



Molecular and Phenotypic Characterization of Fertility Restorer Genes (*Rf-3*, *Rf-4*) for the Development of Rice Hybrids

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ABSTRACT

Background: In rice cultivation, the wild abortive (WA) cytoplasmic male sterile (CMS) system is predominantly utilized for producing hybrids. The fertility restoration within this system is governed by two independent dominant genes, *Rf-3* and *Rf-4*. Effective identification and utilization of these genes are crucial for enhancing hybrid rice production.

Methods: In the present study, 79 elite rice genotypes were screened for the presence of fertility restorer genes (*Rf-3* and *Rf-4*) using candidate gene-based markers. These 79 genotypes were crossed with 21 different WA-CMS lines to generate a total of 109 test cross hybrids. Pollen and spikelet fertility were studied for 109 testcrosses.

Result: Out of the 79 genotypes, 8 (10%) had a dominant functional allele for *Rf-3* (*Rf3Rf3/rf4rf4*), while 44 (56%) exhibited the dominant allele for *Rf-4* (*rf3rf3/Rf4Rf4*). Additionally, 8 (10%) were positive for both alleles (*Rf3Rf3/Rf4Rf4*) and 19 (24%) showed recessive alleles for both genes (*rf3rf3/rf4rf4*). Notably, the *Rf-4* gene was found to be more predominant than *Rf-3* in the studied material. In the testcross hybrids, pollen fertility varied significantly, ranging from 0% (completely sterile) to 100% (completely fertile), while spikelet fertility ranged from as low as 0% to as high as 93.33%. Based on spikelet fertility, the genotypes were classified, identifying 17 maintainers, 17 partial maintainers, 18 partial restorers and 37 restorers. The findings revealed differential fertility behaviour of the genotypes when crossed with different CMS lines. The identified maintainers and restorers have the potential to be utilized effectively in hybrid breeding programs for Punjab, India.

Key words: Fertility restoration, Hybrid rice, Maintainer lines, *Rf3* and *Rf4*, WA-CMS.

INTRODUCTION

In the hybrid rice system, several types of cytoplasmic male sterility (CMS) systems are known, but only a few are used for commercial hybrid seed production (Sankar Deepa *et al.*, 2019). The Wild Abortive (WA), Bao Tai (BT) and Honglian (HL) are commonly used for commercial hybrid seed production. Amongst the three, WA system is the most stable, showing complete pollen sterility and is the most commonly used CMS system (Shinjyo and Omura, 1966). The genetics and inheritance of fertility restoration (*Rf* genes), particularly in WA-CMS system has been significantly reviewed (Bharaj *et al.*, 1995; Zhang *et al.*, 1997; Govindaraj and Virmani, 1988; Yao *et al.*, 1997; Ali *et al.*, 2003). Although several restorer genes have been reported in various restorer lines, most of the time either or both dominant restorer alleles *i.e.*, *Rf-3* and *Rf-4* were suspected to be responsible for the fertility restoration in the majority of restorer lines for WA-CMS lines (Tan *et al.*, 1998). The use of CMS system in developing hybrids in crops is possible only when stable CMS lines, their effective maintainer and restorer lines with high pollen load and spikelet fertility are identified (Hasan *et al.*, 2015). The CMS lines introduced from elsewhere may not be well adapted in the target area. Therefore, it becomes imperative to identify maintainers and restorers from well adapted local germplasm for the development of component lines in a hybrid program (Rajkumar and Ibrahim, 2019). Traditionally, maintainers and restorers are identified through evaluating the pollen or spikelet fertility or both of the testcross hybrids

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(Sutaryo *et al.*, 1989). Traditional method of identifying maintainers and restorers is tedious and time consuming. Revathi *et al.* (2013) have reported efficiency of 85-92% of tightly linked markers of *Rf-3* and *Rf-4* genes in identifying maintainers and restorers. The use of molecular markers linked to *Rf* genes can enhance the selection efficiency, save time and avoid the complications associated with phenotype-based screening (Singh *et al.*, 2025).

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 a commercial hybrid breeding program. Thus, the overall objective of the study was to evaluate the efficiency of restorer genes and identify locally adapted maintainers and restorers using molecular markers and testcrossing for development of rice hybrids suitable to Punjab.

