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Evaluation of some elite parental lines for maintainer/restorer reaction for WA-CMS system in rice (*Oryza sativa* L.)

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Abstract
The contemporary study was organised to identify the potential maintainers/restorers of rice parental lines for CMS system in. During *kharif* 2019 seven WA-CMS lines were crossed to 33 elite lines. A total of 52 F_1s were evaluated during *kharif* 2020. Among these 13 F_1s recorded 100% pollen sterility, 27 F_1s recorded effective restorability (>75% spikelet and pollen fertility), 10 F_1s recorded partial restorability (50-75% pollen and spikelet fertility) and 2 F_1s (1-50% pollen and spikelet fertility) observed as partial maintainers. The male parents NTCN1, NTCN 107, NTCN112 and NTCN 120 can be developed into new CMS lines through recurrent back-cross breeding. The male parents NTCN22, NTCN37, NTCN52, NTCN57, NTCN58, NTCN72, NTCN118, NTCN151 and NTCN153 can be used to develop medium to long duration hybrids in rice.

Keywords: Hybrid rice, cytoplasmic male sterility (CMS), pollen fertility %, spikelet fertility %, restorers, maintainers

1. Introduction
It is estimated that hybrid rice provides 15-20% advantage in yield over traditional rice cultivation. China has more area i.e., 56% under hybrid rice production compared to conventional rice area followed by USA, Vietnam, Bangladesh, Philippines, India, and Indonesia with area of 14.5%, 9.4, 6.8%, 4.3%,3.2%, and 0.5% (Barclay, 2010) [4]. Identification of effective maintainers/restorers is the initial step of the three-line system in hybrid rice. The key features of three-line system consists of a male sterile line (A-line), a maintainer line (B-line) which maintains A-line and a restorer line (R-line) which provides fertile pollen. Careful selection of parental lines in developing rice hybrids is most important for achieving decent amount of heterosis. Development and cultivation of heterotic rice hybrids will help in increasing the productivity per unit area.
Deficiency of effective superior CMS lines, maintainer lines and non-availability of restorer lines, high seed cost, meager quality of grains of hybrids remains some of the glitches limiting the extension of hybrid rice technology. Hybrid rice requires skill-orientation, knowledge and intensive labour. Hybrid rice seeds are more susceptible to seed borne diseases than traditional rice varieties (Ilyas Ahamed *et al.*, 2006) [7].
Rice being a self-pollinated crop, comprises use of an effective three-line system to develop F_1 hybrids. Successful breeding programme requires a stable CMS line, restorer and acceptable magnitude of heterosis. Identification and careful evaluation of the restorers and maintainers from local elite lines is the most critical step the for development of locally adaptable heterotic rice hybrids.
Hybrid rice under three-line system based on WA-CMS lines shows 20% improvement in yield when compared to improved varieties (Yaun and Virmani, 1994) [25]. Hence, Identification of maintainers/restorer lines from test cross nursery in developing potential hybrids is a pre-requisite.

2. Materials and Methods
The current investigation was carried out at Regional Agricultural Research Station (RARS), Nandyal, Andhra Pradesh located at 15° N, 77.59° E, 78.8° N, at an altitude of 289 m above MSL (Mean Sea Level) with an average rainfall of 780 mm and categorized under scarce rainfall zone.

A total of seven WA-CMS lines (APMS 10A, CMS 16A, CMS 23A, IR 79156A, IR 6888A, IR 68897A and APMS 9A) were crossed with 33 elite male parental lines from germplasm collection present in the research station. Both the CMS lines and elite lines were transplanted in a 5 m row length by a spacing of 20 cm × 20 cm in two replications during *kharif* 2019. Healthy CMS lines with emerging panicles were uprooted and placed in the plastic pots in crossing chamber. Panicles which were going to emerge next day were selected and top 1/3rd part of florets was clipped carefully during sunset hours and enclosed with butter paper bags. Next day morning pollen dust from the male parents was collected and pollinated with CMS lines panicles, covered and labelled carefully. After attaining maturity, the seeds were collected and raised in test cross nursery, studied during *kharif* 2020.

