



Identification of Potential Maintainer and Restorer Lines Using Testcross Hybrids

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ABSTRACT

Five CMS lines were crossed with 49 genotypes as 'testers' to get 245 hybrids. The 245 hybrids were subjected to pollen and spikelet fertility analysis. Among the 245 hybrids 21 hybrids were expressed as restorers, 24 as maintainers, and 200 intermediate types. Six tester's viz., BR6592-4-6-4, BR6839-41-5-1, IR74052-184-3-3, IR72593-B-3-2-2-2, BR7011-37-1-2, and IR72049-B-R-22-3-1-1 were identified as restorers for highest 2 lines out of the five CMS lines, differently. None of them were found to be restorer for all the five CMS lines. BAU581 was found to be maintainer for four lines except for D.ShanA. Purbachi was found to be maintainer for three lines except for D.ShanA and IR73328A out of the five CMS lines. Out of 245 crosses only 45 crosses contributed directly to the identification of maintainer and restorer. Other crosses were more or, less of intermediate types which indicated neither maintainer nor restorer. It is well known that, pollen fertility is controlled by one dominant gene (*R/Rf*). If pollen fertility is governed by only one gene the product would be either restorer or, maintainer where will be no existence of intermediate types. So, there might be modifier genes in different genotypes which interacted with male sterile nuclear genes that resulted in intermediate male sterility in the crosses.

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Introduction

Increased and sustainable rice production must be achieved in less land in near future due to higher population pressure on ever decreasing resources (Rothschild, 1997). So, there is no other alternative but to increase per unit production. But the yield potential of modern rice varieties reached the plateau. Under such situation, alternative approaches should be implemented for development and adoption of yield enhancing technologies. Presence of hybrid vigor and availability of pollination control mechanism such as male sterility in crop plants has been proved to be an efficient tool for obtaining higher returns in advance agricultural economy (Hawlder et al., 1997). Use of male sterility system would be appropriate approach for commercial exploitation of heterosis in rice. Though, several male sterility systems are known to occur in rice, of which cytoplasmic genetic male sterility (CMS) has been widely used for developing rice hybrids. CMS system is controlled by the interaction of cytoplasmic genes with nuclear genes (Kaul, 1997). Development of a hybrid rice using CMS requires development of a male sterile line (A-line), a maintainer line (B-line) and a restorer line (R-

line). Where, A-line is male sterile line which is used as female line in commercial hybrid production plot; B-line is isogenic line of A-line which is needed to maintain A-line; and R-line is male fertile line which is used as pollen parent in commercial seed production plot. B-line is considered as key line without which A-line cannot be maintained. Test cross programs help to identify maintainers as well as restorers. Maintainer lines are converted into new CMS lines and restorer lines are subsequently used as male parent in hybrid development program. IRRI initiated hybrid rice program in Bangladesh in late eighties through BRRI. But the program did not gain momentum. CMS lines introduced from elsewhere were not well adapted to the given target area. Successful use of hybrid vigor in rice largely depends on availability of locally developed cytoplasmic genetic male sterile (CMS) and restorer lines (Kumar et al. 1996). Julfikar et al. (2002) evaluated some Chinese CMS lines e.g. V20A and Zhen Shan97A along with their maintainer for adaptability and performance but those were not adaptable to Bangladesh condition as they become highly susceptible to disease and insects.

Moreover, breeders need wide range of CMS lines to produce desired hybrids. Therefore, it is necessary to transfer available CMS system into local elite breeding lines. So, use of local CMS line would be helpful to alleviate this problem and to develop adaptable, heterotic hybrids. Kumar et al. (1996) identified two effective maintainers for WA cytoplasmic source and successfully converted them into local lines through backcrossing. But it is not complete process in searching maintainer and restorer lines from rice elite breeding lines against different local and exotic cytosources. So, the present investigation was undertaken with the following objectives: to identify maintainers and restorers from elite lines based on test-cross pollen performance and to initiate backcrossing for conversion of maintainers into new CMS lines.

Materials and Methods

Five alien male sterile lines (IR58025A, BRR11A, D.ShanA, BRR19A and IR73328A) and 49 elite lines were grown in the field during Boro season. Three sets of elite lines were sown in 5 days interval. Similarly, four sets of CMS lines were sown in 5, 3 and 4 days interval, respectively. First set of elite lines was 5 days earlier than the first set of CMS lines. Second and third set of elite lines were sown on the same date of sowing of first and second set of CMS lines, respectively. Unit plot size for each CMS and elite lines was 2.72 m² (3.4 m × 0.8 m). Twenty one (21) days old seedlings of the tested entries were transplanted in separate strips with spacing of 20 cm between plant to plant and 20 cm between row to row. Unit plot consists of 68 plants. Adequate soil fertility was ensured by applying Urea-TSP-MP-Gypsum-ZnSO₄ at the rate of 150-100-70-60-10 kg/ha, respectively during T. Aman season. All the TSP, MP, Gypsum and ZnSO₄ were applied during final land preparation. Total Urea was applied in three installments, at 15 days after transplanting (DAT), 30 DAT, and 45 DAT. Necessary intercultural operations were carried out during the cropping period for proper growth and development of plants. For facilitating hybridization, at flowering stage, several sterile plants from CMS lines were transferred in pots which were previously filled with soil and crossed with pollens from plants of 49 elite lines (Table 1). Clipping of CMS panicles was done in the afternoon and hand pollination was carried out in the following morning by dusting pollen from selected elite lines. Adequate measures were taken to avoid undesirable pollination. The clipped and crossed panicles were tagged and bagged properly with white and brown papers, respectively. After pollination, bagging continued for 3-4 days to avoid unwanted pollination. After maturity sufficient number of spikelet from panicles of 245 crosses were collected. Properly dried and cool seeds were kept in store for further study. All F₁s along with their pollen parents were grown during both T. Aman and Boro seasons for testing pollen viability status of F₁s. Around, 20 spikelet were randomly selected which were collected from newly emerged panicle of F₁ plant in a vial containing 70% ethanol for pollen viability test. At laboratory, one drop of 1% Iodine Potassium Iodide (IKI) stain (the stain is

prepared by dissolving 1 g Iodine and 2 g Potassium iodide in 100 ml water) was put on a glass slide.

