.* (2010) and Satyapal Singh *et al.* (2014). The identified restorers, ADT 43, ADT 37, ASD 16 and Anna(R) 4 were released by Tamil Nadu Agricultural University. ADT 43 is a popular early duration variety with good grain quality and AS 10070 is an elite breeding line with short duration and good cooking quality. Among the restorers identified, the restorers ADT 43 and AS 10070 could be utilized to develop good grain quality hybrids with medium slender grains.

The pollen parents PMK 3, TKM 9, CO 51 and AS 10036 were identified as maintainers for COMS 24A. Likewise, TKM 9, ADT 47, IET 1308 and AS 10062 were classified as maintainers for TNAU CMS 2A (Table 3.). Many earlier investigators identified maintainers for WA-CMS lines in rice (Sharma *et al.*, 2011 and Banumathy *et al.*, 2013).

The identified maintainers, CO 51, TKM 9, ADT 47 and PMK 3 were released by Tamil Nadu Agricultural University. CO 51 is a short duration variety with good grain quality; TKM 9 is a short duration, drought tolerant variety; ADT 47 is another early duration variety with good grain quality; PMK 3 is a drought tolerant, short duration variety suitable for semi dry conditions.

Higher frequency of maintainers than restorers was observed in this study which was reported by many earlier workers, namely, Sabar *et al.* (2007) and Akhter *et al.* (2008).

The fertility restoration performance of the genotypes varies with genetic background of CGMS lines. More emphasis should be given to utilize proper rice genotypes, as parental lines to achieve the goal of developing superior hybrids with better grain quality. The identified restorers and maintainers from this study are locally adapted. The restorer lines *viz.*, ADT 43 and AS 10070 could be utilized to develop good grain quality hybrids with medium slender grains and ADT 37 can be used to develop coarse grain hybrids. New restorer can be developed through recombinant breeding which can expand the genetic base of restorer by pyramiding complementary traits from diverse sources according to breeding objectives. The maintainer lines identified could be utilized in back cross breeding programme, for development of their male sterile versions. Being the popular and well adapted varieties, conversion of CO 51 and ADT 47 into CGMS lines could be useful to develop rice hybrids with marketable grain quality and consumer preference. Conversion of the popular and well adapted varieties like TKM 9 and PMK 3 could be of great use in the development of drought tolerant rice hybrids. Many CGMS lines were developed in rice through back cross breeding by earlier workers namely, Shen Xian-hua *et al.* (2013) and Thiyagarajan *et al.* (2013).

On the basis of spikelet fertility six heterotic hybrid combinations, namely COMS 24A x ADT 43, COMS 24A x Anna(R) 4, TNAU CMS 2A x ADT 37, COMS 24A x AS 10070, TNAU CMS 2A x ASD 16 and COMS 24A x ADT 37 were identified. Among them, COMS 24A x ADT 43 and COMS 24A x AS 10070 were the best hybrids based on grain quality traits. The hybrid COMS 24A x ADT 43 exhibited better performance for quantitative traits like, days to 50 % flowering, total number of tillers per plant, panicle length, number of spikelets per panicle, spikelet fertility, 100 grain weight and grain yield per plant over the popular check variety ADT 43 (Table 4.). The performance of this hybrid for grain quality traits like, hulling per cent, milling per cent, kernel length, kernel length/ breadth ratio, kernel length after cooking, linear elongation ratio and volume expansion ratio were also better than ADT 43 (Table 5.). The results indicate that, only parents with acceptable grain quality need to be chosen to produce hybrids with good grain quality.

The investigation resulted in the identification of potential restorers, *viz*., Anna(R) 4, ADT 37, ADT 43, ASD 16 and AS 10070 which can be used for developing high yielding rice hybrids. The identified maintainers like, PMK 3, TKM 9, CO 51, ADT 47, IET 1308, AS 10036 and AS 10062 which could be utilized in back cross breeding programme, for development of their male sterile versions.