MATERIALS AND METHODS

In the present study, a set of 79 promising rice genotypes was used for molecular characterization of *Rf-3* and *Rf-4* genes. Candidate gene-based markers DRRM-RF3-10 for *Rf-3* and DRCG-RF4-14 for *Rf-4* genes (Balaji *et al.*, 2012) were used. Twenty-one different CMS lines were crossed in various combinations with 79 promising lines to generate 109 test crosses in *kharif* 2024 (Table 1) in the Rice experimental farm, Punjab Agricultural University (PAU). These test crosses were evaluated in the field along with the checks viz., PR 126, to identify potential maintainers and restorers in *kharif* 2025. The material was planted in an augmented design comprising of 8 blocks. Observation was recorded on 10 randomly selected plants for various characters such as plant height and days 50% flowering. Pollen fertility and spikelet fertility were recorded to identify maintainers and restorers. Genotypes categorized as Maintainers exhibit pollen fertility between 0-1% and spikelet fertility of less than 5%. Genotypes classified as Partial Maintainers show pollen fertility ranging from 1.1% to 50%, with spikelet fertility between 5% and 20%. In contrast, Partial Restorer genotypes achieve pollen fertility levels from 50.1% to 80% and exhibit spikelet fertility from 20% to 70%. Finally, genotypes categorized as Restorers display pollen fertility greater than 80% and spikelet fertility exceeding 70% (Virmani *et al.*, 1997; Mallikarjuna *et al.*, 2013).

RESULTS AND DISCUSSION

Screening for restorer genes (*Rf-3* and *Rf-4*) using candidate gene-based markers

The marker DRRM-RF3-10 amplified 210 bp for *Rf3* allele and 155 bp for *rf3* allele, likewise DRCG-RF4-14 amplified 800 bp for *Rf-4* allele and 900 bp for *rf4* (Fig 1 and 2). Out of 79 elite genotypes, 8 (10%) had dominant functional allele for *Rf-3* (*Rf3Rf4*), 44 (56%) had dominant allele *Rf-4* (*Rf3Rf4*), 8 (10%) positive for both the alleles (*Rf3Rf4*) and 19 (24%) had recessive allele for both genes (*rf3rf4*). The *Rf-4* genes were predominant than *Rf-3* in the studied material. The reason for this probably lies in the pedigree relationship. The parents with *rf3rf3/Rf4Rf4* genotype have been used for developing most of these lines (Katara *et al.*, 2017). *Rf4* gene on an average, showed higher efficiency in restoring male fertility as compared to *Rf3* gene. Relatively, genotypes carrying only *Rf4* when crossed with different CMS lines restored the fertility fully to partially in more than 70% testcross hybrids, while genotypes carrying only *Rf3* gene when crossed with the CMS line could restore fertility fully or partially only in 54% of the testcross hybrids. Interestingly, it was also observed that, absence of *Rf3* and *Rf4* genes did not affect the restoration ability of some lines such as in PCMS 18A/HHZ 23-DT16-DT1-DT1, PCMS 22A/IR 12L144 and PCMS 23A/ HHZ 4-SAL-12-LI1-LI1 which had spikelet fertility of 87.61%, 82.42% and 77.99% and lines like IR 62829A/NVSR-V-2057, PCMS 27A/HHZ17-DT6-SAL3-DT1 and PCMS 31A/HHZ 3-SAL 6-Y1-Y1 acted like maintainer despite the presence of *Rf*-genes indicating there might be other minor/modifier genes present in the pollinator which restores the fertility (Yadav *et al.*, 1997).

Evaluation of the test cross nursery

Based on pollen fertility and spikelet fertility, these genotypes were grouped into maintainers, partial maintainers, partial restorers and restorers. Pollen fertility

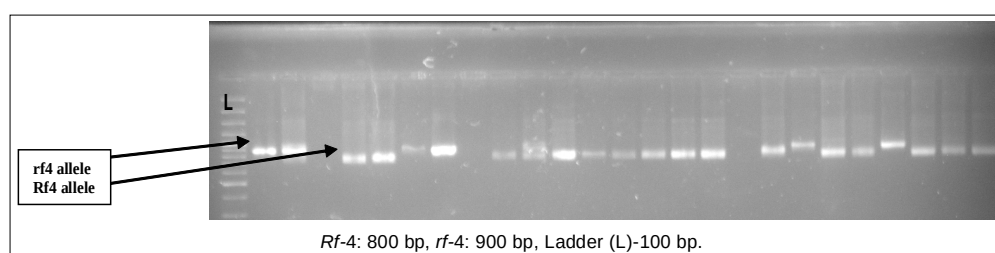


Fig 1: Representative PCR amplification of DRCG-RF4-14.