2.1 Pollen Viability Test

During *kharif* 2020 a total of 52 F_1s were raised and 15-20 spikelets were collected from just emerged panicles and kept in a vail containing 70% ethanol for this test. Anthers from four to six spikelets were taken and one droplet of 1% Iodine Potassium Iodine (IKI) stain and placed on a glass slide. Anthers were placed on the stain on glass slide and gently crushed to separate the pollen from anthers and pollen fertility/sterility was observed in three random fields under compound microscope as shown in the Figure 1. Based on pollen reaction the male parent of particular cross can be classified as fertile/sterile according to Virmani *et al.* (1997) [24].

Pollen fertility %: Pollen collected from random spikelets of hybrids and tested with 1% IKI solution using formula

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

Spikelet fertility %: It can be calculated at the time of maturity from the outcrossed panicles using formula

$$\text{Spikelet fertility} = \frac{\text{No.of filled grains per panicle}}{\text{Total no,of grains per panicle}} \times 100$$

According to Virmani *et al.* (1997) [24] based on pollen and spikelet fertility the hybrids can be classified into:

Category	Pollen fertility%	Spikelet fertility%
Restorers	>80	>75
Partial Restorers	50.1-80	50.1-75
Partial Maintainers	1.1-50	0.1-50
Maintainers	0-1	0

3. Results and Discussion

A total of 52 test cross hybrids were evaluated during *kharif* 2020 for the identification of maintainer/restorer reaction furnished in Table 1. The pollen fertility% was ranged from 0.0 to 90.4% and spikelet fertility was ranged from 18.9 to 96.3%. Pollen sterility for 13 (25%) hybrids was 100% and exhibited maintainer reaction NTCN01 with APMS 10A, NTCN01 and NTCN114 with CMS 16A, NTCN01 with CMS 23A, NTCN105 and NTCN107 with IR 79156A, NTCN01, NTCN108 and ntcn113 with IR 68888A, NTCN112 and NTCN120 with IR 68897A and NTCN69 and NTCN76. with APMS 9A. Similar results were reported by Gayathri *et al.* (2021) [5], Rasim (2021) [17], Satyendra *et al.* (2020) [19], Rashid *et al.* (2019) [16], Shama 2018 *et al.* (2018) [20], Anis (2017) [2], Rajendra prasad *et al.* (2017) [15], Geeta Pandey *et al.* (2016) [6], Pankaj Kumar *et al.* (2015) [9] and Ali *et al.* (2014) [1].

The CMS line IR 68888A has more frequency of maintainers. There was a slight spikelet fertility existed in the test crosses exhibiting 100% pollen sterility and this was due to natural outcrossing. The male parent NTCN01 was recognized as effective maintainer line. It showed maintainer reaction with the CMS lines CMS 16A, CMS 23A and IR 68888A. The identified completely sterile test crosses via. NTCN01, NTCN107, NTCN112 and NTCN120 having excellent outcrossing per cent (i.e., 27.7 to 28.3%) should be used to transfer the sterility and conversion into new Cyto-sterile lines through recurrent backcrossing in the breeding programme having pollen donor as recurrent parent. Out of 27 restorers 21 restorers having high spikelet fertility per cent and can be converted into commercial hybrids by using these restorers to cross with the traits like filled grains panicle^{-1}, productive tillers, maturity duration and disease resistance should also be considered.

