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Evaluation of Test Crosses for Identification of Potential Restorers and Maintainers for Development of Rice Hybrids (*Oryza sativa* L.)

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A B S T R A C T

The investigation was carried out to identify the potential maintainers and restorers in rice by crossing the one CMS line with 65 selected genotypes during *rabi* 2012-13. The resultant 65 test crosses were evaluated during *kharif* 2013. A high range of spikelet fertility was recorded among the hybrids *i.e.*, from 0 to 92.4 per cent which indicated that restorability varies depending on male parent. Among the 65 test crosses studied, 28 genotypes exhibited high spikelet fertility (>75%), 20 were partial fertile (50 to 75 %), whereas 14 genotypes were partial maintainers (1 to 50 %) and three genotypes were found to be completely sterile (0 %). The identified maintainers can be developed as new cytoplasmic male sterile lines by repeated back cross breeding with recurrent parent. Among the 28 restorers identified, 12 best restorers with more than 80 per cent spikelet fertility were selected for further use in hybrid rice breeding.

Introduction

The development and use of hybrid rice varieties on commercial scale using male sterility and fertility restoration system has proved to be a breakthrough in rice improvement. The availability of stable cytoplasmic male sterility and fertility restoring system is vital for commercial exploitation of heterosis in rice. Cytoplasmic genetic male sterile lines introduced from elsewhere may not be well adapted to a given target area. Successful use of hybrid vigour in rice largely depends on the availability of locally developed cytoplasmic genetic male sterile and restorer lines (Kumar *et al.*, 1996). Identification of locally adapted maintainers and restorers which show complete sterility and consistently high degree of restoration of CMS lines would be of great value in commercial hybrid programme, if restoring ability is combined with high combining ability. Earlier researchers *viz.*, Hussain and Sanghera (2012); Singh *et al.*, (2013);

Veeresha *et al*., (2013); Bhati *et al*., (2014); Parmeshwar Kumar *et al*., (2014); Jamil Hasan *et al*., (2015); Pankaj Kumar *et al*., (2015) and Ramesh *et al*., (2018) evaluated the test crosses in rice to identify the restorer and maintainer reaction and reported varying levels of pollen and spikelet fertility percentage. The establishment of test cross nursery to identify restorers and maintainers is the first step in three line heterosis breeding (Akhter *et al.,* 2008). Use of male sterility system would be appropriate approach for commercial exploitation of heterosis in rice. In view of the above, the present study was conducted to identify the effective fertility restorers and maintainers.

Materials and Methods

The basic material for the study consists of one cytoplasmic male sterile (CMS) line of IR-79156A and 65 elite rice genotypes obtained from germplasm collection of Rice Research Centre, Rajendranagar, Hyderabad. The CMS line IR-79156A was crossed with 65 diverse male fertile genotypes during *rabi,* 2012-13. In a crossing block, one CMS line (IR-79156A) and 65 elite rice genotypes were transplanted with a spacing of 20 x 15 cm in a row of 6 m length in three staggered sowings.

Healthy male sterile plant with just emerged panicles were uprooted and potted into plastic buckets filled with mud and were transferred to the crossing chamber. Healthy panicles with florets expected to open on the next day were used for crossing. Top 1/3rd portion of each floret was clipped with scissors during evening hours and covered with butter paper bags. Next day morning, panicles ready for anthesis were collected from male parents and used for pollination with CMS lines (female parent). The pollinated spikelets were then covered and labelled. Crossed seeds were collected after four weeks and seeds were dried and preserved.

The resultant 65 test crosses were evaluated in test cross nursery during kharif, 2013. Pollen fertility test was carried out by using the five randomly selected spikelets from each entry. With the help of forceps, the anthers from the spikelets were gently placed on glass slide containing 2% Iodine Potassium Iodide (IKI) solution. Then the anthers were gently crushed to release the pollen grains and observed under microscope. The pollen fertility (%) was calculated by using the formula as given below:

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

Five panicles were selected randomly from each entry during maturity and spikelet fertility (%) was calculated by using the following formula:

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

The following criteria for classifying the pollen parents were used as proposed by (Virmani *et al*., 1997).

