

Evaluation Of Genotypes For Fertility Restoring And Maintaining Behaviors In Rice (*Oryza Sativa* L.)

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Abstract: The narrow genetic base and inadequate number of parental lines are the major constrains for the development of location specific hybrid varieties in rice. The commercial exploration of hybrid in rice has been made possible by identification of parental lines i.e maintainers and restorers. A study was conducted on ten cytoplasmic male sterile (CMS) lines and twenty five elite rice genotypes of diverse source of origin to evaluate the genotypes in order to identify potential restorers and maintainers from test crosses. The F₁'s (crossed between genotypes and CMS lines) expressed different fertility reactions. Among the tested genotypes, twelve genotypes expressed restorer (R) reaction and two exhibited maintainer (M) reaction. The identified maintainers and restorers were locally well adopted. The identified genotypes can play a pivotal role in hybrid rice development.

Key words: CMS lines, hybrid rice, maintainer, pollen fertility, restorer, spikelet fertility, test crosses.

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops in the world, consumed daily by more than half of the world population. Rice provides 20 percent of the world's dietary energy supply, while wheat supplies 19 percent and maize 5 percent (FAO, International year of rice, 2004). In concern over the growing population in India, it needs to increase the productivity of rice (Krishnalatha *et al.*, 2012). The exploration of hybrid varieties has potential to overcome the current yield plateaus in rice (Virmani, 1994). Chinese rice scientists already proved the 15-20 % yield advantage of hybrid rice over semi dwarf rice varieties (Yuan, 1977 and Yuan *et al.* 1989). The commercial exploitation of heterosis in rice has been made possible by the use of cytoplasmic genetic male sterility and fertility systems (Krishnalatha *et al.*, 2012). Success and sustenance of hybrid rice technology solely depend on the exploitation of heterosis in F₁ generation (Virmani *et al.*, 1997).

2. Materials and methods

Ten CMS lines and twenty five elite rice genotypes were comprised the materials for present study. Those materials were grown in source nursery at Rice Research Station, Chinsura, West Bengal during boro season 2011-12. The pollen parents i.e the restorer and germplasms were planted in plot with a spacing of 15cm (plant to plant) X 20 cm(row to row). Before crossed, all CMS plants were tested separately by pollen sterility to ensure 100 % pollen sterility of CMS plants. The panicles of CMS plant were bagged with butter paper before anthesis period. Pollen from pollen parent and germplasms were dusted on bagged panicles of CMS lines separately. So all possible 250 cross combinations were attempted and crossed seeds from 224 combinations were collected for evaluation.

Table -1: Origin of Cytoplasmic Male Sterile (CMS) Line or A line and their respective maintainer or B line

Sr. No.	CMS Line (A line) and Maintainer line (B line)	Origin
1	IR 58025A & B	International Rice Research Institute, Philippines
2	IR 68897A & B	International Rice Research Institute, Philippines
3	IR 80555A & B	International Rice Research Institute, Philippines
4	IR 79156 A & B	International Rice Research Institute, Philippines
5	IR 80559A & B	International Rice Research Institute, Philippines
6	PMS 3A & B	Punjab Agricultural University, Ludhiana, India
7	PMS 10A & B	Punjab Agricultural University, Ludhiana, India
8	Satabdi A & B	Rice Research Station, Chinsurah, West Bengal
9	DRR 4A & B	Directorate of Rice Research, Hyderabad, India
10	COMS 15A & B	Tamilnadu Agricultural University, Coimbatore, India

Estimation of pollen fertility: Pollen fertility test of test cross F₁ was carried out for their fertility or sterility responses. The spikelets (5 to10) from the just emerged panicle of 3 randomly selected plants were collected in vial containing 70 percent ethanol. With the help of forceps, the anthers from the spikelets were placed on a glass slide containing 2% Iodine Potassium Iodide (IKI) strain. Then the anthers were gently crushed by using needle to release the

pollen grains. After removing the debris, a cover slip was put on the slide and observed under microscope.