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Table 1. Fertility classification of hybrids involving two Cytoplasmic Genic Male Sterile lines and 20 pollen parents in rice

S.No.	Genotypes	COMS 24A	TNAU CMS 2A
1.	ADT 36	PM	PM
2.	ADT 37	R	R
3.	ADT 43	R	PM
4.	ADT 45	PR	PM
5.	ADT 47	PM	M
6.	ADT 48	PM	PM
7.	ASD 16	PM	R
8.	ASD 18	PR	PR
9.	AS 10036	M	PM
10.	AS 10062	PM	M
11.	AS 10070	R	PM
12.	CB 09538	PR	PM
13.	IET 1308	PM	M
14.	CO 47	PM	PM
15.	CO 51	M	PM
16.	Anna(R) 4	R	PM
17.	MDU 5	PR	PM
18.	PMK 3	M	PR
19.	TKM 9	M	M
20.	TPS 5	PR	PR

M - Maintainer, PM - Partial maintainer, PR - Partial restorer, R – Restorer

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

CGMS used	Effective restores	Pollen fertility (%)	Spikelet fertility (%)
COMS 24A	ADT 43	69.83	85.59
	ADT 37	62.08	79.11
	AS 10070	69.54	75.13
	Anna(R) 4	63.92	75.11
TNAU CMS 2A	ADT 37	61.35	75.77
	ASD 16	60.57	77.15

Table 3. Genotypes identified as maintainers and their pollen and spikelet fertility

CGMS used	Effective maintainers	Pollen fertility (%)	Spikelet fertility (%)
COMS 24A	AS 10036	0.00	0.00
	CO 51	0.00	0.00
	PMK 3	0.00	0.00
	TKM 9	0.00	0.00
TNAU CMS 2A	ADT47	0.00	0.00
	AS 10062	0.00	0.00
	IET 1308	0.00	0.00
	TKM 9	0.00	0.00

Table 4. Morphological attributes of the heterotic rice hybrids identified from test crosses

Hybrids	DFF	PH (cm)	NTP	PL (cm)	NSP	SF (%)	100 GW (g)	GY/P (g)
COMS 24A x ADT 43	87.00	115.98	20.0	27.90	241.00	85.89	1.63	57.35
COMS 24A x Anna(R)4	99.50	127.00	17.5	29.25	209.00	75.11	2.49	56.68
TNAU CMS 2A x ADT 37	85.50	109.99	18.00	24.70	222.00	77.15	2.02	55.30
COMS 24A x AS 10070	90.00	112.09	19.66	26.75	239.00	84.93	1.61	52.29
TNAU CMS 2A x ASD 16	92.50	112.25	16.66	27.67	226.00	75.77	2.25	51.33
COMS 24A x ADT 37	89.00	114.94	17.33	26.19	158.00	79.11	2.05	39.97
ADT 43 (Check)	88.50	113.85	19.33	27.44	205.16	88.31	1.64	29.01

DFF - Days to 50% flowering, PH - Plant height, NTP - Number of tillers per plant, PL - Panicle length, NSP - Number of spikelets per panicle, SF - Spikelet fertility, 100 GW - Hundred grain weight, GY/P - Grain yield per plant

Table 5. Grain quality traits of the heterotic rice hybrids identified from test crosses

Hybrids	H%	M%	KL (mm)	KL/B	KLAC (mm)	VER	LER
COMS 24A x ADT 43	82.62	73.10	6.20	3.17	11.05	3.50	1.78
COMS 24A x Anna(R)4	78.0	67.25	6.50	3.33	10.35	3.40	1.59
TNAU CMS 2A x ADT 37	81.73	67.25	5.15	2.34	8.30	3.44	1.61
COMS 24A x AS 10070	77.25	72.95	6.05	3.07	9.65	3.95	1.59
TNAU CMS 2A x ASD 16	79.75	66.97	5.35	2.14	8.05	3.37	1.50
COMS 24A x ADT 37	82.03	67.69	5.25	2.05	8.45	3.11	1.60
ADT 43 (Check)	80.23	72.75	5.80	3.05	9.35	3.45	1.61

H% - Hulling per cent, M% - Milling per cent, KL - Kernel length, KL/B - Kernel length/ breadth ratio,
KLAC - Kernel length after cooking, LER - Linear elongation ratio , VER - Volume expansion ratio