Table 1 List of elite lines used for identifying maintainer and restorer against five alien CMS lines (IR58025A, BRR11A, D.ShanA, BRR19A, and IR73328A)

Sl. No.	Genotypes	BRR1*
1.	BR368-15-2-2-3	3801
2.	BR601-1-3-3-4-2-4	3802
3.	BR5563-3-3-4-1	dhan48
4.	BR6592-4-6-4	7101
5.	BR6723-1-1-2	-
6.	BR6839-41-5-1	-
7.	BR7011-37-1-2	-
8.	BAU553	BAU
9.	BAU572	BAU
10.	BAU581	BAU
11.	IR7676-12-1-2	3750
12.	IR9202-36-3-2	3756
13.	IR25924-51-2-3	3792
14.	IR61228-3B-10-3-2	-
15.	IR72049-B-R-22-3-1-1	5917
16.	IR74052-184-3-3	-
17.	OM1490	5110
18.	BKNLR-75091-GNT-B3-RST-48-2-2	3832
19.	Moroberekan	-
20.	Hare Bare	3378
21.	Koshinihisini	3397
22.	Yamabiko	3383
23.	Sweon-287	3973
24.	Hunen Wase	3348
25.	Aokaze	3395
26.	Aki Hi Kari	3338
27.	Kogahikari	3399
28.	Chinese Variety 2	3405
29.	Chinese Variety 5	3419
30.	Chinese Variety 8	3409
31.	Purbachi	6207
32.	BR7-51(78)-3	3759
33.	BR11-54-1-1	3970
34.	BR220-1-1-1	3748
35.	BR707C-MR-137	3771
36.	BR7109-5R-2	-
37.	BR7109-5R-4	-
38.	BRR1 dhan29-SC3-27	4410
39.	IR1544-38-2-2	3971
40.	IR11248-148-3-2-33	3770
41.	IR19660-73-4-2	3968
42.	IR19661-13	3966
43.	IR69515-KKN-4-UBN-4-2-1-1	-
44.	IR72022-462-3-3-2	-
45.	IR72593-B-3-2-2-2	-
46.	IR74963-262-5-1-3-3	-
47.	IR77034-B-4-3-3	-
48.	IR9447-P1-P2-4B	3751
49.	IR9708-5-1-2	3826

* BRR1 Accession No./Variety/ Source

All anthers of 5 to 6 spikelet were then taken out with the help of forceps and placed on stain of the glass slide. The 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 status was observed under a compound microscope. The entire slide was scanned and pollen fertility was counted in three random fields. The pollen grains were classified based on

their shape, size and extent of staining. Only dark stained round pollens were classified as fertile while unstained withered, unstained spherical and light stained round were denoted as sterile. Only two characters were recorded to identify maintainers and restorers of CMS lines. Those were pollen sterility percentage and spikelet fertility percentage.

Pollen sterility (%): Sterile pollen of a plant at flowering stage was counted under compound microscope stained with 1 % IKI solution from three random foci. Percentage of sterile pollen to the total pollen under the focus was calculated.

Spikelet fertility (%): The proportion of filled grains to total spikelets of 10 or all (which had less than 10 panicles per hill) panicles was expressed in percentage and average was taken. F₁S were classified as described in Table 2, based on the extent of pollen sterility (Virmani et al., 1997).

Table 2 Pollen fertility category

Pollen sterility (%)	Category
100	Completely sterile (CS)
91-99	Sterile (S)
71-90	Partially sterile (PS)
31-70	Partially fertile (PF)
21-30	Fertile (F)
0-20	Fully fertile (FF)

Results and Discussion

Considering results of two T. Aman and two Boro season on pollen sterility and spikelet fertility percentage of F₁S were classified.

Identification of Maintainer and Restorer Lines Against IR58025A

Pollen sterility and spikelet fertility of forty nine crosses between IR58025A and 49 rice elite breeding lines were determined in T. Aman/2009 and Boro/2009-10 seasons for identification of maintainer and restorer lines from elite breeding lines of rice. Pollen sterility in T. Aman/2009 ranged from 15.73% (IR58025A × BR6839-41-5-1) to 100% (IR58025A × BAU572, IR58025A × BAU581, and IR58025A × Purbachi) and the highest spikelet fertility of the crosses against IR58025A was 74.15% (IR58025A × BKNLR-75091-GNT-B3-RST-48-2-2) in T. Aman/2009 season (Table 3). Considering the results of two seasons, three crosses might be denoted as completely sterile, eight as sterile, thirteen as partially sterile, fourteen as partially fertile, seven as fertile and four as fully fertile crosses (Table 8). The F₁S, IR58025A × BAU572, IR58025A × BAU581, and IR58025A × Purbachi showed 100 % pollen sterility and less than 5% spikelet fertility. Such results indicated that the pollen parents (BAU572, BAU581, and Purbachi) carry maintainer genes. So, BAU572, BAU581, and Purbachi might be denoted as maintainer lines against IR58025A (Table 9). Efforts could be made to convert these F₁S (IR58025A × BAU572, IR58025A × BAU581, and IR58025A × Purbachi) into local cyto-sterile lines in the background of IR58025A through backcrossing technique. In an experiment, Zaman et al. (1998) found Pusa Basmati-1 as a perfect maintainer of wild abortive