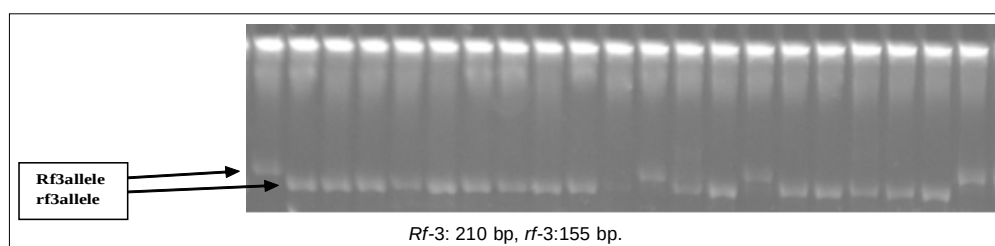


Fig 2: Representative PCR amplification of DRRM-RF3-10.

Table 1: Performance of testcross hybrids for various traits and screening for *Rf-3* and *Rf-4*.

Cross	Pollen fertility (%)	Spikelet fertility (%)	Categories based on both	Restorer genes		Standard heterosis (%) (Yield per plant)		Days to 50% flowering	Plant height (cm)	BB score
				<i>Rf-3</i>	<i>Rf-4</i>	PR 121	PR 126			
BOA /HHZ17-DT6-SAL3-DT1	22.66	0.00	PM/M	-	+	S	S	139	110	7
BOA /HUANGHUZHAN	4.12	0.00	M/M	-	+	S	S	137	100	7
CRMS 8A /2K10-23-451-21-81-5-0-0	0.00	7.68	M/PM	-	+	S	S	109	93	5
CRMS 8A /IR 75478-282-5-1	0.00	6.80	M/PM	-	-	S	S	85	90	9
CRMS 8A /IRMT 4402	0.00	6.47	M/PM	-	-	S	S	102	98	5
CRMS 8A /PAU 5216-10-1-3-1	0.00	0.00	M/M	-	-	S	S	84	77	3
CRMS 8A /Sharbati	0.00	6.56	M/PM	-	+	S	S	108	106	5
IR 62829A /NVSR-V-2057	0.00	0.00	M/M	+	+	S	S	79	90	9
IR 62829A /HHZ17-DT6-SAL3-DT1	55.47	85.46	PR/R	-	+	17.8	-7.7	84	96	9
IR 64607A /PR 121	74.07	83.77	PR/R	+	+	7.3	-15.9	100	99	5
IR 64607A /PR 123	87.74	80.83	R/R	-	+	23.7	-3.0	99	101	3
IR 68281A /PR 121	36.46	78.20	PM/PM	+	+	-22.6	-39.3	92	97	9
IR 68281A /PR 123	88.03	78.95	R/R	-	+	19.6	-6.2	94	105	9
PCMS 10A /HHZ17-DT6-SAL3-DT1	87.41	69.66	R/R	-	+	-4.3	-24.9	87	95	7
PCMS 10A /HUANGHUZHAN	50.47	60.84	PR/PR	-	+	-30.5	-45.5	88	98	7
PCMS 10A /CB 12599	83.04	67.92	R/PR	-	+	-47.7	-58.9	75	92	9
PCMS 10A /PR 124	52.78	59.95	PR/PR	-	+	-37.9	-51.4	79	90	9
PCMS 10A /RP 5940-15-13-2-1-1	64.49	36.89	PR/PR	-	-	-76.1	-81.2	78	91	7
PCMS 11A /HHZ 11-Y6-Y2-SUB1	84.62	64.00	R/PR	-	+	-49.9	-60.7	90	96	9
PCMS 11A /HHZ 1-DT4-LI1-LI1	85.31	82.27	R/R	-	+	28.2	0.5	88	93	9
PCMS 11A /HHZ 1-DT7-LI2-LI1	80.62	81.04	R/R	-	+	8.8	-14.7	87	94	9
PCMS 11A /HHZ 3-SAL13-Y1-SAL 1	94.00	80.43	R/R	-	+	7.7	-15.6	90	95	9
PCMS 11A /HHZ 6-DT1-LI1-LI1	69.53	31.94	PR/PR	-	-	-57.8	-66.9	91	96	7
PCMS 11A /IR 64 Sub 1	58.49	85.69	PR/R	-	+	37.2	7.5	108	116	9
PCMS 11A /IR 71033-4-3	42.69	59.34	PR/PR	-	-	12.2	-12.1	111	105	7
PCMS 11A /PR124	86.49	76.99	R/R	-	+	-4.7	-25.3	100	99	9
PCMS 12A /HHZ 15-SAL 13-Y1	80.23	81.75	R/R	-	+	-40.2	-53.1	63	90	9
PCMS 12A /HHZ 16-SAL 13-LI1-LI1	81.45	86.45	R/R	-	+	32.3	3.7	60	92	7
PCMS 12A /HHZ 22-Y3-DT1-Y1	32.84	87.99	PR/R	+	+	47.3	15.5	89	90	7
PCMS 12A /HHZ 24-DT11-LI1-LI1	92.31	89.23	R/R	-	+	4.4	-18.2	85	94	7
PCMS 12A /HHZ 2-SUB 2-DT1-DT1	8.55	72.79	PR/R	-	+	2.4	-19.7	110	104	9
PCMS 12A /HHZ 4-DT3-Y1-Y1	64.58	78.99	PR/R	-	+	28.2	0.5	72	93	7
PCMS 12A /HUANGHUZHAN	86.67	70.59	R/R	-	+	-17.0	-34.9	88	86	7
PCMS 12A /IRRI 123	38.53	35.12	PM/PR	+	-	-35.3	-49.3	91	89	5