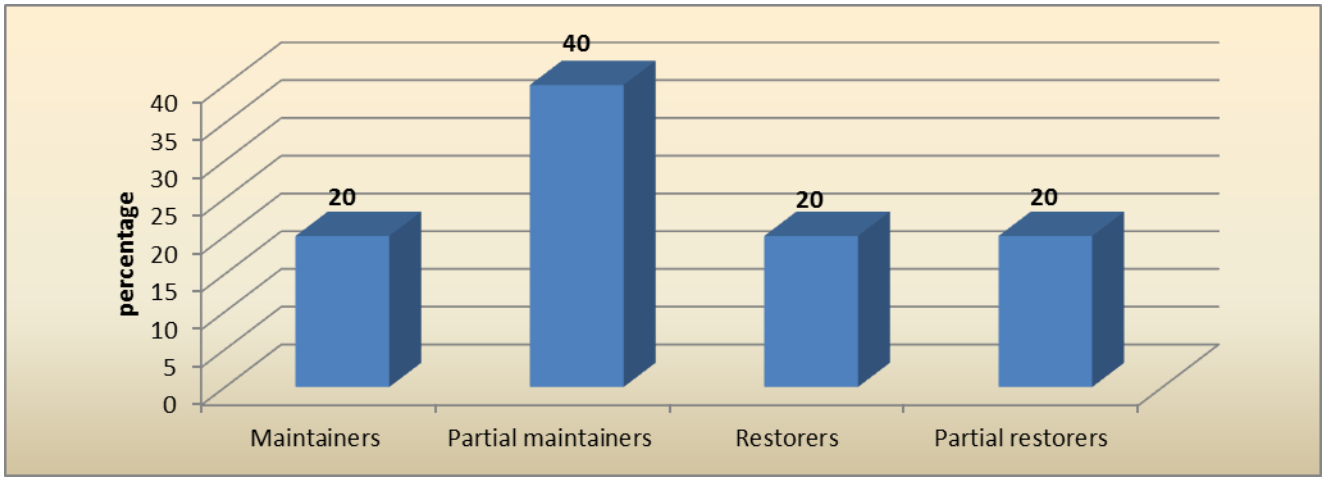

Fig 1. Maintainers, partial maintainers, partial restorers and restorers for COMS 24A based on pollen and spikelet fertility

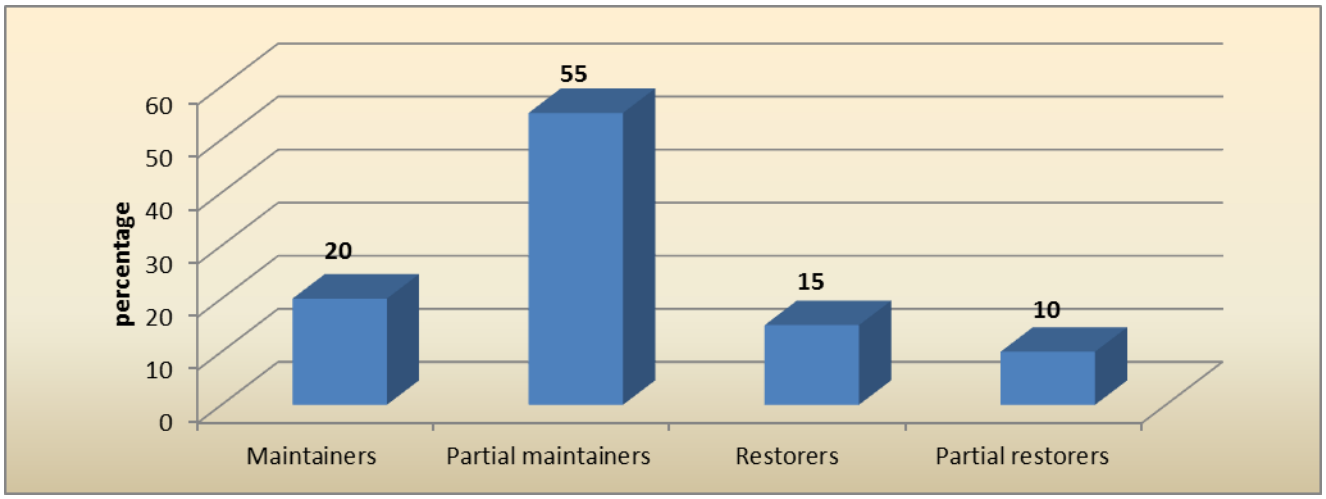

Fig 2. Maintainers, partial maintainers, partial restorers and restorers for TNAU CMS 2A based on pollen and spikelet fertility