(WA) cyto-sterility from test-crosses of various Basmati lines with IR58025A. Ali et al. (2006) identified five suitable maintainer lines through test-crossing with IR58025A. On the other hand, F₁S involving BR6592-4-6-4, BR6839-41-5-1, IR74052-184-3-3 and BKNLR-75091-GNT-B3-RST-48-2-2 produced more than 80% pollen and spikelet fertility in both T. Aman/2009 and Boro/2009-10 seasons indicating that these pollen parents carry restorer genes against IR58025A cytoplasm and BR6592-4-6-4, BR6839-41-5-1, IR74052-184-3-3 and BKNLR-75091-GNT-B3-RST-48-2-2 might be denoted as restorer lines against IR58025A (Table 10). The crosses of IR58025A with restorers could be evaluated for heterosis to develop hybrid rice. Hoan et al. (1998) identified RPW20001 and DRGL30090 as two restorer lines against the CMS line MS577A. Other crosses showed variable percentage of pollen sterility and spikelet fertility indicating that involving pollen parents were identified as heterozygous for sterility or, fertility. Forty three potential maintainers and 10 effective restorers were identified from 264 testcross F₁S using 11 CMS lines including D.ShanA, IR58025A, BRR11A and BRR19A during T. Aman, 2007 (Anon, 2010). Ingale et al. (2008) also found 40 restorers and 77 maintainers among 145 genotypes for 10 CMS lines.

Identification of Maintainer and Restorer Lines Against BRR11A

Pollen sterility of 49 crosses between CMS line BRR11A and rice elite breeding lines ranged from 11.98% (BRR11A × IR72593-B-3-2-2-2) to 100% (BRR11A × BAU553, BRR11A × BAU581, BRR11A × Sweon-287, BRR11A × Aokaze, BRR11A × Aki Hi Kari, BRR11A × Kogahikari, and BRR11A × Purbachi). Likewise, spikelet fertility was ranged from 1.33% (BRR11A × Purbachi) to 83.82% (BRR11A × BR5563-3-3-4-1) in T Aman/2009 season (Table 4). Evaluating the results, seven crosses were identified as completely sterile, five as sterile, fourteen as partial sterile, thirteen as partial fertile, seven as fertile, and three as fully fertile (Table 8). Pollen parents (BAU553, BAU581, Sweon-287, Aokaze, Aki Hi Kari, Kogahikari and Purbachi) of complete sterile crosses were identified as potential maintainers against BRR11A (Table 9). The completely sterile crosses could be converted into CMS lines with background of BRR11A cytoplasm by repeated backcrossing with respective pollen parents as recurrent parent. Male parents (BR5563-3-3-4-1, IR72593-B-3-2-2-2, and IR9708-5-1-2) of full fertile crosses were identified as restorers against BRR11A (Table 10). Forty three potential maintainers and 10 effective restorers were identified from 264 testcross F₁S using 11 CMS lines including D.ShanA, IR58025A, BRR11A, and BRR19A during T. Aman/2007 (Anon, 2010). Again, 2 potential maintainers and 4 restorers were identified from 213 testcrosses using 59 CMS lines including D.ShanA, IR73328A, BRR11A and BRR19A during Boro, 2007-08 (Anon, 2010). Borkakati and Chetia (2000) found Satava as a potential maintainer for IR62829A. Singh et al. (2000) identified Sarjoo52, Saket4, IR24, and Narendra118 as restorers against IR62829A.

Table 3 Sterility maintenance and fertility restoration ability of 49 elite lines against alien CMS line, IR58025A

