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## Test crosses evaluation for identification of maintainer and restorer lines in hybrid rice breeding programme

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### ABSTRACT

*The study was conducted to identify potential maintainers and restorers in developing rice hybrids through three line system. During kharif 2018 six established CMS lines were crossed with 34 elite lines and 87 F1s were evaluated during 2019 in test cross nursery. Among these 49 genotypes recorded effective restorability with more than 75% spikelet fertility, 9 genotypes recorded partial fertility with 50-75% spikelet fertility, 3 genotypes were partial maintainers with 1-50% spikelet fertility and 25 genotypes as maintainers with 100% pollen sterility. In present study high frequency of restorers (56%) were observed than maintainers (29%). The genotypes NTCN1, NTCN 31 and NTCN 93 can be used for development of new CMS lines through recurrent back crossing programme and other potential restorers like NTCN 2, NTCN 3, NTCN 18, NTCN 19, NTCN 31, NTCN 33, NTCN 58, NTCN 78, NTCN 79, NTCN 80, NTCN 85, NTCN 97, NTCN 98, NTCN 101, NTCN 102, NTCN 103, NTCN 104, NTCN 105, NTCN 109, NTCN 111, NTCN 115, NTCN 113, NTCN 116, NTCN 118 and NTCN 119 can be used for developing early to medium duration hybrids in rice.*

**Keywords:** Hybrid Rice, Pollen fertility%, Spikelet fertility%, Maintainers, Restorers.

### 1. INTRODUCTION

Commercialization of hybrid rice technology is possible by identification of effective maintainers and restorers. For development of commercially usable hybrids choice of appropriate parental lines possessing good combining ability, high yield potential, good grain quality and resistance to major pests and diseases is prerequisite. Development of new improved parental lines possessing desirable characteristics and evaluation of them are the major activities of heterosis breeding programme. Identification of maintainers and the restorers from elite breeding lines and germplasm through test crossing and their use in further breeding programme are the initial steps in three line heterosis breeding which is in use in Andhra Pradesh.

Both pollen sterility and spikelet fertility are being used as index to develop maintainers and restorers from test cross nursery programme. Successful hybrid rice breeding programme depends on development of local cytoplasmic genetic male sterile (CMS) and restorer lines as CMS lines introduced from other areas may not adopt well in the target environment. Hence it is essential to develop local CMS lines by transferring available CMS system into local elite breeding lines to develop successfully adoptable heterotic hybrids. So far five maintainer lines (APMS lines) were developed to use in hybrid rice breeding programme from Regional Agriculture Research Station, Maruteru, Acharya N.G.Ranga Agricultural University (Vijaykumar and Murthy 1997). Hence it is a prerequisite to identify maintainer and restorer lines from the test cross nurseries to develop potential rice hybrids.

### 2. MATERIALS AND METHODS

The present experiment was carried out at Regional Agricultural Research Station, Nandyal, Andhra Pradesh located at 15 °N, 77.59 °E, 78.8 °N and at an altitude of 289 m above mean sea level with an average rainfall 780 mm. In the present study seven established CMS lines (APMS 6A, CMS 16A, CMS 23A, IR7 9156 A, IR 68897A, IR 68888A and IR 58025A) were crossed with different elite lines selected from the germplasm collection of the Research Station. In crossing block six CMS line and different rice elite male lines were transplanted in a row of 5 m length with a spacing of 20 x 20 cm in two staggered sowings during kharif 2018. Healthy plants of CMS lines with just emerging panicles were uprooted and potted in plastic pots in crossing chamber. Panicles going to emerge next day were selected for crossing. Top 1/3<sup>rd</sup> part of each floret was clipped carefully during evening hours and covered with butter paper bags. Next day morning pollen from the male parent was collected and pollinated with CMS line panicle covered and labeled. At the time of maturity seed was collected and studied in test cross nursery during kharif 2019.

During kharif 2019 F1s were raised and 20 spikelets were collected randomly from the just emerged panicles in a vial containing 70% ethanol for pollen viability test. One drop of 1% Iodine Potassium Iodide (IKI) stain was put on a glass slide. All anthers of 5-6 spikelets were taken out with the help of forceps and placed on stain of the glass slide. Anthers were crushed gently by using needle to release pollen grains. After removing debris, a cover slip was placed on crushed grains and pollen fertility was recorded in three random fields and classified based on fertility/sterility reaction as per Virmani *et al.* (1997).

Pollen fertility: Pollen was collected from randomly selected spikelets of each cross and tested with IKI solution under microscope and calculated using the formula

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

Spikelet fertility was calculated at the time of maturity from the out crossed panicles using the formula

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

Classification of the above two parameters were done as per Virmani *et al.* (1997).

