



## RESEARCH ARTICLE

### IDENTIFICATION OF DROUGHT TOLERANT RESTORER LINES AND ASSOCIATION BETWEEN POLLEN FERTILITY AND SPIKELET FERTILITY IN CMS BASED RICE (*ORYZA SATIVA* L.) HYBRIDS

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#### ABSTRACT

Hybrid rice systems are based on three line Cytoplasmic Male Sterility (CMS) which has been an efficient tool in commercialization of hybrid rice technology. Therefore, identification of potential restorers in rice is the basic step in development of rice hybrids. In the present study, 70 drought tolerant lines were crossed with two cytoplasmic male sterility lines *viz.*, IR58025A and IR79156A and the F<sub>1</sub>s were analyzed for pollen fertility (1% I-KI Solution) and spikelet fertility. Based on the fertility restoration in F<sub>1</sub>s, 23 genotypes were classified as restorers with IR58025A and 25 genotypes were classified as restorers in IR79156A. Highly significant correlations of 0.88 and 0.93 between pollen and spikelet fertility were obtained in hybrids involving CMS line IR 58025A and CMS IR 79156A respectively. KMR 3, IR 84891-B-112-CRA-15-1 and IR79906-B-192-2-2 produced F<sub>1</sub>s with highest pollen and spikelet fertility and are considered as the most promising restorers.

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#### INTRODUCTION

Hybrid rice technology is one of the potential options for increasing rice yield. Hybrid rice varieties have shown 20-30% yield advantage over conventional inbreds (Virmani *et al.*, 2003). The procedure for developing rice hybrids is quite distinct from those employed for breeding inbred varieties. Nearly about 20 CMS sources were developed through wild species and cultivar rice varieties. (Fuji *et al.*, 2010). The cytoplasmic genetic male sterile system involving a CMS source, a maintainer and a restorer is extensively being used in the production of commercial rice hybrids (Virmani *et al.*, 1997). Development of drought tolerance rice hybrids is the immediate requirement for increasing rice production in the drought prone regions. As drought is a serious threat affecting the rice productivity, breeding for drought tolerant hybrids is one of the practical approach. IRRI Scientists (Virmani *et al.*, 2003) have also observed better adaptation of rice hybrids in aerobic rice cultivation conditions. Availability of rice hybrids for drought prone region is not significant. Most of them have been bred for irrigated condition. Thus, breeding for drought hybrids which confers grain yield advantage under drought

stress condition is one of the priority areas in rice hybrid breeding. Currently, the most popular male sterility system for commercial exploitation of hybrid rice technology is the CMS, popularly known as the three-line system. This utilizes three different lines, namely a cytoplasmic male sterile line (A line), a maintainer (B line), and a restorer (R line). The use of cytoplasmic genetic male sterility system in developing hybrids is possible when effective restorers are identified (Umadevi, *et al.*, 2012). Hybrid rice based on cytoplasmic male sterility (CMS) increases grain yield by more than 20% relative to improved inbred rice varieties (Yuan *et al.*, 1994). Cytoplasmic male sterility in rice was first reported in 1954. (Weeraratne, 1954; Sampath and Mohanthy, 1954). CMS line being exploited for commercial purpose should have a stable male sterility, adaptability to tropical rice growing conditions, good out crossing potential and should be excellent an source for the hybrid seed production (Virmani and Kumar, 2004). Identification of potential restorers in rice is the basic step in development of rice hybrids. Among the various sources of CMS systems, Wild abortive (WA) has been most commonly used for production of hybrids and most of the hybrids have been developed using WA cytoplasm source (Yao *et al.*, 1997). The restoration ability for WA system was reported to be relatively higher in indica cultivars as compared to japonica cultivars (Zhuang *et al.*, 1997). The fertility restoration ability

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for CMS-WA cytoplasm is sporophytic and is governed by two genes, one of which has a strong effect than the other (Govinda Raj and Virmani, 1988). Dominant restorer alleles *Rf3/Rf4* might be responsible for the fertility restoration in WA-CMS (Tan *et al.*, 1998). Among different WA, CMS sources IR58025A has been one of the popular and most extensively used CMS line (Xie, 2009). Sri Krishnalatha and Deepak, 2012, crossed 3 CMS lines to 6 testers and identified two parents as potential restorers and two parents as maintainers. Pollen fertility is an important criteria to identify potential restorers since it helps to know fertility of hybrids one month earlier than spikelet fertility. Umadevi *et al.*, 2012 reported pollen fertility range from 0 to 98.3% among 76 crosses and classified genotypes as maintainers, partial maintainers/restorers and restorers. The present study aims at identification of effective restorers and maintainer lines among a set of drought tolerant genotypes and association between pollen fertility and spikelet fertility in CMS based rice hybrids.