Sl. No.	Pollen parents	Pollen sterility (%) of F ₁		Spikelet fertility (%) of F ₁		Status	Remarks
		T.Aman/09	Boro/09-10	T Aman/09	Boro/09-10		
1.	BR368-15-2-2-3	93.07	91.32	6.79	8.75	S	
2.	BR601-1-3-3-4-2-4	82.32	79.65	22.63	27.95	PS	
3.	BR5563-3-3-4-1 (BRRI dhan48)	95.48	93.22	8.50	11.29	S	
4.	BR6592-4-6-4 (BRRI dhan49)	17.03	14.75	81.37	84.30	FF	Restorer
5.	BR6723-1-1-2	24.95	22.72	54.30	63.97	F	
6.	BR6839-41-5-1	15.73	12.67	80.89	83.35	FF	Restorer
7.	BR7011-37-1-2	26.02	23.79	57.30	64.20	F	
8.	BAU553	70.00	67.83	49.10	57.89	PF	
9.	BAU572	100.00	100.00	1.90	3.45	CS	Maintainer
10.	BAU581	100.00	100.00	2.53	4.95	CS	Maintainer
11.	IR7676-12-1-2	57.49	53.73	42.87	51.21	PF	
12.	IR9202-36-3-2	80.32	75.42	20.50	24.25	PS	
13.	IR25924-51-2-3	47.92	44.75	38.21	46.25	PF	
14.	IR61228-3B-10-3-2	81.64	78.53	21.39	29.72	PS	
15.	IR72049-B-R-22-3-1-1	65.47	60.36	46.63	55.10	PF	
16.	IR74052-184-3-3	18.09	15.73	82.98	85.69	FF	Restorer
17.	OM1490	27.93	23.47	58.07	64.23	F	
18.	BKNLR-75091-GNT-B3-RST-48-2-2	18.78	16.05	83.15	86.91	FF	Restorer
19.	Moroberekan	29.30	26.72	60.12	68.85	F	
20.	Hare Bare	94.82	92.35	6.84	9.75	S	
21.	Koshinihisini	68.75	65.93	47.30	56.86	PF	
22.	Yamabiko	87.92	84.78	27.17	32.97	PS	
23.	Sweon-287	95.79	92.37	7.25	9.32	S	
24.	Hunen Wase	97.33	95.45	10.04	13.07	S	
25.	Aokaze	89.52	87.09	28.38	37.82	PS	
26.	Aki Hi Kari	88.02	86.25	28.10	36.98	PS	
27.	Kogahikari	93.10	91.39	5.67	8.13	S	
28.	Chinese Variety 2	95.07	92.48	8.12	9.30	S	
29.	Chinese Variety 5	87.45	85.30	26.87	33.89	PS	
30.	Chinese Variety 8	96.82	94.50	9.05	11.27	S	
31.	Purbachi	100.00	100.00	2.35	4.05	CS	Maintainer
32.	BR7-51(78)-3	49.59	44.30	38.50	46.93	PF	
33.	BR11-54-1-1	82.40	78.25	22.63	27.98	PS	
34.	BR220-1-1-1	59.95	55.47	43.95	51.72	PF	
35.	BR707C-MR-137	86.42	82.30	25.07	31.13	PS	
36.	BR7109-5R-2	64.97	60.10	45.63	44.65	PF	
37.	BR7109-5R-4	68.72	65.70	47.48	56.80	PF	
38.	BRRI dhan29-SC3-27	59.78	55.80	43.95	52.79	PF	
39.	IR1544-38-2-2	81.20	76.35	20.87	24.95	PS	
40.	IR11248-148-3-2-33	62.73	58.42	45.43	53.75	PF	
41.	IR19660-73-4-2	79.20	75.35	19.97	23.45	PS	
42.	IR19661-13	49.52	45.30	38.77	47.50	PF	
43.	IR69515-KKN-4-UBN-4-2-1-1	27.33	24.20	58.35	67.50	F	
44.	IR72022-462-3-3-2	79.73	75.47	20.09	24.73	PS	
45.	IR72593-B-3-2-2-2	29.32	26.47	60.36	68.90	F	
46.	IR74963-262-5-1-3-3	86.37	83.19	25.62	31.13	PS	
47.	IR77034-B-4-3-3	59.47	55.32	43.08	51.75	PF	
48.	IR9447-P1-P2-4B	69.75	67.22	48.93	57.85	PF	
49.	IR9708-5-1-2	28.30	26.27	59.63	67.50	F	

CS = Completely sterile, S = Sterile, PS = Partially sterile, F = Fertile, and FF = Fully fertile

Identification of Maintainer and Restorer Lines Against D.Shana

Among crosses between D.ShanA and 49 local and exotic rice elite breeding lines the highest (100%) pollen sterility was observed in D.ShanA × BAU553, D.ShanA × Hare Bare, D.ShanA × Koshinihisini, D.ShanA × Yamabiko, D.ShanA × Aokaze, D.ShanA × Aki Hi Kari, and D.ShanA × Kogahikari and the lowest was 14.79 % (D.ShanA × BR368-15-2-2-3) in T. Aman/2009 season. On the other hand, spikelet fertility was the lowest in D.ShanA × Aki Hi Kari (1.34 %) and the highest in D.ShanA × BAU581 (82.50 %) in T. Aman/2009 season (Table 5). After compilation of the results, it appeared

that seven crosses were completely sterile, four crosses sterile, twelve crosses partial sterile, twelve crosses partial fertile, six crosses fertile and eight crosses were fully fertile (Table 8). The pollen parents (BAU553, Hare Bare, Koshinihisini, Yamabiko, Aokaze, Aki Hi Kari, and Kogahikari) of complete sterile crosses might be maintainers against D.ShanA (Table 9) The pollen parent (BR368-15-2-2-3, BR6592-4-6-4, BR6723-1-1-2, BR6839-41-5-1, BR7011-37-1-2, IR9202-36-3-2, IR72049-B-R-22-3-1-1, and IR72593-B-3-2-2-2) of fully fertile cross might be denoted as restorer against D.ShanA (Table 10).

Table 4 Sterility maintenance and fertility restoration ability of 49 elite lines against alien CMS line, BRR1A