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

Results and Discussion

A total of 65 test crosses were evaluated for fertility restorer and maintainer reaction. The performance of the hybrids in test cross nursery for fertility restoration is presented in Table 1. The pollen fertility of hybrids varied from 0.2 % (IR-79156A x WGL-13400) to 94.2% (IR-79156A x RNR-2456). A high range of spikelet fertility was recorded among the hybrids *i.e.*, from 0 to 92.4 per cent which indicated that restorability varies depending on male parent. Among the 65 test crosses studied, 28 genotypes considered as restorers which exhibited high spikelet fertility (>75%), 20 genotypes found to be partial fertile (50 to 75 %), whereas, 14 genotypes were partial maintainers (1 to 50 %) and three genotypes (IR-79156A x JGL-11727, IR-79156A x Sumathi and IR-79156A x WGL-13400) showed complete spikelet sterility (0 %). Ali *et al.*, (2014); Pankaj Kumar *et al.*, (2015) and Srijan *et al.*, (2015) reported that fertility restoration reaction of the genotypes varies with genetic background of CMS lines. Umadevi *et al.*, (2010) also reported that this variation may be due to the pollen fertility / restoring genes differ or their penetrance or expressivity differed with genotypes or due to existence of modifiers genes.

Table.1 Fertility restoration study for identification of restorers and maintainers in rice

S.No.	Crosses	Days to 50% flowering	Pollen fertility (%)	No. of unfilled grains / panicle	No. of filled grains / panicle	Spikelet fertility (%)	Fertility reaction
1	IR-79156A x RNR-17462	90	89.5	19.7	239.4	92.4	R
2	IR-79156A x RNR-15351	99	85.4	19.5	235.4	92.3	R
3	IR-79156A x RNR-6378	99	82.6	19.8	221.5	91.8	R
4	IR-79156A x RNR-15038	91	92.8	23.5	258.3	91.7	R
5	IR-79156A x WGL-583	100	86.0	18.6	183.3	90.8	R
6	IR-79156A x WGL-48684	92	84.3	19.1	184.7	90.6	R
7	IR-79156A x NLR-33358	81	80.7	20.7	192.5	90.3	R
8	IR-79156A x RNR-883	113	87.5	19.7	175.6	89.9	R
9	IR-79156A x RNR-2456	100	94.2	21.0	189.7	90.0	R
10	IR-79156A x 6527	94	86.1	23.3	195.4	89.3	R
11	IR-79156A x WGL-451	89	90.2	16.7	135.7	89.1	R
12	IR-79156A x RNR-17473	92	82.5	18.0	142.3	88.8	R
13	IR-79156A x WGL-3962	90	86.3	28.6	216.4	88.3	R
14	IR-79156A x WGL-347	92	88.4	31.2	219.2	87.5	R
15	IR-79156A x NLR-3042	88	90.0	28.6	198.4	87.4	R
16	IR-79156A x RNR-15398	99	83.9	29.5	201.6	87.2	R
17	IR-79156A x RNR-2781	91	91.5	36.5	245.7	87.1	R
18	IR-79156A x IR-83142-B-57-B	91	84.9	36.1	238.4	86.8	R
19	IR-79156A x D-4098	91	90.5	29.7	184.8	86.2	R
20	IR-79156A x WGL-14	93	83.7	32.6	198.5	85.9	R
21	IR-79156A x RNR-2458	92	89.3	35.6	208.2	85.4	R
22	IR-79156A x RNR-15048	99	87.9	36.7	215.0	85.4	R
23	IR-79156A x RNR-15028	90	93.5	36.5	204.9	84.9	R
24	IR-79156A x NWGR-3132	97	86.7	42.5	254.9	85.7	R
25	IR-79156A x MTU-1081	89	90.0	26.7	136.4	83.6	R
26	IR-79156A x RNR-2465	100	92.5	23.7	118.0	83.3	R