Table 1: Continue...

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PCMS 12A /PR121	97.41	67.11	R/PR	+	+	35.7	6.4	101	95	5
PCMS 13A /HHZ 15 SAL 13-Y3	21.69	5.37	PM/PM	-	-	S	S	87	86	7
PCMS 13A /HHZ 3-SAL 13-Y2-DT1	50.29	72.11	PR/R	-	+	-13.2	-32.0	88	97	7
PCMS 13A /PR 121	98.00	77.98	R/R	+	+	32.7	4.0	107	97	7
PCMS 13A /PR 123	96.84	83.79	R/R	-	+	39.8	9.6	108	102	7
PCMS 15A / NPT 14-10-2	73.43	69.97	R/PR	-	-	-8.8	-28.5	110	131	7
PCMS 15A /IR 11A106	43.86	68.19	PR/PR	-	+	12.5	-11.8	110	116	7
PCMS 15A /IR 11A293	32.97	29.74	PM/PM	-	+	-77.0	-82.0	110	94	7
PCMS 15A /IR 11A534	55.21	65.25	PR/PR	-	+	0.2	-21.5	112	105	7
PCMS 15A /PR 121	55.47	75.60	PR/R	+	+	28.9	1.2	109	91	5
PCMS 16A /RP 5898-101-9-3-1-1	45.34	60.40	PR/PR	-	+	4.3	-18.2	119	96	9
PCMS 18A /HHZ 10-DT5-LI-1-LI1	91.56	83.88	R/R	-	+	16.6	-8.6	89	95	9
PCMS 18A /HHZ 15-DT7-SAL 2	71.43	67.53	PR/PR	-	+	49.5	17.2	94	97	7
PCMS 18A /HHZ 23-DT16-DT1-DT1	85.83	87.61	R/R	-	-	66.7	30.7	97	110	5
PCMS 19A /BP 11820-5F-KN-10-2	70.91	9.67	PM/PM	+	-	S	S	111	98	5
PCMS 19A /HHZ 14-SAL 13-LI2-DT1	78.29	79.91	PR/R	-	+	39.1	9.0	101	110	5
PCMS 19A /HHZ 4-SAL 12-LI1-LI1	72.97	87.60	PR/R	+	-	37.6	7.8	110	103	5
PCMS 19A /IR 04A381	84.44	70.20	R/R	+	-	45.0	13.7	114	109	7
PCMS 19A /IR 11A 476	83.95	0.00	R/M	-	-	S	S	111	112	5
PCMS 19A /IR 11A501	73.08	83.39	PR/R	-	-	41.3	10.7	110	119	7
PCMS 19A /IR 12L159	90.29	85.20	R/R	-	+	44.6	13.4	110	110	7
PCMS 19A /IRRI 123	89.92	77.56	R/R	+	-	46.2	14.6	114	114	7
PCMS 19A /Peell Pusa 1	84.88	90.20	R/R	-	+	37.2	7.5	101	107	7
PCMS 22A /BP 12816F-KN-7-1	54.97	14.63	PM/PM	-	-	-70.8	-77.1	104	108	5
PCMS 22A /HHZ17-DT6-SAL3-DT1	28.07	66.29	PR/PR	-	+	33.5	4.6	93	101	5
PCMS 22A /HUANGHUAZHAN	61.45	76.96	PR/R	-	+	98.1	55.3	92	96	5
PCMS 22A /IR 11A410	78.09	18.22	PM/PM	-	-	-64.5	-72.2	105	105	5
PCMS 22A /IR 11N187	93.22	36.99	R/PR	-	+	37.6	7.8	108	117	5
PCMS 22A /IR 11N313	69.74	72.45	PR/PR	-	+	42.8	11.9	111	112	5
PCMS 22A /IR 12L125	82.69	78.10	R/R	-	+	40.6	10.2	111	108	7
PCMS 22A /IR 12L144	85.00	82.42	R/R	-	-	48.8	16.6	108	118	7
PCMS 22A /PANT DHAN 19	86.75	69.55	R/PR	-	-	58.5	24.2	93	104	7
PCMS 23A /BP 10618F-BB8-18-BB4	82.91	93.33	R/R	-	-	-3.6	-24.4	113	117	9
PCMS 23A /HHZ 4-SAL 12-LI1-LI1	88.41	77.99	R/R	+	-	15.1	-9.7	111	121	9
PCMS 23A /IR 10F379	16.11	4.94	PM/M	-	-	S	S	113	106	7
PCMS 23A /IR 11A108	82.25	73.25	R/R	-	+	136.6	85.5	111	112	7
PCMS 23A /IR 11A316	78.87	71.81	R/R	-	+	0.2	-21.5	106	113	5
PCMS 23A /IR 11A318	33.71	85.85	PR/R	-	+	46.2	14.6	111	110	5