Sl. No.	Pollen parents	Pollen sterility (%) of F ₁		Spikelet fertility (%) of F ₁		Status	Remarks
		T.Aman/09	Boro/09-10	T.Aman/09	Boro/09-10		
1.	BR368-15-2-2-3	69.78	65.32	47.83	56.01	PF	
2.	BR601-1-3-3-4-2-4	81.22	78.07	20.50	27.16	PS	
3.	BR5563-3-3-4-1 (BRR1 dhan48)	18.38	15.73	83.82	85.50	FF	Restorer
4.	BR6592-4-6-4 (BRR1 dhan49)	25.03	22.49	55.27	64.45	F	
5.	BR6723-1-1-2	96.47	93.21	8.20	10.79	S	
6.	BR6839-41-5-1	26.37	22.44	56.12	64.25	F	
7.	BR7011-37-1-2	79.25	76.09	20.54	24.90	PS	
8.	BAU553	100.00	100.00	2.75	4.36	CS	Maintainer
9.	BAU572	86.20	83.82	25.70	32.19	PS	
10.	BAU581	100.00	100.00	1.98	3.75	CS	Maintainer
11.	IR7676-12-1-2	78.95	75.32	19.30	23.25	PS	
12.	IR9202-36-3-2	89.21	86.07	28.45	34.19	PS	
13.	IR25924-51-2-3	84.44	81.25	23.89	29.50	PS	
14.	IR61228-3B-10-3-2	47.09	44.32	37.20	47.07	PF	
15.	IR72049-B-R-22-3-1-1	24.49	21.25	54.29	63.54	F	
16.	IR74052-184-3-3	39.07	35.62	33.95	42.51	PF	
17.	OM1490	26.74	23.70	55.83	65.28	F	
18.	BKNLR-75091-GNT-B3-RST-48-2-2	44.92	40.07	37.21	45.20	PF	
19.	Moroberekan	30.00	27.32	61.12	71.75	F	
20.	Hare Bare	95.60	91.30	8.03	10.13	S	
21.	Koshinihisini	98.25	94.68	10.41	12.39	S	
22.	Yamabiko	97.72	93.57	9.10	11.50	S	
23.	Sweon-287	100.00	100.00	1.39	3.78	CS	Maintainer
24.	Hunen Wase	75.38	72.85	16.25	20.63	PS	
25.	Aokaze	100.00	100.00	2.75	4.56	CS	Maintainer
26.	Aki Hi Kari	100.00	100.00	1.95	4.27	CS	Maintainer
27.	Kogahikari	100.00	100.00	2.10	3.85	CS	Maintainer
28.	Chinese Variety 2	86.20	83.82	25.70	32.19	PS	
29.	Chinese Variety 5	94.79	91.30	8.35	10.12	S	
30.	Chinese Variety 8	87.99	85.35	27.10	38.25	PS	
31.	Purbachi	100.00	100.00	1.33	3.57	CS	Maintainer
32.	BR7-51(78)-3	41.53	38.93	34.75	43.79	PF	
33.	BR11-54-1-1	49.37	45.04	39.50	47.30	PF	
34.	BR220-1-1-1	56.79	53.07	41.87	51.20	PF	
35.	BR707C-MR-137	79.27	76.23	19.72	24.25	PS	
36.	BR7109-5R-2	86.98	83.07	25.75	31.82	PS	
37.	BR7109-5R-4	47.92	44.63	37.26	46.50	PF	
38.	BRR1 dhan29-SC3-27	56.87	52.29	41.87	52.10	PF	
39.	IR1544-38-2-2	80.09	76.31	20.65	24.09	PS	
40.	IR11248-148-3-2-33	81.26	77.25	21.37	25.25	PS	
41.	IR19660-73-4-2	26.79	23.21	56.21	64.23	F	
42.	IR19661-13	68.42	65.35	47.08	56.10	PF	
43.	IR69515-KKN-4-UBN-4-2-1-1	61.73	58.21	45.01	53.27	PF	
44.	IR72022-462-3-3-2	26.25	23.47	55.92	64.25	F	
45.	IR72593-B-3-2-2-2	14.56	11.98	82.75	84.39	FF	Restorer
46.	IR74963-262-5-1-3-3	41.97	38.21	35.74	44.13	PF	
47.	IR77034-B-4-3-3	82.46	79.47	21.80	27.58	PS	
48.	IR9447-P1-P2-4B	65.96	62.10	46.63	56.10	PF	
49.	IR9708-5-1-2	15.79	13.75	80.75	82.50	FF	Restorer

CS = Completely sterile, S = Sterile, PS = Partially sterile, F = Fertile, and FF = Fully fertile

Forty three potential maintainers and 10 effective restorers were identified from 264 testcross F₁s using 11 CMS lines including D.ShanA, IR58025A, BRR1A and BRR1A during T. Aman, 2007 (Anon, 2010). Again, 2 potential maintainers and 4 restorers were identified from 213 testcrosses using 59 CMS lines including D.ShanA, IR73328A, BRR1A and BRR1A during Boro/2007-08 (Anon, 2010). Jayamani et al. (1998) found F₁ of V20A × Dular as completely sterile and used for subsequent backcrossing up to BC₆ generation. Singh (2005) identified 41 potential restorers and 11 effective maintainers from 75 F₁s against different CMS lines.

Identification of Maintainer and Restorer Lines Against BRR1A

Among crosses between BRR1A and 49 rice elite breeding lines, the highest (100%) pollen sterility was observed in BRR1A × BAU581, BRR1A × Chinese Variety 5, and BRR1A × Purbachi and the lowest was 15.47% (BRR1A × IR25924-51-2-3) in T. Aman/2009 season. On the other hand, spikelet fertility was the lowest in BRR1A × BAU581 (1.35%) and the highest in BRR1A × Moroberekan (83.80%) in T. Aman/2009 season (Table 6).