## MATERIALS AND METHODS

The experimental study was conducted at Barwale Foundation Research Farm, Hyderabad located at the latitude of 17°24'20"N longitude of 78°13'31"E and altitude of 536m on vertisol/clay loam soil having pH more than 7. The material comprises of two cytoplasmic male sterile (CMS) lines IR58025A and IR79156A with wild abortive (WA) source. 65 identified drought tolerant lines received under IIRRI- India drought breeding network project as coordinating centre and 5 commercial varieties were used as pollen parents. Seventy drought tolerant lines were crossed to two CMS lines IR58025A and IR79156A during *Kharif* 2009 and 140 F<sub>1</sub>s were obtained. The derived F<sub>1</sub>s were grown under irrigated condition and were evaluated for pollen fertility and spikelet fertility during Rabi 2009-10. Pollen fertility was studied for the 140 F<sub>1</sub>s. Three spikelets were collected randomly from each entry. The anthers from each spikelet were collected on a clean glass slide and crushed using 1% Iodine-Potassium Iodide solution (Rosamma *et al.*, 2005); the slides were examined under microscope by using 10X magnification. The pollen with deeply stained and round was considered as fertile and the pollen stained with medium brown was considered as partial fertile and the pollen with pale stain and shriveled was considered as sterile. For calculating spikelet fertility percentage six panicles were collected from each the entry and average the number of filled grains and unfilled grains per panicle were counted and expressed as per cent.

$$\text{Spikelet Fertility (SF\%)} = \frac{\text{No. of filled spikelets}}{\text{Total no. of spikelets}} \times 100$$

Based on spikelet fertility percentage in F<sub>1</sub>s, drought tolerant genotypes were classified as maintainers ( $\leq 10\%$ ), partial maintainers (11-50%), partial restorers (51-74%) and restorers ( $\geq 75\%$ ). Correlation between pollen and spikelet fertility was calculated using the standard formula in F<sub>1</sub>s involving CMS lines IR58025A and IR79156A separately.

## RESULTS AND DISCUSSION

### Spikelet fertility

Spikelet fertility in F<sub>1</sub>s ranged from 8.37% to 91.05%, when drought tolerant genotypes were crossed with CMS line IR 58025A, highest being produced by KMR 3 (91.05%) followed by IR 84891-B-112-CRA-15-1 (89.44%) and average being 59.45% (Table 1). When same set of genotypes were crossed to CMS line IR 799156A, spikelet fertility in F<sub>1</sub>s ranged from 12.1% to 88.3% highest being produced by KMR 3 (88.3%), followed by IR79906-B-192-2-2 (86.84%), and average being 58.89% (Table 1). In both the CMS lines, KMR 3 produced highest spikelet fertility in F<sub>1</sub>s suggesting that it is a very good restorer. KMR 3 is already used as male parent (R line) in developing KRH 2 rice hybrid, which is used as national check for hybrids due to its wider adaptability, higher yield and tolerance to pests and diseases. This infers that KMR3 may be having more number of fertility restoration (*Rf*) genes than other genotypes studied. Based on the earlier findings, it shows that hybrid vigour in a self pollinated crop will be yielding more than 25 percent than the best commercial entry (Swaminathan *et al.*, 1972). Increase in hybrids yield were due to increase in spikelet number per panicle (Virmani *et al.*, 1982; Ponnuthurai *et al.*, 1984), which was also confirmed by Patel *et al.* (1994) and Reddy (1996) and Yield was highly associated with spikelet fertility ( $r = 0.74^{***}$ ) (Hong, HE. and Serraj, 2012). Drought tolerant genotypes are grouped into different classes based on their ability to restore fertility in F<sub>1</sub>s when crossed to CMS lines. In case of IR 58025A CMS line, the genotypes were grouped into 4 classes, maintainer (1), partial maintainer (20), partial restorer (24) and restorer (25). When drought tolerant genotypes were crossed to CMS line IR 79156A, based on the spikelet fertility in F<sub>1</sub>s, the genotypes were grouped into three classes, i.e., partial maintainer (23), partial restorer (22) and restorer (25) (Table 2). 25 genotypes which grouped into restorer class when crossed to IR 58025A, were also grouped into restorer class, when crossed with IR 79156A. This suggests that there could be a single mechanism (same set of genes) which regulates fertility restoration in both the CMS lines.