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

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Table 2 : Origin of genotypes

Sr. No.	Genotypes	Origin
1	Gajapati	Orissa University of Agricultural and Technology
2	Khandagiri	Orissa University of Agricultural and Technology
3	Pratiksha	Orissa University of Agricultural and Technology
4	Sidhant	Orissa University of Agricultural and Technology
5	PNR 546	Indian Agricultural Research Institute, New Delhi,
6	MTU 1010	Agricultural Research Station, Maruteru, A.P
7	Swarna Sub 1	Central Rice Research Institute, Orissa
8	Swarna Dhan (IET 5656)	Agricultural Research Station, Maruteru, A.P
9	Basmati 370	Haryana
10	Nayanmoni	Traditional rice genotype
11	Dular	Traditional rice genotype
12	CN 1719-4	Rice Research Station, Chinsurah, West Bengal
13	CN 915	Rice Research Station, Chinsurah, West Bengal
14	CNR 33	Rice Research Station, Chinsurah, West Bengal
15	CNR 45	Rice Research Station, Chinsurah, West Bengal
16	CNR 47	Rice Research Station, Chinsurah, West Bengal
17	CNR 55-3	Rice Research Station, Chinsurah, West Bengal
18	CNR 55-6	Rice Research Station, Chinsurah, West Bengal
19	CNR 55-10	Rice Research Station, Chinsurah, West Bengal
20	CNR 55-15	Rice Research Station, Chinsurah, West Bengal
21	CNR 57	Rice Research Station, Chinsurah, West Bengal
22	CNR 77	Rice Research Station, Chinsurah, West Bengal
23	CNR 93	Rice Research Station, Chinsurah, West Bengal
24	CNR 98	Rice Research Station, Chinsurah, West Bengal
25	CNR 102	Rice Research Station, Chinsurah, West Bengal

Estimation of spikelet fertility: Estimation was done on three panicles per plant (two selected at random and one from the main culm) from five randomly selected plants for each testcross hybrid at maturity. Spikelet fertility of hybrids was assessed by taking the count of well filled and chaffy spikelets in each panicle.

$$\text{Spikelet fertility (\%)} = \frac{\text{No. of total filled spikelets per panicle}}{\text{Total no. of spikelet per panicle}} \times 100$$

Classification of pollen parents : The pollen parents were classified into four categories - Maintainers (M), Partial Maintainers (PM), Partial Restorer (PR) and Restorer (R) according to *Virmani et al.*, 1997 (Table -3)

Table -3. : Classification of elite lines into maintainers and restorers (Virmani et al., 1997)

Pollen Fertility % (PF%)	Category	Spikelet Fertility % (SF %)
0 – 1	Maintainers (M)	0
1.1 – 50	Partial Maintainers (PM)	0.1 – 50
50.1 – 80	Partial Restorer (PR)	50.1 – 75
>80	Restorer (R)	> 75

3. Results and Discussion

The establishment of test cross nursery to identify restorer and maintainer is the initial step in three line hybrid rice breeding (*Akhter et al.*, 2008, *Ikehashi and Araki*, 1984; *Virmani*, 1996). The result showed that out of 250 attempted test crosses, 224 test crossed were successfully evaluated (Table 4). The frequency of restorers, maintainers, partial restorers and partial maintainers reaction were 51.3 %, 6.3%, 22.8% and 15.2% respectively. *McWilliam et al.*(1995) also reported higher frequency of restorers (21%) than that of maintainers (11%) from evaluation of 6000 testcrosses. But *Akhter et al.* (2008) reported higher frequency of maintainers (17%) than that of restorers (11 %) from 65 testcrosses. The pollen fertility per cent of hybrids were varied from 0% to 100%. Pertaining from table- 4, some genotypes namely Khandagiri, MTU 1010, CN 915, CNR 93 and CNR 102 were exhibited different reaction with different CMS lines. *Virmani et al.* (1997) also reported this kind of differential reaction of rice genotypes with different CMS lines of the same cytoplasmic source. Similar observations have been reported by *Hemareddy et al.* (2000), *Gannamani* (2001) and *Bisne and Motiramani* (2005). This variation may be due to the pollen fertility – restoring genes differ or their penetrance or expressivity differed with genotypes (*Umadevi et al.*, 2010) or due to existence of modifiers genes (*Pande et al.*, 1990). It has been also reported that restoration reaction also influenced