Table 1: Continue...

Table 1: Continue...

PCMS 23A/IR 11N169	94.44	74.03	R/R	+	+	15.9	-9.2	113	111	7
PCMS 23A/IR 11N231	90.59	77.04	R/R	+	-	36.4	6.9	110	109	7
PCMS 23A/IR11L236	14.01	0.00	PM/M	-	+	S	S	113	98	5
PCMS 23A/IRRI 102	94.71	0.00	R/PR	-	+	18.1	-7.4	111	101	5
PCMS 23A/IRRI 154	92.75	64.58	R/PR	-	+	15.5	-9.5	115	113	9
PCMS 23A/PR 124	81.98	35.93	R/PR	-	+	-49.9	-60.7	100	105	9
PCMS 24A/HHZ 14-SAL 19-Y1	0.00	2.54	M/M	-	+	S	S	74	102	9
PCMS 24A/HHZ 21-Y4-Y2-Y1	0.00	8.74	M/PM	+	+	S	S	104	109	9
PCMS 24A/IR 10M179	0.00	6.00	M/PM	-	-	-77.6	-82.4	104	98	9
PCMS 24A/IRRI 105(RC 18)	0.00	6.81	M/PM	-	+	S	S	110	103	7
PCMS 24A/R-RHZ-R56	0.00	5.85	M/PM	+	-	S	S	101	96	7
PCMS 27A/PAU 2k3-322-5-1-1-18-1-1-2-1-10-00	0.00	5.20	M/PM	-	-	S	S	84	78	5
PCMS 27A/PAU 2k3-322-5-1-1-9-1-1-1-1-1-1	0.00	3.69	M/M	-	+	S	S	83	78	5
PCMS 27A/HHZ17-DT6-SAL3-DT1	0.00	0.00	M/M	-	+	S	S	84	81	7
PCMS 27A/HUANGHUAZHAN	0.00	1.24	M/M	-	+	S	S	84	76	7
PCMS 27A/PAU 4320-21-1-3-1	0.00	0.00	M/M	-	+	S	S	84	76	7
PCMS 27A/PR 124	0.00	0.53	M/M	-	+	S	S	83	80	5
PCMS 29A/HHZ 14-SAL 19-Y1	6.84	1.66	PMM	-	+	S	S	104	99	5
PCMS 29A/HHZ 4-SAL12-LI1-LI1	3.33	0.00	PMM	+	-	S	S	100	98	9
PCMS 30A/HHZ17-DT6-SAL3-DT1	74.29	52.36	PR/PR	-	+	-32.3	-46.9	87	96	5
PCMS 31A/HHZ 21-DT7-Y1-Y1	0.00	0.00	M/M	-	+	S	S	85	88	7
PCMS 31A/HHZ 3-SAL 6-Y1-Y1	0.00	2.32	M/M	+	+	S	S	99	100	5
PCMS 31A/PR 124	0.00	2.83	M/M	-	+	S	S	84	79	7
PCMS 32A/BP 10618F-BB8-18-BB4	82.39	92.53	R/R	-	-	54.4	21.0	109	107	7
PCMS 32A/HHZ 1-DT3-Y1-Y1	80.49	85.55	R/R	-	+	25.6	-1.5	106	104	7
PCMS 32A/HHZ 4-DT3-Y1-Y1	82.35	72.16	R/R	-	+	20.4	-5.6	90	103	7
PCMS 32A/HHZ 24-DT11-LI1-LI1	71.52	6.40	PM/PM	-	+	S	S	87	91	7
PCMS 32A/HHZ 4-SAL12-LI1-LI1	54.92	70.32	PR/PR	+	-	16.6	-8.6	104	99	7
PCMS 32A/IR 10A270	41.45	86.27	PR/R	-	+	148.6	94.9	114	122	7
PCMS 32A/IR 10N389	44.90	3.56	PMM	+	-	S	S	115	103	5
PCMS 32A/IR 11A346	100.0	82.88	R/R	+	+	52.5	19.6	112	112	7
PCMS 32A/IR 11N191	95.56	84.85	R/R	-	+	27.5	-0.1	108	107	9
PCMS 32A/HHZ 15-DT7-SAL2	69.59	63.03	PR/PR	-	+	10.7	-13.3	99	100	9
PCMS 32A/PR 121	73.03	83.42	PR/R	+	+	37.6	7.8	107	100	9
PCMS 32A/IRP 5163-102-3-5-2-2	48.92	28.72	PM/PM	+	+	-63.7	-71.6	112	106	5
PCMS 32A/IRP 5940-96-7-2-1-1	0.00	2.40	M/M	-	-	S	S	112	103	7
PCMS 32A/IRP 5949-29-5-1-1-1	75.33	53.40	PR/PR	-	+	-39.1	-52.2	97	112	7
PR 121 (Check 1)	97.2	92.06	PR/R	+	+	-	-	111	92	3
PR126 (Check 2)	96.8	93.26	R/R	-	+	-	-	92	87	3
CD at 5%	-	-	-	-	-	NS	NS	6	6	-