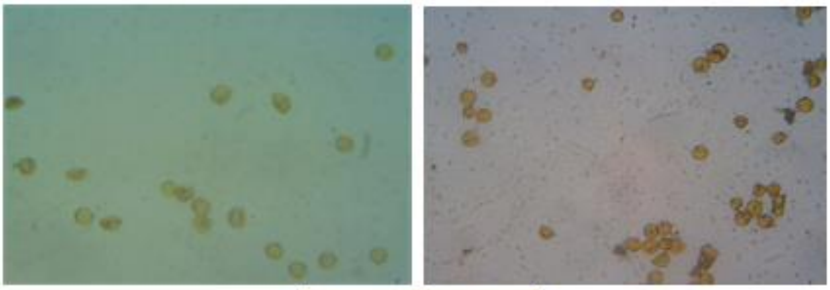
Frequency of restorers was more in the present study i.e., 27 (52%) restorers. Highest restorers were recorded with maintainer line CMS 23A followed by APMS 10A and CMS 16A. Among the 27 restorers 21 test crosses hybrids showed >80% pollen and spikelet fertility *via.* the male parents NTCN54, NTCN58, NTCN151, NTCN154 with APMS 10A, NTCN52, NTCN72, NTCN152, NTCN153 with CMS 16A, NTCN22, NTCN52, NTCN54, NTCN151, NTCN152, NTCN153, NTCN157 with CMS 23A, NTCN118 with IR 79156A and NTCN02, NTCN28, NTCN37 with APMS 6A. Similar results reported by Srinivas Rao *et al.* (2021) [23], Parimala *et al.* (2019) [10], Pushpam *et al.* (2019) [13], Priyantha *et al.* (2018) [12], Riaz *et al.* (2017) [18], Prathima and Goswami (2017) [11], Madhukar *et al.* (2017) [8], Srikanth *et al.* (2017) [22], Srijan *et al.* (2015) [21] and Raghavendra and Shailaja (2015) [14].

Table 1: Evaluation of test crosses with elite parental lines for maintainer/restorer reaction in rice (*Oryza sativa* L.) conducted at RARS, Nandyal during *kharif* 2020.

S. No	Genotype	Pollen fertility% of F_1s	Spikelet fertility% of F_1s	Classification
		APMS 10A		
1.	NTCN-2	0.0	*27.1	Maintainer
2.	NTCN-52	86.3	85.3	Restorer
3.	NTCN-54	85.3	84.7	Restorer
4.	NTCN-55	82	68.8	Partial restorer
5.	NTCN-57	81.6	88.2	Restorer
6.	NTCN-58	85.3	92.7	Restorer
7.	NTCN-151	90.4	91.8	Restorer
8.	NTCN-153	81.4	76.0	Restorer
9.	NTCN-154	90.0	86.8	Restorer
10.	NTCN-156	50.6	52.7	Partial restorer

CMS 16A				
11.	NTCN-01	0.0	*27.7	Maintainer
12.	NTCN-02	81.9	85.5	Restorer
13.	NTCN-52	80.1	85.1	Restorer
14.	NTCN-54	62.6	68.7	Partial restorer
15.	NTCN-57	86.3	85.1	Restorer
16.	NTCN-72	85.9	94.5	Restorer
17.	NTCN-114	0.0	*22.8	Maintainer
18.	NTCN-152	80.2	82.2	Restorer
19.	NTCN-153	90.0	93.7	Restorer
CMS 23A				
20.	NTCN-01	0.0	*29.1	Maintainer
21.	NTCN-101	71.1	68.2	Partial restorer
22.	NTCN-02	58.2	54.7	Partial restorer
23.	NTCN-10	62.5	68.5	Partial restorer
24.	NTCN-22	87.0	84.5	Restorer
25.	NTCN-52	82.3	87.4	Restorer
26.	NTCN-54	84.4	84.0	Restorer
27.	NTCN-55	62.4	64.7	Partial restorer
28.	NTCN-57	75.0	96.3	Restorer
29.	NTCN-62	81.6	86.4	Restorer
30.	NTCN-151	70.5	73.3	Partial restorer
31.	NTCN-152	82.5	84.5	Restorer
32.	NTCN-153	82.5	88.2	Restorer
33.	NTCN-154	81.6	77.1	Restorer
34.	NTCN-155	40.2	46.6	Partial maintainer
35.	NTCN-157	85.0	80.35	Restorer
IR 79156A				
36	NTCN-105	0.0	*28.2	Maintainer
37	NTCN-107	0.0	*27.0	Maintainer
38	NTCN-118	89.1	90.3	Restorer
IR 68888A				
39	NTCN-1	0.0	*27.4	Maintainer
40	NTCN-108	0.0	*28.2	Maintainer
41	NTCN-113	0.0	*28.2	Maintainer
IR 68897A				
42	NTCN-112	0.0	*28.3	Maintainer
43	NTCN-120	0.0	*25.8	Maintainer
APMS 6A				
44	NTCN-01	82.0	75.8	Restorer
45	NTCN-02	90.0	82.6	Restorer
46	NTCN-16	18.0	38.0	Partial maintainer
47	NTCN-28	80.0	83.9	Restorer
48	NTCN-37	85.3	89.2	Restorer
49	NTCN-47	70.0	73.3	Partial restorer
50	NTCN-59	68.5	63.0	Partial restorer
51	NTCN-69	0.0	*18.9	Maintainer
52	NTCN-76	0.0	*19.1	Maintainer
*Indicates spikelet fertility due to outcrossing				