### Pollen fertility

Pollen fertility is one of the important traits in three line heterosis breeding especially at test cross nursery stage which is a first step. Higher temperatures reduce the pollen fertility which in turn affects the spikelet fertility (Tsutomu *et al.*, 1997). Pollen fertility showed significant correlation with spikelet fertility in F<sub>2</sub> population of a CMS based hybrid KCMS 26A x IET 19886 (Naresh Babu *et al.*, 2010). Pollen fertility is a genetically controlled trait and is less influenced by environment; however spikelet fertility is influenced by environmental factors like nutrition, abiotic stresses like drought, salinity and extreme temperature (Naresh Babu *et al.*, 2010). CMS lines derived from the WA cytoplasm were found to be most stable in terms of pollen sterility (Brar *et al.*, 1998). Highest pollen fertility was observed in three genotypes; KMR 3, IR 84891-B-112-CRA-15-1, IR 82870-58 (90%) followed by IR78878-53-2-2-2 (87.5%) in F<sub>1</sub>s obtained by crossing drought tolerant genotypes with CMS line IR 58025A (Fig 1).

Table 1. Spikelet fertility and pollen fertility of F<sub>1</sub>s produced by crossing 70 drought tolerant genotypes (Male) with CMS lines IR58025A (Female) and IR79156A (Female)