M- Maintainer; PM- Partial maintainer, PR- Partial restorer, R- Restorer, NS- Non-significant, S- Sterile, - (absent), + (present).

ranged from 0% (completely sterile) to 100% (completely fertile), while spikelet fertility ranged from as low as 0% to as high as 93.33%. The results of pollen and spikelet fertility showed that among the testcrosses, 17 were completely sterile, 37 were completely fertile while rest showed partial fertility. It was also observed that some testcrosses with medium pollen fertility, such as IR 62829A/HHZ 17-DT6-SAL3-DT1, PCMS 12A/HHZ 22-Y3-DT1-Y1, PCMS 12A/HHZ 2-SUB 2-DT1-DT1, PCMS 13A/HHZ 15 SAL 13-Y3, PCMS 23A/IR 11A318 also showed high spikelet fertility. This could be explained as each spikelet may receive many pollens but one fertile pollen would be sufficient to fertilize the spikelet (Joshi *et al.*, 2007). Hence, for identification of restorer lines, spikelet fertility is potential trait than pollen fertility (Babu *et al.*, 2010).

An interesting observation was made on fertility restoration behaviour such as genotype HHZ17-DT6-SAL3-DT1, behaved as restorer for PCMS 10 A and IR 62829A giving spikelet fertility of 69.99% and 85.46%, respectively but as maintainer for BOA and PCMS 27A showing complete sterility in testcross hybrid, whereas PR 124 behaved as maintainer for PCMS 27A and PCMS 31A showing complete sterility and as restorer for PCMS 11A giving 76.99% spikelet fertility. The genotypes showed differential fertility behaviour with different CMS lines. The variation in behaviour of fertility restoration may be attributed to varied penetrance and expressivity of restorer genes in the different female backgrounds, as also reported in earlier studies (Wilson, 1968; Virmani *et al.*, 1997). This could also be due to the effect of the environment on the restoration ability of genotypes (Virmani and Edwards, 1983) or it may also be attributed to the presence of modifier genes (Ngangkham *et al.*, 2010). Similar results have been reported in the study conducted by Hasan *et al.* (2015) and Hariprasanna *et al.* (2005), wherein the same genotypes behaved differently for different CMS line.