Among the crosses evaluated 10 (20%) were detected as partial restorers having the spikelet fertility between 50 to 75% and only 2 crosses were observed as partial maintainers. This can be due to variation occurred in fertility restoration of different restorer lines when crossed with different CMS genotypes as stated by Ali *et al.* (2014) and Pankaj Kumar *et al.* (2015). The partial restorers have no practical use in hybrid rice breeding programme as quoted by Ashish *et al.* (2004) [3].



Sterile Hybrids

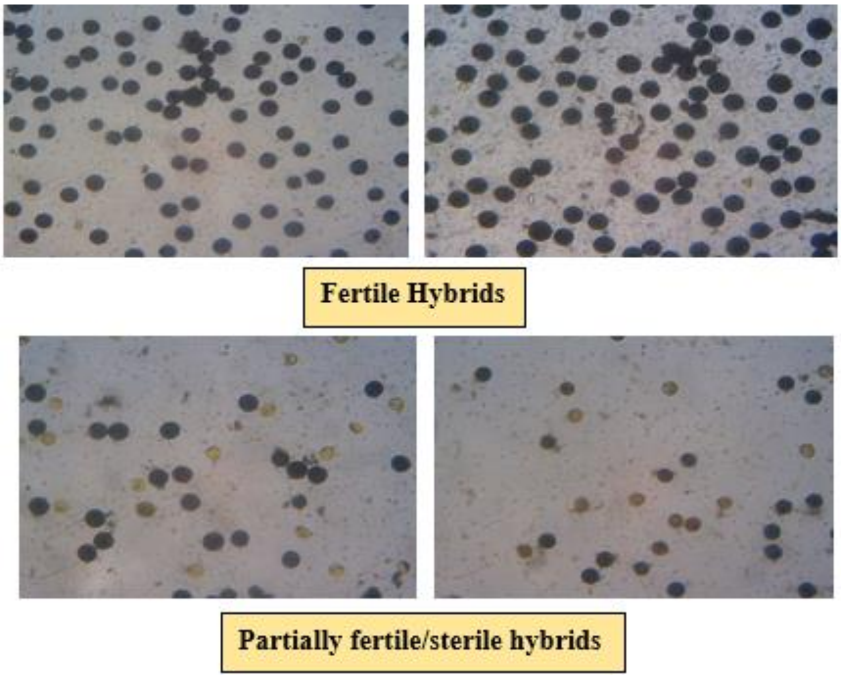


Fig 1: Pollen study of some hybrids evaluated during kharif 2020.

4. Conclusion

Based on the above analysis, it is recommended that four male parents showed maintainer reaction *via*. NTCN1, NTCN 107, NTCN112 and NTCN 120 having 100% pollen sterility and excellent outcrossing per cent, they can be further developed into new Cyto-sterile lines through recurrent back-cross breeding programme and nine male parents *via*. NTCN22, NTCN37, NTCN52, NTCN57, NTCN58, NTCN72, NTCN118, NTCN151 and NTCN153 having effective restorability *i.e.*, >80% pollen and spikelet fertility can be further tested to develop into medium to long duration hybrids in rice.

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