

**Research Article****Identification of usable maintainers and restorers for new CMS lines of rice (*O. sativa* L.)**

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**Abstract**

The success of hybrid rice technology depends on the extent of heterosis realized and the grain quality of rice. Seven new CMS lines developed at Zonal Agricultural Research Station, Mandya through backcross breeding were evaluated along with two checks CRMS31A and CRMS 32A for their morphological and floral traits besides aroma during *kharif* 2008. All the CMS lines evaluated exhibited complete pollen sterility and spikelet sterility. KCMS 29A recorded the higher stigma exertion (30.31%) and natural outcrossing (19.76%). KCMS 31A had the longest style (1.26 mm) and stigma (1.20 mm) while the angle between stigma lobes was wider (65.48 (φ)) in KCMS 37A which also had the best panicle exertion (79.28%). All the nine CMS lines had no aroma. Based on morphological and floral traits CMS lines KCMS 29A, KCMS 31A and KCMS 37A were better compared to the checks CRMS 31A and CRMS 32A. To identify usable maintainers and restorers for these CMS lines eleven testers were crossed with all the nine CMS lines in a line x tester fashion. Based on the percent spikelet fertility in hybrids, 60 showed restorer reaction and eight had maintainer reaction while the rest 31 showed partial maintainer / restorer reaction. Among the testers MSN-20-13, MSN-62, MSN-63, MSN-64 and MSN 68 were identified as good maintainers while KMR-3, KMR-4, MSN-67 and MSN-69 as good restorers.

**Key words:** CMS lines, Back cross breeding, Pollen sterility, Spikelet fertility, Stigma exertion, maintainer and restorer lines

**Introduction**

The challenge of increasing the rice production up to 120 million tons by the year 2020 calls for both short and long term planning encompassing genetic as well as crop management options. Among the innovative genetic options available, hybrid rice technology is practically feasible and readily adoptable. However, commercial success of hybrid rice technology mainly depends on the extent of heterosis realized and the quality of rice. So far, 43 rice hybrids have been released for commercial cultivation in the country by both public and private sectors. These hybrids have yield advantage of 1.0-1.5 tons/ha over the highest yielding inbred varieties. But this moderate yield advantage is not economically attractive to the farmers. Currently the yield levels in seed production plots is also low (1.0-1.2 tons/ha) leading to high costs of hybrid seeds.

Development of new cytoplasmic male sterile (CMS) lines with better floral traits like stigma exertion and natural out crossing percentage would therefore be of paramount importance to ensure better seed set and seed yield in large scale seed production and commercial cultivation of the hybrids. Similarly, identification of a better restorer for obtaining a good hybrid combination with higher seed yield and grain quality will help in popularizing the hybrids. Hence, a study was undertaken to evaluate the new CMS lines developed and to identify usable restorers and maintainers for them.

**Material and methods**

Seven cytoplasmic male sterile (CMS) lines developed at Zonal Agricultural Research Station (University of Agricultural Sciences, Bangalore), V.C. Farm, Mandya and two male sterile lines from Central Rice Research Institute, Cuttack were evaluated for their morphological and floral traits during *kharif* 2008. The 25 days old seedlings of CMS lines were planted at a spacing of 20 × 15 cm

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and crop was raised following all the package of practices. Observations on days to 50 per cent flowering, pollen fertility (%), style length (mm), stigma length (mm), angle between stigma lobes ( $\theta$ ), stigma exertion (%), seed set on bagging (%), natural out crossing (%), plant height (cm), number of panicles per plant, panicle length (cm), panicle exertion (%) and aroma were recorded on five randomly selected plants.

For pollen fertility assessment, about two to three spikelets from the top, middle and bottom of freshly emerged panicles were collected and examined under microscope with one per cent Iodine-Potassium Iodide (IKI) solution. Style length and stigma length were measured by using ocular micro meter and expressed in mm. The angle between stigma lobes was measured by using formula

$$\text{Angle between stigma lobes } \theta = 2 \tan^{-1} [\frac{1}{2}h/v]$$

Stigma exertion was assessed by number of spikelets with exerted stigma (one or both lobes extended) to the total number of spikelets in a panicle at full blooming and expressed in per cent. Panicles emerging from the sheath were bagged with butter paper bags prior to anthesis to prevent cross pollination. The bagged panicles were harvested to assess per cent seed setting. Similarly, seed set through natural out crossing was also recorded on five uncovered panicles. Panicle exertion, the ratio of number of spikelets out side the flag leaf to the total number of spikelets in the panicle was expressed in per cent. To identify the presence or absence of aroma, leaf bits cut into one cm size were taken in a petri dish and added with 1.76 per cent potassium hydroxide solution. After ten minutes the content was smelt and classified.