S. No	Drought tolerant genotype	Source*	AFG		AUG		ATGN		ASF		APF		GRADE	
			IR58025A/IR79156A	IR58025A/IR79156A	IR58025A/IR79156A	IR58025A/IR79156A	IR58025A/IR79156A	IR58025A/IR79156A	IR58025A/IR79156A	IR58025A/IR79156A	IR58025A/IR79156A	IR58025A/IR79156A		
1	IR79906-B-192-2-1	IRRI	206.67	146.75	38.33	35.75	245.00	182.50	84.36	80.41	85.00	80.00	R	R
2	IR78878-53-2-2-2	IRRI	217.33	169.00	31.50	36.50	248.83	205.50	87.34	82.24	87.50	82.50	R	R
3	Minghui63	IRRI	178.00	157.00	32.35	31.80	210.35	188.80	84.62	83.16	85.00	80.00	R	R
4	IR80411-B-49-1	IRRI	159.75	159.75	35.75	35.25	195.50	195.00	81.71	81.92	85.00	80.00	R	R
5	IR82870-58	IRRI	131.95	141.00	31.33	39.33	163.28	180.33	80.81	78.19	90.00	80.00	R	R
6	IR78581-12-3-2-2	IRRI	162.50	159.95	38.75	41.33	201.25	201.28	80.75	79.47	85.00	80.00	R	R
7	KMR3	UASB	213.75	198.67	21.00	26.25	234.75	224.92	91.05	88.33	90.00	85.00	R	R
8	IR 84891-B-112-CRA-15-1	IRRI	180.75	166.00	21.35	39.50	202.10	205.50	89.44	80.78	90.00	85.00	R	R
9	IR62036-222-3-3-1-2R	IRRI	182.50	159.00	56.35	46.75	238.85	205.75	76.41	77.28	78.00	80.00	R	R
10	MTU9992	ANGARU	195.00	188.00	46.75	40.25	241.75	228.25	80.66	82.37	85.00	85.00	R	R
11	IR79906-B-192-2-2	IRRI	185.25	189.75	37.25	28.75	222.50	218.50	83.26	86.84	85.00	90.00	R	R
12	IR36	IRRI	148.50	129.95	35.50	34.85	184.00	164.80	80.71	78.85	80.00	80.00	R	R
13	IR 79899-B-179-2-3	IRRI	156.75	139.00	35.00	40.50	191.75	179.50	81.75	77.44	80.00	75.00	R	R
14	IR 83614-203-B	IRRI	158.25	148.70	29.30	40.30	187.55	189.00	84.38	78.68	80.00	75.00	R	R
15	IR 83614-315-B	IRRI	168.75	143.25	41.75	43.58	210.50	186.83	80.17	76.67	80.00	75.00	R	R
16	IR 83614-61-B	IRRI	163.00	150.96	37.50	35.96	200.50	186.92	81.30	80.76	80.00	80.00	R	R
17	IR 80013-B-141-4-1	IRRI	158.00	143.50	38.75	43.75	196.75	187.25	80.30	76.64	80.00	75.00	R	R
18	IR80461-B-7-1	IRRI	164.33	150.00	26.17	36.83	190.50	186.83	86.26	80.29	80.00	80.00	R	R
19	IR 79956-B-60-2-3	IRRI	147.33	133.75	35.34	39.61	182.67	173.36	80.65	77.15	77.50	78.00	R	R
20	IR 78937-B-4-B-B-B	IRRI	169.25	132.33	24.50	40.17	193.75	172.50	87.35	76.71	85.00	75.00	R	R
21	IR 83614-46-B	IRRI	179.95	151.25	36.00	45.75	215.95	197.00	83.33	76.78	82.50	75.00	R	R
22	IR78875-18-B-2-B	IRRI	148.95	176.25	45.13	39.95	194.08	216.20	76.75	81.52	78.00	82.00	R	R
23	IR80408-B-43-3	IRRI	139.95	125.75	37.95	33.50	177.90	159.25	78.67	78.96	80.00	80.00	R	R
24	IR81430-B-B-94	IRRI	168.33	154.95	39.17	28.35	207.50	183.30	81.12	84.53	85.00	80.00	R	R
25	IR79956-B-60-2-3-B	IRRI	176.25	138.35	44.00	35.95	220.25	174.30	80.02	79.37	80.00	80.00	R	R
26	IR84894-B-143-CRA-17-1	IRRI	116.50	88.50	83.75	124.75	200.25	213.25	58.18	41.50	85.00	65.00	PR	PR
27	NDR 1119	IRRI	123.25	97.35	117.00	102.75	240.25	200.10	51.30	48.65	65.00	55.00	PR	PR
28	IR84896-159-CRA-12-1	IRRI	126.00	107.50	118.67	102.33	244.67	209.83	51.50	51.23	60.00	50.00	PR	PR
29	MAS 961	UASB	141.00	126.75	59.33	58.67	200.33	185.42	70.38	68.36	70.00	70.00	PR	PR
30	IR 83614-511-B	IRRI	90.50	160.00	85.67	79.33	176.17	239.33	51.37	66.85	55.00	65.00	PR	PR
31	IR 83614-976-B	IRRI	102.83	136.00	91.83	113.33	194.66	249.33	52.83	54.55	50.00	50.00	PR	PR
32	IR 79913-B-221-B-2	IRRI	82.50	75.50	61.83	51.00	144.33	126.50	57.16	59.68	60.00	60.00	PR	PR
33	IR 83614-813-B	IRRI	99.95	126.75	45.70	67.00	145.65	193.75	68.62	65.42	70.00	70.00	PR	PR
34	IR 83614-798-B	IRRI	126.00	115.00	78.30	57.00	204.30	172.00	61.67	66.86	70.00	70.00	PR	PR
35	IR79975-B-83-4-3	IRRI	122.95	109.00	56.75	68.33	179.70	177.33	68.42	61.47	60.00	60.00	PR	PR
36	IR 83614-688-B	IRRI	122.50	136.00	87.33	92.33	209.83	228.33	58.38	59.56	60.00	60.00	PR	PR
37	IR81039-B-173-U-3-3	IRRI	118.95	133.17	59.33	51.33	178.28	184.50	66.72	72.18	30.00	70.00	PR	PR
38	IR 80508-B-57-2-B	IRRI	138.67	196.67	66.33	77.67	205.00	274.34	67.64	71.69	65.00	70.00	PR	PR
39	IR79907-B-406-B-B	IRRI	102.33	114.75	89.35	76.35	191.68	191.10	53.39	60.05	60.00	65.00	PR	PR
40	IR59682-132-1-1-2R	IRRI	95.35	84.00	90.35	69.00	185.70	153.00	51.35	54.90	55.00	60.00	PR	PR
41	IR 83614-438-B	IRRI	169.00	154.00	134.00	142.00	303.00	296.00	55.78	52.03	65.00	60.00	PR	PR
42	IR 78908-126-B-2-B	IRRI	108.67	119.00	83.75	105.00	192.42	224.00	56.48	53.13	60.00	55.00	PR	PR
43	IR 77298-14-1-2	IRRI	142.00	107.75	104.25	103.25	246.25	211.00	57.66	51.07	75.00	75.00	PR	PR
44	IR 79970-B-47-1	IRRI	98.33	71.00	79.67	74.00	178.00	145.00	55.24	48.97	60.00	50.00	PR	PM
45	IR 83614-673-B	IRRI	117.50	109.00	111.35	178.33	228.85	287.33	51.34	37.94	50.00	30.00	PR	PM
46	IR 83614-281-B	IRRI	117.33	78.83	112.33	94.45	229.66	173.28	51.09	45.49	50.00	40.00	PR	PM
47	IR84894-B-139-CRA-8-1	IRRI	153.00	89.75	86.33	95.00	239.33	184.75	63.93	48.58	55.00	45.00	PR	PM