Table 4 : Test cross nursery

Sl. No		IR 58025A			IR 68897A			IR 80555A			IR 79156 A			IR 80559A		
		PF %	SF %	Categories	PF %	SF %	Categories	PF %	SF %	Categories	PF %	SF %	Categories	PF %	SF %	Categories
1	Gajapati	100.0	97.0	R	100.0	89.5	R	100	92.0	R	99.0	80.0	R	100.0	91.0	R
2	Khandagiri	70.0	52.8	PR	50.6	60.0	PR	56.0	60.5	PR	51.0	62.0	PR	25.6	9.8	PM
3	Pratiksha	56.5	60.8	PR	50.7	62.5	PR	60.2	68.0	PR	56.8	55.0	PR	58.0	66.0	PR
4	Sidhant	60.0	65.5	PR	55.2	40.0	PR/PM	70.0	56.0	PR	54.5	65.5	PR	69.0	51.0	PR
5	PNR 546	52.0	30.6	PR/PM	50.5	35.8	PR/PM	54.0	50.0	PR	-	-	-	-	-	-
6	MTU 1010	82.0	72.0	R/PR	80.0	65.0	R/PR	60.0	55.0	PR	56.0	51.2	PR	54.0	61.3	PR
7	Swarna Sub 1	36.0	42.0	PM	38.0	15.0	PM	15.0	8.0	PM	32.0	38.0	PM	38.5	13.9	PM
8	Swarna Dhan (IET 5656)	60.8	72.5	PR	65.0	70.0	PR	22.0	17.5	PM	65.0	51.2	PR	-	-	-
9	Basmati 370	0.0	0.0	M	1.0	0	M	0.0	0.0	M	2.5	0.0	PM/M	0.0	0.0	M
10	Nayanmoni	15.0	26.8	PM	31.5	20.0	PM	25.0	32.0	PM	22.0	8.0	PM	35.8	12.0	PM
11	Dular	70.0	56.5	PR	68.8	56.0	PR	67.0	55.0	PR	71.0	76.0	PR	72.0	57.0	PR
12	CN 1719-4	-	-	-	-	-	-	20.5	10.0	PM	0.0	0.0	M	15.0	2.0	PM
13	CN 915	1.0	4.0	M/PM	0.0	0.0	M	0.0	0.0	M	0.0	0.0	M	0.0	0.0	M
14	CNR 33	97.5	93.8	R	91.8	95.8	R	97.0	97.0	R	99	96.5	R	-	-	-
15	CNR 45	96.4	94.0	R	92.7	87.4	R	97.8	96.5	R	100	99.0	R	92.0	80.0	R
16	CNR 47	92.1	94.5	R	89.0	87.5	R	99.0	94.5	R	98.7	97.0	R	91.0	82.5	R
17	CNR 55-3	93.0	92.6	R	87.8	94.4	R	96.5	92.6	R	100	92.6	R	89.8	88.6	R
18	CNR 55-6	92.8	90.1	R	-	-	-	93.9	94.5	R	98.7	97.0	R	100.0	80.5	R
19	CNR 55-10	100.0	92.3	R	92.5	99.0	R	100.0	92.7	R	95.8	90.0	R	88.8	81.5	R
20	CNR 55-15	100.0	96.2	R	97.5	94.2	R	90.4	93.8	R	97.8	92.8	R	100.0	88.0	R
21	CNR 57	93.0	94.8	R	99.0	98.2	R	100.0	99.0	R	95.5	94.5	R	89.6	78	R
22	CNR 77	94.5	91.3	R	100.0	96.2	R	95.5	97.0	R	97.4	95	R	88.0	94.0	R
23	CNR 93	95.8	91.8	R	97.8	94.0	R	60.8	73.8	PR	60.2	74.2	PR	87.0	96.2	R
24	CNR 98	94.0	90.0	R	94.5	96.7	R	97.8	90.0	R	100	87	R	90.0	97.4	R
25	CNR 102	86.2	73.0	R/PR	92.7	89.2	R	-	-	-	90.0	68.0	R/PR	52.0	66.4	PR

Table 4: Contd.