All the nine CMS lines were crossed with eleven testers viz., KMR-3, KMR-4, KMR-12, Thanu, MSN-62, MSN-63, MSN-64, MSN-67, MSN-68, MSN-20-13 and MSN-69 during *Kharif* 2008 following line  $\times$  tester crossing design to obtain 99 hybrid combinations. These hybrids were raised during summer 2009 in Randomized Block Design with two replications following 20  $\times$  15 cm spacing under normal agronomic practices.

Fertility restoration ability of the testers and the spikelet fertility of the hybrids were assessed at maturity by taking count of well filled and chaffy spikelets in each panicle. The proportion of well filled spikelets was calculated as percentage to the total number of spikelets per panicle. Based on the

spikelet fertility in the hybrids, the testers that could produce more than 70 per cent seed set were classified as restorers, 20 to 70 per cent as partial restorers, 5 to 20 per cent as partial maintainers and 0 to 5 per cent as maintainers.

### Results and discussion

The results showed that (table 1) all the nine CMS lines evaluated exhibited complete pollen sterility and complete spikelet sterility as indicated by no seed set on bagging confirmed this. Maximum style length was recorded in KCMS 31A (1.26 mm) followed by KCMS 33A (1.16 mm) and KCMS 37A (1.07 mm). Maximum stigma length was in KCMS 34A (1.28 mm) followed by KCMS 31A (1.20 mm) and KCMS 29A (1.15 mm). Angle between stigma lobes was wider in KCMS 35A (74.90), KCMS 37A (65.48) and KCMS 33A (64.52). Stigma exertion of about 30.31 per cent was recorded by KCMS 29A followed by KCMS 37A (20.44%) and KCMS 35A (20.24%). All the above floral traits offer better scope for effective pollination as they expose more stigmatic surface area for pollen to drop on stigma which in turn helps in better seed set. In a similar study, Ashish *et al.*, (2006) felt that higher stigma exertion per cent had direct relationship with high natural out crossing rate and hence it is highly desirable feature in hybrid rice breeding programmes. All the nine CMS lines studied were dwarf (< 90 cm) in stature and possessed no aroma. As non-aromatic hybrids with medium slender grains are preferred, these CMS lines will be of use in future breeding programmes.

Further, the CMS lines, KCMS 29A, KCMS 31A, KCMS 33A, KCMS 35A and KCMS 37A were found to be more promising with favorable morpho-floral characters viz., plant height(cm), pollen fertility (%), style length (mm), stigma length (mm), angle between stigma lobes( $\theta$ ), stigma exertion (%), panicles per plant, panicle length(cm), seed set on bagging (%), natural out crossing(%) and panicle exertion(%). Hence, they can be effectively utilized for developing three line rice hybrids.

Among the 99 hybrids evaluated, the spikelet fertility of hybrids ranged between 2.42 (CRMS 32A/ MSN-68) and 97.05 (KCMS 38A/ MSN-63) as shown in table 2. Out of the 99 hybrids, 60 had restorer reaction having spikelet fertility more than 70 per cent, eight had maintainer reaction having spikelet fertility less than five per cent while remaining thirty one hybrids exhibited either partial restorer or partial maintainer reaction with spikelet fertility ranging from five to seventy per cent.

In the present study, the CMS lines KCMS 29A and CRMS 31A had two maintainers each from the testers evaluated while, KCMS 33A, KCMS 34A, KCMS 38A and CRMS 32A had one maintainer each. However, KCMS 31A, KCMS 35A and KCMS 37A had no maintainers. The CMS line KCMS 31A had five restorers, KCMS 33A had six while all others had seven restorers each. Among the testers, MSN 20-13, MSN-62, MSN-63, MSN-64 and MSN-68 were identified as good maintainers (tables 2 and 3).

There was variation in fertility restoration ability of different testers for a CMS line which could be due to differential interaction between cytoplasm of different male sterile lines with individual pollinator variety. As opined by Virmani, (1987) lower effectiveness of some restorer lines in relation to a CMS line could be attributed to the presence of nuclear genes for sterility in female parent which might inhibit the pollen fertility restoration in  $F_1$  generation. Such differential fertility restoration of R lines for the same source of cytoplasm was also reported by Bobby and Nadarajan, (1994) and Asish et al. (2006).

The testers KMR 3 and KMR 4 were proved to be effective as indicated by fertility restorer reaction with all the CMS lines they were crossed with. Similarly, MSN 67 and MSjN 69 were also good restorers for majority of the CMS lines.