48	IR 83614-643-B	IRRI	187.50	73.50	166.00	79.67	353.50	153.17	53.04	47.99	50.00	50.00	PR	PM
49	IR58103-62-3R	IRRI	95.50	105.33	86.50	142.33	182.00	247.66	52.47	42.53	60.00	35.00	PR	PM
50	IR 78908-105-B-2-B	IRRI	85.67	132.00	91.50	72.33	177.17	204.33	48.35	64.60	30.00	65.00	PM	PR
51	IR 70215-70-CPA-3-4-1-3	IRRI	99.33	108.35	100.50	56.75	199.83	165.10	49.71	65.63	20.00	60.00	PM	PR
52	IR84899-B-183-CRA-19-1	IRRI	74.50	89.35	96.17	45.33	170.67	134.68	43.65	66.34	45.00	70.00	PM	PR
53	MTU1010	ANGARU	49.33	127.45	98.75	67.50	148.08	194.95	33.31	65.38	50.00	65.00	PM	PR
54	IR 83614-349-B	IRRI	78.65	119.00	125.33	120.67	203.98	239.67	38.56	49.65	20.00	50.00	PM	PM
55	IR 83614-513-B	IRRI	53.33	68.00	142.33	149.00	195.66	217.00	27.26	31.34	30.00	30.00	PM	PM
56	IR 78875-131-B-1-4	IRRI	39.75	34.88	159.00	143.00	198.75	177.88	20.00	19.61	70.00	60.00	PM	PM
57	IR55419-04	IRRI	49.00	58.75	189.50	89.55	238.50	148.30	20.55	39.62	20.00	40.00	PM	PM
58	IR 78875-190-B-4-B	IRRI	39.83	32.67	135.33	237.33	175.16	270.00	22.74	12.10	30.00	30.00	PM	PM
59	IR 83614-564-B	IRRI	65.00	75.00	74.35	107.00	139.35	182.00	46.65	41.21	50.00	50.00	PM	PM
60	IR 79913-B-176-B-239-B-4	IRRI	66.75	34.00	166.75	230.00	233.50	264.00	28.59	12.88	30.00	35.00	PM	PM
61	IR 72667-16-1-B-B-3	IRRI	58.50	56.67	105.83	116.50	164.33	173.17	35.60	32.73	40.00	40.00	PM	PM
62	IR 82873-9	IRRI	65.67	76.50	118.50	157.00	184.17	233.50	35.66	32.76	40.00	40.00	PM	PM
63	IR80416-B-152-4	IRRI	49.46	73.13	125.62	132.17	175.08	205.30	28.25	35.62	35.00	40.00	PM	PM
64	IR 80021-B-86-3-4	IRRI	103.67	93.50	115.55	108.33	219.22	201.83	47.29	46.33	50.00	55.00	PM	PM
65	IR 79966-B-2-52-2	IRRI	78.63	66.00	94.33	87.67	172.96	153.67	45.46	42.95	40.00	40.00	PM	PM
66	IR74371-70-1-1	IRRI	30.00	65.50	155.00	123.75	185.00	189.25	16.22	34.61	20.00	35.00	PM	PM
67	IR 81025-B-425-B	IRRI	127.33	48.00	126.66	119.33	253.99	167.33	50.13	28.69	50.00	35.00	PM	PM
68	IR 81025-B-311-B	IRRI	144.33	87.25	196.00	188.33	340.33	275.58	42.41	31.66	40.00	35.00	PM	PM
69	IR 83614-902-B	IRRI	52.33	33.00	168.33	194.67	220.66	227.67	23.72	14.49	30.00	10.00	PM	PM
70	IR 78908-142-B-3-B	IRRI	9.00	26.00	98.50	118.00	107.50	144.00	8.37	18.06	10.00	20.00	M	PM