Thirty-seven restorers were identified with the highest spikelet fertility of 93.33% recorded for testcross hybrid PCMS 23A/BP 10618F-BB8-18 followed by PCMS 19A/Peeli Pusa 1 with 90.20%, which were comparable with the checks. Other top performing restorers were HHZ 24-DT11-LI1-LI1 and HHZ 22-Y3-DT1-Y1 with 89.23% and 87.99% of spikelet fertility in their respective testcross PCMS 12A/HHZ 24-DT11-LI1-LI1 and PCMS 12A/HHZ 22-Y3-DT1-Y1. The identified restorers can be put into restorer improvement program by crossing with the landraces or other elite restorers and selection in advanced generation, which will lead to locally adapted restorers for hybrid rice production. The potential maintainers identified include IR 75478-282-5-1, IRMT 4402, PAU 5216-10-1-3-1, NVSR-V-2057, PR 121, IRRI 123, HHZ 15 SAL 13-Y3, HHZ17-DT6-SAL3-DT1, HHZ 14-SAL 19-Y1, R-RHZ-R56, 2k3-322-5-1-1-18-1-1-1-2-1-1, 2k3-322-5-1-1-9-1-1-1-1-1-1, HUANGHUAZHAN, PAU 4320-21-1-3-1, PR 124, HHZ 4-SAL12-LI1-LI1, HHZ 21-Y4-Y2-Y1, HHZ 3-SAL 6-Y1-Y1 and HHZ 24-DT11-LI1-

LI. These identified maintainers showed complete pollen sterility to <5% pollen fertility and 0 to <10% spikelet fertility in the respective test cross. These identified maintainers as well be put into conversion backcross breeding programs for its diversification by backcrossing with their respective test cross hybrids to develop new CMS lines. The maintainers and restorers identified can be directly used in strengthening the hybrid breeding programme in PAU for Punjab.

CONCLUSION

The comprehensive screening of promising genotypes for restorer genes *Rf-3* and *Rf-4*, combined with the evaluation of test cross nurseries, has yielded significant insights into the potential for enhancing hybrid rice production in Punjab. The results indicate a predominance of the *Rf-4* gene, which demonstrated higher efficiency in restoring male sterility compared to *Rf-3*. A total of 37 restorer lines, 17 maintainers, 17 partial maintainers and 18 partial restorers were identified. In conclusion, the findings of this study lay a robust foundation for the ongoing hybrid rice improvement program at Punjab Agricultural University (PAU) by integrating high-performing restorer and maintainer lines.

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Disclaimers

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

REFERENCES

- Ali, A.J., Mohammed, S.E.N., Rajagopalan, R. and Vijaykumar, C.H.M. (2003). Inheritance of Fertility Restoration WA Cytoplasm in Sodic Tolerant Rice Hybrids. In: *Advances in Rice Genetics. Supplement to Rice Genetics IV. Proceedings of the Fourth International Rice Genetics Symposium, 22-27 October 2000, Los Banos, Philippines.* [Khush GS, Brar DS, Hardy B (eds)], International Rice Research Institute, Manila, Philippines. pp 19-20.