Category	Pollen Fertility %	Spikelet Fertility%
Maintainers	0-1	0
Partial Maintainer	1.1-50	0.1-50
Partial Restorer	50.1-80	50.1-75
Restorer	>80	>75

### 3. RESULTS AND DISCUSSION

A total of 109 crosses were attempted during kharif 2018 out of which 87 test crosses were evaluated successfully during kharif 2019 for maintainer and restorer reaction (Table 1). The pollen fertility ranged between 0 to 100% and spikelet fertility ranged from 32.14% to 95.16% based on fertility data. Restorability depends on the male parent used in the cross which is due to pollen fertility restoring genes differing in their expressivity with genotypes. Similar results were earlier reported by Ali *et al.* (2014) and Rajendra Prasad *et al.* (2017). 100% pollen sterility was recorded in 25 hybrids APMS 6A with NTCN 31, NTCN 101; CMS16A with NTCN 31, NTCN 93, NTCN 97, NTCN 111; CMS23A with NTCN 1; IR 79156A with NTCN 1, NTCN 33, NTCN 80, NTCN 93, NTCN 97, NTCN 101, NTCN 102, NTCN 103, NTCN 108, NTCN 114; IR 68897A with NTCN 1, NTCN 3, NTCN 93, NTCN 104, NTCN 110, NTCN 115, NTCN 117 and IR 58025A with NTCN 1.

Based on hybrid evaluation 49 genotypes were found to have effective restorability with >75% spikelet fertility while 9 were partial fertile with 50 to 75% spikelet fertility, 3 genotypes were partial maintainers with 1 to 50% spikelet fertility and 25 genotypes showed complete sterility with 100% pollen sterility. This might be due to variation in the fertility restoration reaction of the genotypes with the genetic background of CMS lines as reported by Ali *et al.* (2014), Pankaj kumar *et al.* (2015) and Srijan *et al.* (2015). This variation is due to difference in the penetrance or expressivity of restoring genes present in the genotypes due to presence of modifying genes according to Umadevi *et al.* (2010). Partial restorers have no utility in hybrid rice breeding programme as per Gautam and Singh (2004).

In the present study high frequency of restorers (56%) were observed than that of maintainers (29%). Similar reports presented by Shama *et al.* (2018) and Parimala *et al.* (2019). Among 34 restorers 25 genotypes found to be good with more than 80% fertility restorability viz NTCN1, NTCN 2, NTCN 3, NTCN 18, NTCN 19, NTCN 31, NTCN 33, NTCN 58, NTCN 78, NTCN 79, NTCN 80, NTCN 93, NTCN 97, NTCN 102, NTCN 103, NTCN 104, NTCN 105, NTCN 106, NTCN 109, NTCN 110, NTCN 111, NTCN 113, NTCN 115, NTCN 116, and NTCN 119.

Out of twenty five identified maintainers two are with APMS 6A, four for CMS 16A, one for CMS 23 A, ten for IR 79156 A, seven for IR 68897 A and one for IR 58025 A. Frequency of maintainers were more with IR 79156A followed by IR 68897A and CMS 16A. Identified complete sterile crosses should be utilized for transfer of male sterility and conversion into new CMS line through subsequent back crossing breeding programme with their respective pollen parent as recurrent parent. Superior hybrids can be developed by using popular rice cultivars as parental lines in hybrid rice breeding programme. In addition to spikelet fertility other characters like pollen fertility%, flowering duration, number of filled grains per panicle were also to be considered in selecting restorers. Using of diverse CMS lines in test crosses give validity to fertility restoration studies according to Das *et al.* (2013). The genotype NTCN1 was identified as effective maintainer for four CMS lines CMS 23A, IR 79156A, IR 68897A and IR 58025A

followed by NTCN 93 for three CMS lines CMS 16 A, IR 79156A, IR 68897A and NTCN 31 for two CMS lines APMS 6A and CMS 16A.

The present study suggested that three effective maintainers viz NTCN1, NTCN 93 and NTCN 31 can be exploited for development of new CMS lines through recurrent back crossing programme while potential restorers NTCN 2, NTCN 3, NTCN 18, NTCN 19, NTCN 31, NTCN 33, NTCN 58, NTCN 78, NTCN 79, NTCN 80, NTCN 85, NTCN 97, NTCN 98, NTCN 101, NTCN 102, NTCN 103, NTCN 104, NTCN 105, NTCN 109, NTCN 111, NTCN 115, NTCN 113, NTCN 116 and NTCN 119 are useful for developing early to medium duration hybrids in rice using new CMS lines.

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**APPENDIX**

Table 1. Evaluation of Test Cross entries for maintainer and restorer reaction at RARS, Nandyal during kharif 2019.