Note: AFG-Average Filled Grains/panicle, AUG-Average Unfilled Grains/panicle, ATGN- Average Total Grain Number/panicle, ASF-Average Spikelet Fertility, APF-Average Pollen Fertility, R-Restorer, PR- Partial Restorer, PM-Partial Maintainer, M-Maintainer. \*IRRI: International Rice Research Institute, Philippines ; UAS B: University of Agricultural Sciences, Bengaluru, India; ANGRAU: Acharya N G Ranga Agricultural University, India.

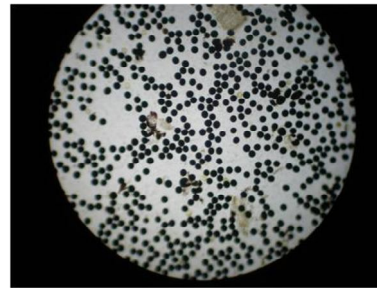


Fig. 1: IR 58025A x IR 84891-B-112-CRA-15-1 (Restorer)

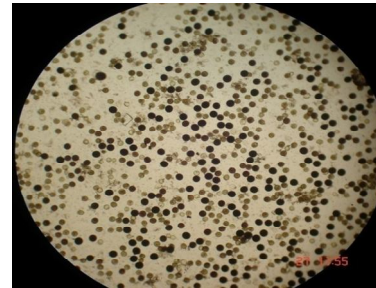


Fig. 2: IR 58025A x IR79975-B-83-4-3 (Partial Restorer)

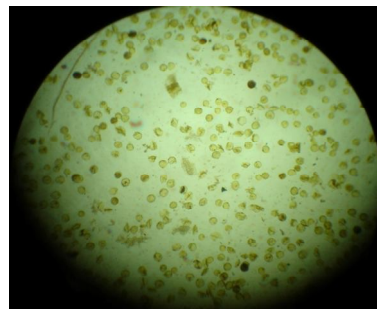


Fig. 3: IR 79156A x IR55419-04 (Partial Maintainer)

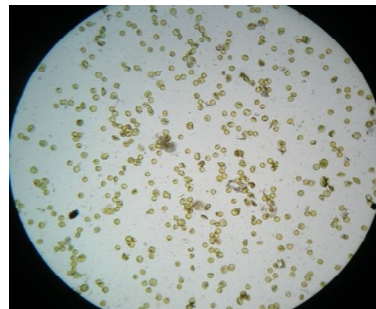


Fig. 4: IR 79156A x IR 83614-902-B (Maintainer)

Lowest pollen fertility was observed in IR 78908-142-B-3-B (10%), IR79975-B-83-4-3 (60%) as partial restorer (Fig 2) and IR 83614-349-B, IR 55419-04 and IR 70215-70-CPA-3-4-1-3 (20%) in partial maintainer category (Table 1). When genotypes were crossed to CMS line IR 79156A, highest F<sub>1</sub> pollen fertility was observed in IR79906-B-192-2-2 (90%) followed by KMR 3, IR 84891-B-112-CRA-15-1, MTU9992 (85%), and IR78878-53-2-2-2 (82.5%). IR55419-04 (40%) as partial maintainer (Fig 3), lowest pollen fertility was observed in IR 83614-902-B (10%) (Fig 4). When both the CMS lines were considered together, KMR 3 and IR 84891-B-112-CRA-15-1 produced F<sub>1</sub>s with high pollen fertility. Pollen fertility of many of the F<sub>1</sub>s in both the CMS lines was in the range of 40-60% which produced partial maintainers and partial restorers (Fig 2 & 3).