Table 5 Sterility maintenance and fertility restoration ability of 49 elite lines against alien CMS line, D.ShanA

Sl. No.	Pollen parents	Pollen sterility (%) of F ₁		Spikelet fertility (%) of F ₁		Status	Remarks
		T.Aman/09	Boro/09-10	T.Aman/09	Boro/09-10		
1.	BR368-15-2-2-3	14.79	12.78	80.25	83.31	FF	Restorer
2.	BR601-1-3-3-4-2-4	58.74	55.32	42.97	52.79	PF	
3.	BR5563-3-3-4-1 (BRR1 dhan48)	61.23	67.09	45.02	58.20	PF	
4.	BR6592-4-6-4 (BRR1 dhan49)	16.03	14.09	80.35	83.59	FF	Restorer
5.	BR6723-1-1-2	19.50	16.78	81.14	84.71	FF	Restorer
6.	BR6839-41-5-1	17.35	15.09	80.05	83.98	FF	Restorer
7.	BR7011-37-1-2	19.03	17.03	81.90	85.40	FF	Restorer
8.	BAU553	100.00	100.00	1.49	3.02	CS	Maintainer
9.	BAU572	88.95	86.20	27.34	34.75	PS	
10.	BAU581	87.95	84.52	27.20	32.20	PS	
11.	IR7676-12-1-2	69.75	65.20	48.71	57.50	PF	
12.	IR9202-36-3-2	17.39	15.37	80.80	83.74	FF	Restorer
13.	IR25924-51-2-3	25.09	22.76	55.67	64.25	F	
14.	IR61228-3B-10-3-2	29.53	27.20	60.14	70.75	F	
15.	IR72049-B-R-22-3-1-1	16.07	13.45	80.08	83.25	FF	Restorer
16.	IR74052-184-3-3	27.33	25.37	58.10	67.50	F	
17.	OM1490	49.47	44.35	38.17	46.06	PF	
18.	BKNLR-75091-GNT-B3-RST-48-2-2	28.74	26.30	28.10	68.93	F	
19.	Moroberekan	29.32	24.75	29.57	66.24	F	
20.	Hare Bare	100.00	100.00	1.37	2.95	CS	Maintainer
21.	Koshinihisini	100.00	100.00	2.09	4.67	CS	Maintainer
22.	Yamabiko	100.00	100.00	1.98	3.49	CS	Maintainer
23.	Sweon-287	97.43	95.83	10.10	12.70	S	
24.	Hunen Wase	88.95	86.20	27.34	34.75	PS	
25.	Aokaze	100.00	100.00	2.47	5.09	CS	Maintainer
26.	Aki Hi Kari	100.00	100.00	1.34	3.29	CS	Maintainer
27.	Kogahikari	100.00	100.00	2.33	4.87	CS	Maintainer
28.	Chinese Variety 2	87.09	85.38	27.26	33.80	PS	
29.	Chinese Variety 5	94.30	92.45	6.78	9.30	S	
30.	Chinese Variety 8	95.92	94.38	7.25	10.10	S	
31.	Purbachi	98.75	96.87	10.26	13.89	S	
32.	BR7-51(78)-3	76.78	72.30	17.97	20.63	PS	
33.	BR11-54-1-1	62.09	56.70	45.30	53.50	PF	
34.	BR220-1-1-1	53.23	48.20	41.71	48.20	PF	
35.	BR707C-MR-137	86.27	82.49	27.03	32.37	PS	
36.	BR7109-5R-2	61.20	58.30	44.95	53.20	PF	
37.	BR7109-5R-4	86.27	83.92	25.87	32.17	PS	
38.	BRR1 dhan29-SC3-27	54.82	50.37	40.87	50.12	PF	
39.	IR1544-38-2-2	87.95	84.52	27.20	32.20	PS	
40.	IR11248-148-3-2-33	81.76	78.22	21.50	27.15	PS	
41.	IR19660-73-4-2	60.07	56.30	44.35	53.25	PF	
42.	IR19661-13	24.95	21.27	55.60	63.89	F	
43.	IR69515-KKN-4-UBN-4-2-1-1	79.97	75.60	19.80	23.25	PS	
44.	IR72022-462-3-3-2	49.49	45.30	39.05	47.50	PF	
45.	IR72593-B-3-2-2-2	18.47	15.03	81.26	84.50	FF	Restorer
46.	IR74963-262-5-1-3-3	69.03	66.20	48.20	58.10	PF	
47.	IR77034-B-4-3-3	81.23	76.95	21.09	24.58	PS	
48.	IR9447-P1-P2-4B	85.69	82.30	24.93	30.10	PS	
49.	IR9708-5-1-2	47.93	43.78	38.85	46.05	PF	

CS = Completely sterile, S = Sterile, PS = Partially sterile, F = Fertile, and FF = Fully fertile

After compilation of the results, it appeared that three crosses were completely sterile, seven crosses sterile, nine crosses partial sterile, nineteen crosses partial fertile, six crosses fertile and five crosses were fully fertile (Table 8). The pollen parents (BAU581, Chinese Variety 5 and Purbachi) of complete sterile crosses might be maintainers against BRR19A (Table 9). The pollen parent (IR25924-51-2-3, IR72049-B-R-22-3-1-1, IR74052-184-3-3, OM1490, and Moroberekan) of fully fertile cross might be denoted as restorer against BRR19A (Table 10). Forty three potential maintainers and 10 effective restorers were identified from 264 testcross F₁s using 11

CMS lines including D.ShanA, IR58025A, BRR11A, and BRR19A during T. Aman/2007 (Anon, 2010). Again, 2 potential maintainers and 4 restorers were identified from 213 testcrosses using 59 CMS lines including D.ShanA, IR73328A, BRR11A, and BRR19A during Boro/2007-08 (Anon., 2010). Jayamani et al. (1998) found F₁ of V20A × Dular as completely sterile and used for subsequent backcrossing up to BC₆ generation. Umadevi et al. (2010) identified 10 restorers from 248 testcross hybrids made by crossing 8 CMS lines with 31 genotypes.