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**Table 1: Mean values of new CMS lines for heir morphological and floral characters.**

<b>CMS lines</b>	<b>Days to 50 per cent flowering</b>	<b>Plant Height (cm)</b>	<b>Panicles per plant</b>	<b>Panicle length (cm)</b>	<b>Seed set on bagging (%)</b>	<b>Stigma exertion (%)</b>	<b>Natural out crossing (%)</b>	<b>Panicle exertion (%)</b>	<b>Stigma length (mm)</b>	<b>Style length (mm)</b>	<b>Angle between Stigma lobes (°)</b>	<b>Pollen fertility (%)</b>	<b>Aroma</b>
KCMS 29A	104.00	87.08	12.35	24.40	0	30.31	19.76	79.20	1.15	0.91	43.79	0	Absent
KCMS 31A	106.50	74.96	10.28	22.83	0	18.07	12.95	71.88	1.20	1.26	49.60	0	Absent
KCMS 33A	108.00	90.78	13.85	26.78	0	17.67	10.52	79.95	1.10	1.16	64.52	0	Absent
KCMS 34A	110.50	119.89	19.09	23.20	0	15.56	9.50	74.73	1.28	0.94	53.68	0	Absent
KCMS 35A	108.00	87.00	15.28	23.75	0	20.24	15.19	71.60	0.82	0.81	74.90	0	Absent
KCMS 37A	106.00	78.76	14.65	25.27	0	20.44	13.52	79.28	1.08	1.07	65.48	0	Absent
KCMS 38A	111.50	74.88	12.34	23.45	0	15.67	18.97	57.72	1.08	0.81	43.11	0	Absent
CRMS 31A	122.00	81.04	11.35	24.41	0	14.33	8.75	71.62	1.11	0.93	50.36	0	Absent
CRMS 32A	115.50	85.04	11.76	22.55	0	15.63	16.02	65.47	1.08	1.01	49.14	0	Absent
<b>Mean</b>	<b>110.22</b>	<b>86.60</b>	<b>13.44</b>	<b>24.07</b>	<b>0</b>	<b>17.77</b>	<b>8.91</b>	<b>72.38</b>	<b>1.11</b>	<b>0.99</b>	<b>54.95</b>	<b>0</b>	

**Table 2. Spikelet fertility percentage in the CMS based hybrids in rice**

Sl. no	Crosses	Spikelet fertility percentage (%)	Classification	Sl. no	Crosses	Spikelet fertility percentage (%)	Classification
1	KCMS 29A/ KMR-3	93.95	R	51	KCMS 35A/MSN-64	60.40	PR
2	KCMS 29A/ KMR-4	89.27	R	52	KCMS 35A/MSN-67	88.08	R
3	KCMS 29A/ KMR-12	70.67	R	53	KCMS 35A/MSN-68	67.68	PR
4	KCMS 29A/ Thanu	90.29	R	54	KCMS 35A/MSN-20-13	10.43	PM
5	KCMS 29A/MSN-62	5.43	PM	55	KCMS 35A/MSN-69	77.01	R
6	KCMS 29A/MSN-63	3.20	M	56	KCMS 37A/KMR-3	70.20	R
7	KCMS 29A/MSN-64	7.09	PM	57	KCMS 37A/KMR-4	86.64	R
8	KCMS 29A/MSN-67	88.43	R	58	KCMS 37A/KMR-12	73.50	R
9	KCMS 29A/MSN-68	83.79	R	59	KCMS 37A/Thanu	95.04	R
10	KCMS 29A/MSN-20-13	3.53	M	60	KCMS 37A/MSN-62	14.15	PM
11	KCMS 29A/MSN-69	76.14	R	61	KCMS 37A/MSN-63	14.22	PM
12	KCMS 31A/KMR-3	77.08	R	62	KCMS 37A/MSN-64	55.33	PR
13	KCMS 31A/KMR-4	73.66	R	63	KCMS 37A/MSN-67	77.18	R
14	KCMS 31A/KMR-12	48.33	PR	64	KCMS 37A/MSN-68	74.96	R
15	KCMS 31A/Thanu	94.06	R	65	KCMS 37A/MSN-20-13	14.69	PM
16	KCMS 31A/MSN-62	6.91	PM	66	KCMS 37A/MSN-69	73.37	R
17	KCMS 31A/MSN-63	10.16	PM	67	KCMS 38A/KMR-3	80.50	R
18	KCMS 31A/MSN-64	14.71	PM	68	KCMS 38A/KMR-4	80.55	R
19	KCMS 31A/MSN-67	10.17	PM	69	KCMS 38A/KMR-12	76.48	R
20	KCMS 31A/MSN-68	81.39	R	70	KCMS 38A/Thanu	57.21	PR