Sl. No		PMS 3A			PMS 10A			Satabdi A			DRR 4A			COMS 15A		
		PF %	SF %	Categories	PF %	SF %	Categories	PF %	SF %	Categories	PF %	SF %	Categories	PF %	SF %	Categories
1	Gajapati	100.0	94.0	R	100.0	95.0	R	98.9	88.5	R	100.0	96.0	R	89.0	88.0	R
2	Khandagiri	38.6	15.8	PM	35.5	20.8	PM	28.9	12.5	PM	-	-	-	-	-	-
3	Pratiksha	68.7	55.9	PR	75.0	66.5	PR	60.8	58.5	PR	56.5	41.2	PR/PM	58.5	51.0	PR
4	Sidhant	67.5	51.5	PR	78.9	58.0	PR	70.0	66.0	PR	65.0	66.0	PR	70.0	50.8	PR
5	PNR 546	-	-	-	36.0	34.5	PM	42.0	36.0	PM	-	-	-	-	-	-
6	MTU 1010	77.0	55.8	PR	70.0	68.5	PR	70.0	62.0	PR	80.0	78.0	R	-	-	-
7	Swarna Sub 1	25.4	12.0	PM	40.0	19.0	PM	26.8	13.0	PM	24.8	13.5	PM	-	-	-
8	Swarna Dhan (IET 5656)	62.0	51.5	PR	74.0	68.0	PR	68.5	59.0	PR	-	-	-	55.5	51.0	PR
9	Basmati 370	0.0	0.0	M	0.0	0.0	M	0.0	0.0	M	0.0	0.0	M	0.0	0.0	M
10	Nayanmoni	34.2	13.8	PM	36.8	23.5	PM	-	-	-	23.8	9.8	PM	34.0	14.5	PM
11	Dular	56.0	66.5	PR	56.6	51.0	PR	68.0	65.0	PR	68.7	56.0	PR	65.0	60.0	PR
12	CN 1719-4	-	-	-	8.0	3.0	PM	9.0	6.0	PM	15.4	6.8	PM	8.0	5.0	PM
13	CN 915	8.0	2.1	PM	4.0	1.0	PM	-	-	-	12.0	3.0	PM	-	-	-
14	CNR 33	94.8	92.5	R	97.4	97.6	R	95.6	88	R	90.0	97.0	R	95.0	96.0	R
15	CNR 45	97.7	94.5	R	99.0	96.8	R	100.0	91.8	R	92.0	96.4	R	92.0	98.0	R
16	CNR 47	92.9	90.6	R	86.0	97.0	R	96.8	92.5	R	97.8	97.5	R	80.0	85.4	R
17	CNR 55-3	88.8	89.9	R	98.7	94.9	R	97.0	94.5	R	97.5	90.0	R	92.8	94.5	R
18	CNR 55-6	92.5	82.0	R	96.5	90.8	R	97.5	90.6	R	-	-	-	92.5	95.0	R
19	CNR 55-10	-	-	-	97.8	94.5	R	100	89.9	R	98.5	95.0	R	89.5	87.0	R
20	CNR 55-15	99.0	91.2	R	97.8	90.0	R	97.0	96.8	R	90.3	87.0	R	98.0	95.5	R
21	CNR 57	97.6	94.4	R	-	-	-	100.0	98.0	R	94.8	78.8	R	55.0	74.0	PR
22	CNR 77	94.3	92.2	R	98.0	99.6	R	100.0	89.0	R	97.8	88.0	R	-	-	-
23	CNR 93	82.0	89.8	R	98.0	96.3	R	60.0	69.5	PR	96.5	76.5	R	94.5	88.8	R
24	CNR 98	96.8	88	R	98.5	84.0	R	-	-	-	98	77.0	R	94.4	89.0	R
25	CNR 102	99.7	91.8	R	98.7	80.0	R	100.0	90.0	R	-	-	-	96.0	92.8	R

by environmental factors (Govindraj *et al.*, 1984). Virmani and Edwards (1983) reported that the restoration ability of some genotypes was found to be site specific. There were some instances in which the classification of tester based on the pollen fertility did not correlate with the classification based on the spikelet fertility. For example, PNR 546 was categorized as partial restorers by pollen fertility and as partial maintainer by spikelet fertility analysis. Some evidences were also observed for Sidhant, CN 915, MTU 1010, Pratiksha etc. The genotypes Basmati 370 was behaved complete maintainer for almost all CMS lines. CN 915 was exhibited maintainer reaction with four CMS and partial maintainer reaction with others. These genotypes can contribute to develop new maintainer lines through breeding programme. The genotypes Gajapati, CNR 33, CNR 45, CNR 47, CNR 55-3, CNR 55-6, CNR 55-10, CNR 55-15, CNR 57, CNR 77, CNR 93 and CNR 98 were exhibited restorer reactions with almost all CMS lines.

4. Conclusion

The findings of present study evident that fertility restoration reaction of the genotypes varies with genetic background of CMS lines. More emphasis should be given to utilize popular rice cultivars in hybrid rice breeding as parental lines to achieve the goal of superior hybrid with better grain quality. The identified maintainers and restorers are locally adopted. The identified maintainers namely Basmati 370 and CN 915 can be developed as a new members in CMS family by repeated back cross breeding. The identified restorer lines were Gajapati, CNR 33, CNR 45, CNR 47, CNR 55-3, CNR 55-6, CNR 55-10, CNR 55-15, CNR 57, CNR 77, CNR 93 and CNR 98. The identified restorer lines can be used as pollen parent in developing new commercial hybrid variety. New restorer may be also developed through crossing programme which can expand the genetic base of restorer by pyramiding complementary traits from diverse sources according to breeding objectives.

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6. References

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