Table 6 Sterility maintenance and fertility restoration ability of 49 elite lines against alien CMS line, BRRI9A

Sl. No.	Pollen parents	Pollen sterility (%) of F ₁		Spikelet fertility (%) of F ₁		Status	Remarks
		T.Aman/09	Boro/09-10	T.man/09	Boro/09-10		
1.	BR368-15-2-2-3	49.09	44.75	39.72	50.60	PF	
2.	BR601-1-3-3-4-2-4	79.22	75.37	21.93	28.78	PS	
3.	BR5563-3-3-4-1 (BRRI dhan48)	96.57	93.49	8.56	12.03	S	
4.	BR6592-4-6-4 (BRRI dhan49)	25.39	22.57	54.74	66.07	F	
5.	BR6723-1-1-2	96.45	93.21	7.98	10.54	S	
6.	BR6839-41-5-1	49.30	45.92	40.17	49.35	PF	
7.	BR7011-37-1-2	59.27	55.03	43.28	52.87	PF	
8.	BAU553	65.79	62.13	46.39	57.23	PF	
9.	BAU572	68.90	63.80	48.08	58.07	PF	
10.	BAU581	100.00	100.00	1.35	2.89	CS	Maintainer
11.	IR7676-12-1-2	49.07	44.79	40.30	47.93	PF	
12.	IR9202-36-3-2	64.09	61.20	46.74	53.89	PF	
13.	IR25924-51-2-3	15.47	12.38	80.65	83.78	FF	Restorer
14.	IR61228-3B-10-3-2	59.61	55.30	44.31	52.39	PF	
15.	IR72049-B-R-22-3-1-1	16.89	13.72	81.07	84.75	FF	Restorer
16.	IR74052-184-3-3	17.76	15.85	82.10	85.87	FF	Restorer
17.	OM1490	16.75	14.78	82.85	85.02	FF	Restorer
18.	BKNLR-75091-GNT-B3-RST-48-2-2	26.09	23.18	56.25	66.74	F	
19.	Moroberekan	18.25	16.09	83.80	86.20	FF	Restorer
20.	Hare Bare	89.75	87.73	28.50	36.51	PS	
21.	Koshinihisini	95.86	93.38	7.96	12.69	S	
22.	Yamabiko	94.52	91.26	6.72	10.83	S	
23.	Sweon-287	87.58	85.47	26.85	34.62	PS	
24.	Hunen Wase	86.92	84.35	25.10	33.07	PS	
25.	Aokaze	95.03	92.97	7.89	11.52	S	
26.	Aki Hi Kari	96.45	93.87	8.12	12.07	S	
27.	Kogahikari	95.30	91.42	7.82	11.51	S	
28.	Chinese Variety 2	68.24	65.15	47.20	56.39	PF	
29.	Chinese Variety 5	100.00	100.00	2.15	4.02	CS	Maintainer
30.	Chinese Variety 8	89.20	85.92	27.92	32.10	PS	
31.	Purbachi	100.00	100.00	1.97	3.87	CS	Maintainer
32.	BR7-51(78)-3	49.23	45.60	40.12	48.59	PF	
33.	BR11-54-1-1	59.07	56.21	42.67	51.33	PF	
34.	BR220-1-1-1	65.34	62.13	45.89	55.10	PF	
35.	BR707C-MR-137	62.44	59.25	43.10	53.79	PF	
36.	BR7109-5R-2	69.35	67.22	48.30	58.18	PF	
37.	BR7109-5R-4	39.74	35.24	34.75	42.27	PF	
38.	BRRI dhan29-SC3-27	29.37	25.66	60.29	67.50	F	
39.	IR1544-38-2-2	77.49	73.20	18.72	22.85	PS	
40.	IR11248-148-3-2-33	89.94	85.74	28.79	33.75	PS	
41.	IR19660-73-4-2	76.22	73.21	17.25	23.25	PS	
42.	IR19661-13	58.47	55.30	43.03	52.97	PF	
43.	IR69515-KKN-4-UBN-4-2-1-1	70.00	68.10	49.01	58.23	PF	
44.	IR72022-462-3-3-2	27.95	25.67	57.45	67.50	F	
45.	IR72593-B-3-2-2-2	25.07	21.45	55.87	63.28	F	
46.	IR74963-262-5-1-3-3	76.75	73.21	16.25	23.76	PS	
47.	IR77034-B-4-3-3	66.27	63.97	45.67	56.30	PF	
48.	IR9447-P1-P2-4B	27.10	23.09	58.25	65.89	F	
49.	IR9708-5-1-2	39.57	35.30	34.73	43.70	PF	

CS = Completely sterile, S = Sterile, PS = Partially sterile, F = Fertile, and FF = Fully fertile

Identification of Maintainer and Restorer Lines Against IR73328A

Among crosses between IR73328A and 49 local and exotic rice elite breeding lines, the highest (100%) pollen sterility was observed in IR73328A × BAU553, IR73328A × BAU572, IR73328A × BAU581, and IR73328A × Chinese Variety 5 and the lowest was 19.72% (IR73328A × BR7011-37-1-2) in T. Aman/2009 season. On the other hand, spikelet fertility was the lowest in IR73328A × BAU572 (1.53%) and the highest in IR73328A × BR7011-37-1-2 (80.92%) in T. Aman/2009 season (Table 7). After compilation of the results, it

appeared that four crosses were completely sterile, six crosses sterile, eleven crosses partial sterile, seventeen crosses partial fertile, ten crosses fertile and a single cross was fully fertile (Table 8). The pollen parents (BAU553, BAU572, BAU581, and Chinese Variety 5) of complete sterile crosses might be maintainers against IR73328A (Table 9). The pollen parent (BR7011-37-1-2) of fully fertile cross might be denoted as restorer against IR73328A (Table 10). Two potential maintainers and 4 restorers were identified from 213 testcrosses using 59 CMS lines including D.ShanA, IR73328A, BRRI1A, and BRRI9A during Boro, 2007-08 (Anon, 2010).