27	IR-79156A x Vajram	90	85.2	56.4	203.5	78.3	R
28	IR-79156A x Akshayadhan	95	86.9	60.1	182.4	75.2	R
29	IR-79156A x Zhonghuai	93	72.6	55.6	159.6	74.2	PR
30	IR-79156A x IR-83142-B-21-B	87	79.6	58.2	166.7	74.1	PR
31	IR-79156A x NP-6226	102	82.0	62.8	175.9	73.7	PR
32	IR-79156A x JGL-1798	102	62.5	64.5	179.8	73.6	PR
33	IR-79156A x RNR-10291	97	84.6	56.4	154.6	73.3	PR
34	IR-79156A x JGL-11470	108	71.9	48.2	132.0	73.3	PR
35	IR-79156A x NLR-40058	93	86.4	58.4	159.6	73.2	PR
36	IR-79156A x RNR-17438	89	68.2	53.4	145.0	73.1	PR
37	IR-79156A x NLR-145	94	50.0	62.7	169.0	72.9	PR
38	IR-79156A x MGD-103	94	83.4	29.7	78.7	72.6	PR
39	IR-79156A x IR-64	104	50.0	53.7	132.7	71.2	PR
40	IR-79156A x RNR-898	99	55.0	79.3	184.6	70.0	PR
41	IR-79156A x C-26	82	61.8	66.7	153.3	69.7	PR
42	IR-79156A x RNR-17420	102	56.3	61.5	124.6	67.0	PR
43	IR-79156A x RNR-17494	92	73.5	95.6	184.6	65.9	PR
44	IR-79156A x RNR-17472	96	59.4	74.8	143.2	65.7	PR
45	IR-79156A x NSN-20114	79	52.0	92.2	148.7	61.7	PR
46	IR-79156A x NPG-210	94	64.8	102.4	124.5	54.9	PR
47	IR-79156A x TCA-80-A	94	73.5	116.7	126.3	52.0	PR
48	IR-79156A x JGL-11118	94	38.6	107.6	112.9	51.2	PR
49	IR-79156A x Pushyami	97	68.9	133.7	122.8	47.9	PM
50	IR-79156A x P-35	91	42.5	171.9	110.5	39.1	PM
51	IR-79156A x Champakali	90	56.9	87.7	47.7	35.2	PM
52	IR-79156A x IR-83142-B-61-B	91	34.4	163.7	83.9	33.9	PM
53	IR-79156A x RNR-11636	93	35.2	95.6	36.8	27.8	PM
54	IR-79156A x NSN-20894	102	43.6	182.4	64.5	26.1	PM
55	IR-79156A x JGL-11690	82	23.4	66.7	19.4	22.5	PM
56	IR-79156A x Dembersali	88	42.5	116.7	16.0	12.1	PM
57	IR-79156A x HonneKattu	81	17.5	224.5	26.7	10.6	PM
58	IR-79156A x CT-18664-9-10-5-6-3	100	40.5	133.3	15.7	10.5	PM
59	IR-79156A x CSR-23	93	17.2	123.4	5.0	3.9	PM
60	IR-79156A x IR-79216-141-1-3-3-3	99	1.6	127.6	3.0	2.3	PM
61	IR-79156A x WGL-20471	107	1.5	128.6	3.0	2.3	PM
62	IR-79156A x JGL-384	106	0.5	142.0	2.0	1.4	PM
63	IR-79156A x JGL-11727	112	1.0	114.6	0.0	0.0	M
64	IR-79156A x Sumathi	104	0.5	125.6	0.0	0.0	M
65	IR-79156A x WGL-13400	102	0.2	137.6	0.0	0.0	M

R-Restorer, **PR**-Partial Restorer, **PM**-Partial Maintainer,**M**-Maintainer

Akhter *et al.*, (2008) observed higher frequency of maintainers (17%) than that of restorers (11%) in their studies. Hence, more emphasis should be given to utilize popular rice cultivars in hybrid rice breeding as parental lines to achieve the goal of superior hybrids. Among the 28 restorers, 12 genotypes were found to be good with more than 80 per cent fertility restorability (RNR-15351, WGL-3962, IR-83142-B-57-B, RNR-15398, D-4098, NWGR-3132, RNR-15028, RNR-15038, RNR-2458, RNR-2456, RNR-17462 and RNR-2781). In addition to spikelet fertility the characters like pollen fertility (%), flowering duration, plant stature and number of filled grains per panicle were taken into consideration while selection of restorers. The hybrids IR-79156A x RNR-2781, IR-79156A x NWGR-3132 and IR-79156A x RNR-15038 exhibited more number of filled grains per panicle. The identified maintainers can be developed as new cytoplasmic male sterile lines by repeated back cross breeding. Twelve good restorer lines found in this study could be utilized as pollen parent for the development of new rice hybrids.

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