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21	KCMS 31A/MSN-20-13	8.91	PM	71	KCMS 38A/MSN-62	4.96	M
22	KCMS 31A/MSN-69	78.79	R	72	KCMS 38A/MSN-63	97.05	R
23	KCMS 33A/KMR-3	72.10	R	73	KCMS 38A/MSN-64	74.41	R
24	KCMS 33A/KMR-4	94.60	R	74	KCMS 38A/MSN-67	72.28	R
25	KCMS 33A/KMR-12	33.13	PR	75	KCMS 38A/MSN-68	58.60	PR
26	KCMS 33A/Thanu	18.66	PM	76	KCMS 38A/MSN-20-13	16.49	PM
27	KCMS 33A/MSN-62	6.38	PM	77	KCMS 38A/MSN-69	75.41	R
28	KCMS 33A/MSN-63	4.29	M	78	CRMS 31A/KMR-3	91.20	R
29	KCMS 33A/MSN-64	84.38	R	79	CRMS 31A/KMR-4	93.70	R
30	KCMS 33A/MSN-67	86.73	R	80	CRMS 31A/KMR-12	82.35	R
31	KCMS 33A/MSN-68	73.49	R	81	CRMS 31A/Thanu	89.81	R
32	KCMS 33A/MSN-20-13	7.46	PM	82	CRMS 31A/MSN-62	4.53	M
33	KCMS 33A/MSN-69	83.18	R	83	CRMS 31A/MSN-63	10.36	PM
34	KCMS 34A/KMR-3	93.85	R	84	CRMS 31A/MSN-64	4.74	M
35	KCMS 34A/KMR-4	90.88	R	85	CRMS 31A/MSN-67	90.00	R
36	KCMS 34A/KMR-12	10.62	PM	86	CRMS 31A/MSN-68	84.43	R
37	KCMS 34A/Thanu	85.66	R	87	CRMS 31A/MSN-20-13	76.91	R
38	KCMS 34A/MSN-62	14.60	PM	88	CRMS 31A/MSN-69	6.75	PM
39	KCMS 34A/MSN-63	73.47	R	89	CRMS 32A/KMR-3	84.07	R
40	KCMS 34A/MSN-64	61.94	PR	90	CRMS 32A/KMR-4	77.04	R
41	KCMS 34A/MSN-67	79.88	R	91	CRMS 32A/KMR-12	88.47	R
42	KCMS 34A/MSN-68	77.61	R	92	CRMS 32A/Thanu	91.30	R
43	KCMS 34A/MSN-20-13	3.36	M	93	CRMS 32A/MSN-62	63.05	PR
44	KCMS 34A/MSN-69	88.72	R	94	CRMS 32A/MSN-63	5.80	PM
45	KCMS 35A/KMR-3	89.93	R	95	CRMS 32A/MSN-64	71.37	R

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46	KCMS 35A/KMR-4	86.59	R	96	CRMS 32A/MSN-67	79.66	R
47	KCMS 35A/KMR-12	79.83	R	97	CRMS 32A/MSN-68	2.42	M
48	KCMS 35A/Thanu	85.49	R	98	CRMS 32A/MSN-20-13	16.14	PM
49	KCMS 35A/MSN-62	6.37	PM	99	CRMS 32A/MSN-69	86.06	R
50	KCMS 35A/MSN-63	74.19	R				

**Table 3: Maintainer and restorer reaction of some elite lines for nine CMS lines**

Lines Testers	KC MS 29A	KCMS 31A	KCMS 33A	KCMS 34A	KCMS 35A	KCMS 37A	KCMS 38A	CRMS 31A	CRMS 32A
KMR-3	R	R	R	R	R	R	R	R	R
KMR-4	R	R	R	R	R	R	R	R	R
KMR-12	R	PR	PR	PM	R	R	R	R	R
Thanu	R	R	PM	R	R	R	PR	R	R
MSN-62	PM	PM	PM	PM	PM	PM	<b>M</b>	<b>M</b>	PR
MSN-63	<b>M</b>	PM	<b>M</b>	R	R	PM	R	PM	PM
MSN-64	PM	PM	R	PR	PR	PR	R	<b>M</b>	R
MSN-67	R	PM	R	R	R	R	R	R	R
MSN-68	R	R	R	R	PR	R	PR	R	<b>M</b>
MSN 20-13	<b>M</b>	PM	PM	<b>M</b>	PM	PM	PR	R	PM
MSN-69	R	R	R	R	R	R	R	PM	R

M: Maintainer ; PM: Partial maintainer ; PR: Partial restorer ; R: Restorer