S.No	Genotype	Days to 50% Flowering	Pollen Fertility% of F1	Spikelet fertility% of F1	Seed yield /plant (g)	Remarks
APMS 6A						
1	NTCN 1	100	92	84.94	175	R
2	NTCN 2	100	89	90.87	95	R
3	NTCN 3	100	85	94.02	219	R
4	NTCN 19	108	88	50.29	32	PR
5	NTCN 31	103	0	24.82*	24	M
6	NTCN 33	103	89	92.08	135	R
7	NTCN 78	103	88	95.16	108	R
8	NTCN 80	100	90	98.76	115	R
9	NTCN 93	111	92	88.13	92	R
10	NTCN 97	108	89	92.30	98	R
11	NTCN 101	97	0	23.42*	27	M
12	NTCN 102	103	90	71.23	112	PR
13	NTCN 103	103	95	94.89	96	R
14	NTCN 104	103	92	41.86	97	PM
15	NTCN 105	103	93	87.84	58	R
16	NTCN 106	97	50	68.43	69	PR

17	NTCN 107	103	25	32.14	82	PM
18	NTCN 108	103	20	67.18	42	PR
19	NTCN 109	103	85	95.72	88	R
CMS 16A						
20	NTCN 1	102	88	23.88	26	PM
21	NTCN 31	107	0	31.67*	41	M
22	NTCN 33	103	87	91.19	222	R
23	NTCN 79	105	85	93.26	62	R
24	NTCN 93	103	0	22.16*	36	M
25	NTCN 97	103	0	27.92*	41	M
26	NTCN 103	108	10	72.21	27	PR
27	NTCN 104	103	90	95.3	130	R
28	NTCN 106	103	90	94.63	119	R
29	NTCN 111	108	0	27.67*	21	M
CMS 23A						
30	NTCN 1	99	0	17.71*	16	M
31	NTCN 2	101	80	90.28	165	R
32	NTCN 3	105	75	87.93	131	R
33	NTCN 33	106	85	86.03	120	R
34	NTCN 58	106	90	92.67	179	R
35	NTCN 79	99	90	93.65	127	R
36	NTCN 97	99	60	67.69	105	PR
37	NTCN 102	106	88	89.32	103	R
38	NTCN 103	103	80	78.05	79	R
39	NTCN 104	101	10	84.95	66	R
40	NTCN 111	100	25	80.00	117	R
IR 79156A						
41	NTCN 1	101	0	8.83*	30	M
42	NTCN 2	99	50	84.80	96	R
43	NTCN 3	113	90	96.79	168	R
44	NTCN 19	105	90	95.49	87	R
45	NTCN 31	97	92	95.08	97	R
46	NTCN 33	93	0	55.70*	31	M
47	NTCN 78	99	100	74.00	58	PR
48	NTCN 79	106	80	92.52	132	R
49	NTCN 80	100	0	65.22*	29	M
50	NTCN 93	106	0	42.85*	19	M
51	NTCN 97	105	0	18.45*	31	M
52	NTCN 101	106	0	39.08*	31	M
53	NTCN 102	93	0	30.03*	25	M
54	NTCN 103	106	0	40.29*	28	M
55	NTCN 104	103	90	99.17	99	R
56	NTCN 106	103	80	83.28	127	R
57	NTCN 108	106	0	26.32*	29	M
58	NTCN 110	99	100	98.32	94	R
59	NTCN 111	93	75	86.86	135	R
60	NTCN 114	97	0	23.15*	25	M
61	NTCN 115	97	100	87.21	126	R
IR 68897A						
62	NTCN 1	97	0	7.94*	12	M
63	NTCN 3	97	0	12.15*	27	M
64	NTCN 18	97	50	87.63	95	R
65	NTCN 31	112	82	95.14	58	R
66	NTCN 33	100	85	89.62	84	R
67	NTCN 78	112	87	95.15	116	R
68	NTCN 79	106	90	94.27	105	R
69	NTCN 85	97	60	86.52	142	R

70	NTCN 93	111	0	3.98*	45	M
71	NTCN 97	93	82	78.59	52	R
72	NTCN 103	113	82	88.23	102	R
73	NTCN 104	97	0	28.57*	31	M
74	NTCN 109	106	90	93.62	113	R
75	NTCN 110	113	0	13.69*	33	M
76	NTCN 113	100	80	84.35	61	R
77	NTCN 114	112	40	85.62	89	R
78	NTCN 115	97	0	33.79*	21	M
79	NTCN 116	97	88	89.62	97	R
80	NTCN 117	97	0	6.76*	30	M
IR 58025A						
81	NTCN 1	112	0	7.90*	33	M
82	NTCN 111	106	70	82.34	121	R
83	NTCN 115	97	50	74.85	104	PR
84	NTCN 118	97	70	91.32	94	R
85	NTCN 119	113	85	86.53	89	R
86	NTCN 120	113	50	69.72	96	PR
87	NTCN 121	113	70	82.63	87	R
Note : * denotes spikelet fertility % due to out crossing.						