**Table 2. Grouping of 70 drought tolerant genotypes into different fertility restoration classes**

Class/Group	with IR58025A	with IR79156A
Maintainer	1	0
Partial Maintainer	20	23
Partial Restorer	24	22
Restorer	25	25
Total	70	70

**Table 3. Correlation between pollen fertility and spikelet fertility in F<sub>1</sub>s when IR 58025A and IR IR79156A were used as female parent**

Female line	Correlation between pollen and spikelet fertility
IR58025A	0.8775**
IR79156A	0.9285**

(\*\* Significant at 0.01 level of probability)

### Correlation between pollen fertility and spikelet fertility

Correlation between pollen fertility and spikelet fertility was calculated separately for the hybrids involving CMS line IR 58025A and CMS line IR 79956A. High correlation of 0.88 and 0.93 between pollen fertility and spikelet fertility was obtained in hybrids involving CMS line IR 58025A and CMS IR 79156A, respectively (Table 3). The correlations were highly significant in both the cases and this shows that pollen fertility could be used as a potential trait in identification of restorers during flowering stage. The crop was maintained under well watered condition throughout the crop growth period which in turn could be one of the reason for getting high pollen fertility and high spikelet fertility and significant correlations. In all the F<sub>1</sub>s, where ever there was high pollen fertility, high spikelet fertility was observed except the F<sub>1</sub>s with genotype IR 78875-131-B-1-4 in which high pollen fertility (70% and 60%) was observed but low spikelet fertility (20% and 19.61%) was obtained. This particular genotype may have problem of pollen germination, incompatibility with CMS lines or other post fertilization barriers. Pollen fertility is one of the important traits in three line system heterosis breeding at the initials cycles of the test cross stage. The major cause of cultivar difference was attributed to the difference in number of fertile pollen grains shed on stigma (Tsutomu *et al.*, 1997). Decreased spikelet fertility and cultivar difference at high

temperature were due mainly to decreased pollen production and pollen numbers on stigma (Prasad *et al.*, 2006). Theoretically, only one potential pollen grain is enough for fertilization and set seed, but as per the probability theory, the probability of fertilization and setting seed is more if there is more number of fertile pollen grains. Xiang *et al.* (2005) showed that spikelet fertility is highly significant and about equally correlated with embryo sac fertility ( $r^2$ 0.62) and pollen fertility ( $r^2$ 0.62).

### Conclusion

Among 70 genotypes studied, KMR 3, IR 84891-B-112-CRA-15-1 and IR79906-B-192-2-2 produced F<sub>1</sub>s with highest spikelet fertility and are considered as good restorers. KMR 3, IR 84891-B-112-CRA-15-1, IR 82870-58 and IR79906-B-192-2-2 produced F<sub>1</sub>'s with highest pollen fertility. High correlation of 0.88 and 0.93 between pollen fertility and spikelet fertility was obtained in hybrids involving CMS line IR 58025A and CMS IR 79156A respectively. Therefore pollen fertility can be considered as a parameter to identify restorers in early cycles.

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### REFERENCES

- Brar DS, Zhu YG, Ahmed MI, Jachuk PJ and Virmani SS 1998. Diversifying the CMS system to improve the sustainability of hybrid: *In*: Virmani SS, Siddiq EA, Muralidharn K. Editors Advances in Hybrid Rice Technology. Proceedings of the 3<sup>rd</sup> International Symposium on Hybrid Rice, 14-16 November 1996. Hyderabad, India. Manilla (Phillipines): *International Rice Research Institute*. p 129-146.
- Fujii S, Yamada M, Fujita M, Itabashi E, Hamada K and Yano K, 2010. Cytoplasmic – Nuclear genomic barriers in rice pollen development revealed by comparison of global gene expression profiles among five independent cytoplasmic male sterile lines. *Plant cell Physiol*, 51(4): 610-620.
- Govind Raj K and Virmani S S, 1988. Genetics of fertility restoration of 'WA' type cytoplasmic male sterility in rice. *Crop Sci*. 28:787-792.
- Hong He and Serraj R, 2012. Involvement of peduncle elongation, anther dehiscence and spikelet sterility in upland rice response to reproductive-stage drought stress. *Environmental and Experimental botany*. 75: 120-127.
- Naresh Babu N, N Shivakumar and Shailaja Hittalmani, 2010. Pollen fertility Vs Spikelet fertility in F<sub>2</sub> of a CMS based hybrids in rice (*Oryza sativa* L.) under Aerobic condition. *Electronic Journal of Plant Breeding* 1(4): 789-793.
- Patel SR, Desai NM and Kukadia MU, 1994. Heterosis for yield contributing characters in upland rice. *GAU Research J*. 20:162-163.
- Ponnuthurai S, Viramani SS and Vergara BS, 1984. Comparative studies on the growth and grain yield of some F<sub>1</sub> rice (*Oryza sativa* L.) hybrids. *Philipp. J. Crop Sci*. 9(3):183-193.