- Babu, N.N., Shivakumar, N. and Hittalmani, S. (2010). Pollen fertility vs spikelet fertility in F_2 of a CMS based hybrid in rice under aerobic conditions. *Electronic Journal of Plant Breeding*. **1**: 789-793.
- Balaji, S., Srikanth, P.B., Hemanth, K.V., Subhakara, R.I., Vemireddy, L.R., Dharika, N., Sundaram, R.M., Ramesha, M.S., Sambasiva, R.K.R.S., Viraktamath, B.C. and Neeraja, C.N. (2012). Fine mapping of *Rf3* and *Rf4* fertility restorer loci of WA-CMS of rice (*Oryza sativa* L.) and validation of the developed marker system for identification of restorer lines. *Euphytica*. **187**: 421-435.
- Bharaj, T.S., Virmani, S.S. and Khush, G.S. (1995). Chromosomal location of fertility restoring genes for 'wild abortive' cytoplasmic male sterility using trisomics in rice. *Euphytica*. **83**: 169-73.
- Govindaraj, K. and Virmani, S.S. (1988). Genetics of fertility restoration of 'WA' type cytoplasmic genic male sterility in rice. *Crop Science*. **28**: 787-792.
- Joshi, B.K., Subedi, L.P., Gurung, S.B. and Sharma, R.C. (2007). Pollen and spikelet analysis in F_1 rice hybrids and their parents. *Nepal Agricultural Research Journal*. **8**: 125-131.
- Hariprasanna, K., Zaman, F.U. and Singh, A.K. (2005). Identification of versatile fertility restorer genotypes for diverse CMS lines of rice. *Oryza*. **42**: 20-26.
- Hasan, M.J., Kulsum, U., Rahman, N.M.F., Farhat, T. and Siddique, M.A. (2015). Hybrid rice parental development utilizing different rice germplasms. *Advances in Environmental Biology*. **9**: 24-29.
- Katara, L.J., Verma, R.L., Nayak, D., Ngangkham, U., Ray, S., Subudhi, H., Behera, L., Samantaray, S., Rao, R.N., Singh, O.N. and Mohapatra, T. (2017). Frequency and fertility restoration efficiency of *Rf-3* and *Rf-4* genes in Indian rice. *Plant Breeding*. **136**: 74-82.
- Mallikarjuna, B.P., Shivakumar, N., Prahalada, G.D., Devendrappa, J. and Muttanna, M.D. (2013). Study on fertility restoration of rice (*Oryza sativa* L.) hybrids. *Bioinfolet*. **10**: 647-649.
- Ngangkham, U., Parida, S.K., De, S., Kumar, K.A.R., Singh, A.K., Singh, N.K. and Mohapatra, T. (2010). Genic markers for wild abortive (WA) cytoplasm based male sterility and its fertility restoration in rice. *Molecular Breeding*. **26**: 275-292.
- Rajkumar, S. and Ibrahim, S.M. (2019). Genetic variability in CMS lines of rice (*Oryza sativa* L.) genotypes that influence outcrossing rate percentage. *Indian Journal of Agricultural Research*. **49(2)**: 165-169. doi: 10.5958/0976-058X.2015.00024.4.
- Revathi, P., Medoju, P., Singh, A.K., Sundaram, R.M., Sravan, R., Senguttuvel, P., Kemparaju, K.B., Hariprasad, A.S., Ramesha, M.S., Neeraja, C.N., Rani, N.S. and Viraktamath, B.C. (2013). Efficiency of molecular markers in identifying fertility restoration trait of WA- CMS system in rice. *Indian Journal of Genetics and Plant Breeding*. **73**: 89-93.
- Sankar Deepa, P., Subbaraman, N. and Narayanan Lakshmi, S. (2019). Heterosis, combining ability and gene action studies in TGMS based rice hybrids under normal and salt affected environments. *Indian Journal of Agricultural Research*. **42(3)**: 177-182.
- Singh, S., Singh, S.K., Korada, M., Khaire, A., Singh, D.K., Habde, S.V., Majhi, P.K. and Rai, B. (2025). Morpho-molecular diversity analysis in rice (*Oryza sativa* L.) genotypes using microsatellite markers. *Indian Journal of Agricultural Research*. **59(1)**: 15-22. doi: 10.18805/IJARE.A-5885.
- Shinjo, C. and Omura, T. (1966). Cytoplasmic-genetic male sterility in cultivated rice, I. Fertilities of F_1 , F_2 and offsprings obtained from their mutual reciprocal backcrosses and segregation of completely male sterile plants. *Japanese Journal of Breeding*. **16**: 179-180.
- Sutaryo, B. (1989). Evaluation of some F_1 rice hybrids developed using MB365A as CMS line. *International Rice Research Newsletter*. **14**: 7-8.
- Tan, X.L., Vanavichit, A., Amornsilpa, S. and Tangoonrung, S. (1998). Genetic analysis of rice CMS-WA fertility restoration based on QTL mapping. *Theoretical Applied Genetics*. **96**: 994-999.
- Virmani, S.S. and Edwards, I.B. (1983). Current status and future prospects for breeding hybrid rice and wheat. *Advances in Agronomy*. **36**: 145-214.
- Virmani, S.S., Viraktamath, B.C., Casal, C.L., Toledo, R.S., Lopez, M.T. and Manalo, J.O. (1997). Hybrid Rice Breeding Manual. International Rice Research Institute, Los Banos (Philippines). pp 155.
- Wilson, J.A. (1968). Genetics of fertility restoration of male sterility. *Euphytica (Suppl)*. **6**: 3-33.
- Yadav, S.S., Kaurya, D.M. and Yadav, D.S. (1997). Identification of restorers for some CMS in rice. *Crop Research*. **13**: 225-226.
- Yao, F.Y., Xu, C.G., Yu, S.B., Li, J.X., Gao, Y.J., Li, X.H. and Zhang, Q.F. (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.
- Zhang, G., Bharaj, T.S., Lu, Y., Virmani, S.S. and Huang, N. (1997). Mapping of *Rf-3* nuclear fertility-restoring gene for WA cytoplasm male sterility in rice using RAPD and RFLP markers. *Theoretical Applied Genetics*. **94**: 27-33.