- Prasad PVV, Boote KJ, Allen LH Jr., Sheehy JE and Thomas JMG, 2006. Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. *Field Crops Research* 95: 398–411.
- Reddy JN 1996. Heterosis and inbreeding depression in short duration in rice. *Madras Agric. J.* 83: 390.
- Rosamma CA, and Vijayakumar NK, 2005. Maintainers and restorers for CMS lines of rice. *J. Trop. Agric.* 43:75–77.
- Sampath S and Mohanty HK, 1954. Cytology of semisterile rice hybrids. *Curr.Sci.* 23: 82-183.
- Sri Krishnalatha and Deepak Sharma, 2012. Identification of maintainers and restorers for WA and Kalinga sources of CMS lines in rice (*Oryza sativa* L.), *Electronic Journal of Plant Breeding*, 3(4): 949-951.
- Swaminathan, MS, EA.Siddiq and SD Sharma, 1972. Out look for hybrid rice in India. In 'Rice Breeding', IRRI, Los Banos Philippines, pp .609-13.
- Tan XL and Trangoonrang S 1998. Genetic analysis of rice CMS-WA fertility restoration based on QTL mapping *Theor. Appl.Genet.* 96: 994-999.
- Tsutomu M, Kenji O and Takeshi H, 1997. High temperature induced spikelet sterility of Japonica rice at flowering in relation to air temperature, humidity and wind velocity conditions. *Jpn. J. crop Sci.* 66(3): 449-455.
- Umadevi, M., Manonmani, S., Pushpam, R., Robin, S., Rajeswari, S. and Thiyagarajan, K., 2012. Suitability of Maintainers and Restorers for CMS Lines in Rice, *Madras Agric. J.*, 99 (4-6): 171-173.
- Virmani S.S, Mao CX and Hardy B. 2003 Hybrid Rice food security, poverty alleviation and environmental protection. Proceedings of the fourth International Symposium on Hybrid Rice, Hanoi, Vietnam, 14-17 May 2002. Los Banos (Philippines): International Rice Research Institute. pp 407.
- Virmani SS and Kumar I, 2004. Development and use of hybrid rice technology to increase rice productivity in the tropics. *IRRN.* 29: 10-19.
- Virmani SS, Aquino RC, Khush GS 1982. Heterosis breeding in rice. (*Oryza sativa* L.) *Theor. Appl. Genet.* 63:373-380.
- Virmani SS, Viraktamath BC, Casal CL, Toledo RS, Lopez MT and Manalo JO. 1997. Hybrid Rice Manual. International Rice Research Institute, Philippines. pp. 1-151.
- Weeraratne H, 1954. Hybridization technique in rice. *Trop. Agri.* 110: 93-97.
- Xiang Song, SQ Qiu, CG. Xu, XH Li and Qifa Zhang, 2005. Genetic dissection of embryo sac fertility, pollen fertility, and their contributions to spikelet fertility of inter subspecific hybrids in rice. *Theor. Appl. Genet* 110: 205–211.
- Xie F 2009. Priorities of IRRI hybrid rice breeding. In: Xie F, Hardy B (eds.) Accelerating hybrid rice development, International Rice Research Institute, Los Banos, Philippines. pp 49-61.
- Yao FY, Xu CG, Yu SB, Li JX, Gao YJ, Li XH and Zhang QF, 1997. Mapping and genetic analysis of two fertility restorer loci in the wild abortive cytoplasmic male sterility system of rice (*Oryza sativa* L). *Euphytica.* 98: 183-187.
- Yuan LP and Virmani SS, 1994. Status of hybrid rice research and development. In: Hybrid Rice, IRRI, Manila, Philippines. p. 7-24.
- Zhuang JY, Qian HR, Lu J, Lin HX and Zheng KL, 1997. RFLP variation among commercial rice germplasms in China. *J. Genet. Breed.* 51:263-268.

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