Table 7 Sterility maintenance and fertility restoration ability of 49 elite lines against alien CMS line, IR73328A

Sl. No.	Pollen parents	Pollen sterility (%) of F ₁		Spikelet fertility (%) of F ₁		Status	Remarks
		T.Aman/09	Boro/09-10	T.Aman/09	Boro/09-10		
1.	BR368-15-2-2-3	24.75	21.30	50.12	65.53	F	
2.	BR601-1-3-3-4-2-4	59.34	55.60	32.95	52.78	PF	
3.	BR5563-3-3-4-1 (BRR1 dhan48)	28.90	26.70	60.12	73.50	F	
4.	BR6592-4-6-4 (BRR1 dhan49)	65.09	62.10	33.87	54.17	PF	
5.	BR6723-1-1-2	69.95	67.10	40.12	60.21	PF	
6.	BR6839-41-5-1	59.07	54.20	39.71	55.08	PF	
7.	BR7011-37-1-2	19.72	17.39	80.92	84.20	FF	Restorer
8.	BAU553	100.00	100.00	1.74	3.45	CS	Maintainer
9.	BAU572	100.00	100.00	1.53	4.75	CS	Maintainer
10.	BAU581	100.00	100.00	2.17	4.32	CS	Maintainer
11.	IR7676-12-1-2	60.10	56.70	46.78	55.20	PF	
12.	IR9202-36-3-2	25.07	22.30	57.12	71.85	F	
13.	IR25924-51-2-3	29.30	25.65	60.98	73.12	F	
14.	IR61228-3B-10-3-2	80.30	75.95	24.57	30.13	PS	
15.	IR72049-B-R-22-3-1-1	29.75	27.10	61.00	73.10	F	
16.	IR74052-184-3-3	27.10	23.25	58.29	70.52	F	
17.	OM1490	23.79	21.15	55.67	67.32	F	
18.	BKNLR-75091-GNT-B3-RST-48-2-2	50.10	46.20	41.98	50.91	PF	
19.	Moroberekan	26.07	23.10	55.32	65.78	F	
20.	Hare Bare	78.87	75.83	20.19	29.38	PS	
21.	Koshinihisini	96.89	92.09	8.53	15.45	S	
22.	Yamabiko	87.08	83.25	23.74	32.97	PS	
23.	Sweon-287	95.42	92.07	7.97	14.92	S	
24.	Hunen Wase	81.38	78.26	21.08	32.97	PS	
25.	Aokaze	97.96	93.67	9.12	14.25	S	
26.	Aki Hi Kari	88.90	86.23	27.12	36.57	PS	
27.	Kogahikari	94.70	92.35	7.95	12.57	S	
28.	Chinese Variety 2	86.79	83.40	25.32	33.12	PS	
29.	Chinese Variety 5	100.00	100.00	1.57	2.95	CS	Maintainer
30.	Chinese Variety 8	94.57	91.23	7.89	12.12	S	
31.	Purbachi	96.75	93.60	6.78	10.34	S	
32.	BR7-51(78)-3	59.47	55.30	43.17	51.20	PF	
33.	BR11-54-1-1	83.75	80.04	19.92	31.12	PS	
34.	BR220-1-1-1	67.92	64.25	46.30	55.65	PF	
35.	BR707C-MR-137	56.00	53.21	43.07	51.21	PF	
36.	BR7109-5R-2	79.27	76.92	20.10	31.74	PS	
37.	BR7109-5R-4	66.92	63.10	43.79	54.92	PF	
38.	BRR1 dhan29-SC3-27	28.10	25.60	59.82	73.12	F	
39.	IR1544-38-2-2	47.07	43.20	36.97	50.39	PF	
40.	IR11248-148-3-2-33	86.92	83.10	26.81	36.73	PS	
41.	IR19660-73-4-2	76.22	72.97	19.12	23.04	PS	
42.	IR19661-13	61.95	58.20	46.07	53.17	PF	
43.	IR69515-KKN-4-UBN-4-2-1-1	53.15	50.30	42.39	50.28	PF	
44.	IR72022-462-3-3-2	28.10	26.30	59.10	72.87	F	
45.	IR72593-B-3-2-2-2	39.49	36.10	34.82	43.85	PF	
46.	IR74963-262-5-1-3-3	82.37	79.24	21.95	29.34	PS	
47.	IR77034-B-4-3-3	49.05	45.27	40.12	49.17	PF	
48.	IR9447-P1-P2-4B	62.79	58.20	46.87	51.98	PF	
49.	IR9708-5-1-2	65.10	60.21	47.39	54.32	PF	

CS = Completely sterile, S = Sterile, PS = Partially sterile, F = Fertile, and FF = Fully fertile

Table 8 Pollen sterility or fertility status of F₁s produced by five CMS lines against 49 local and exotic rice elite breeding lines

CMS line	No. of pollen parents accounts for					
	Completely sterile	Sterile	Partially sterile	Partially fertile	Fertile	Fully fertile
IR58025A	3 (6.12)*	8 (16.33)	13 (26.53)	14 (28.57)	7 (14.29)	4 (8.16)
BRR11A	7 (14.29)	5 (10.20)	14 (28.57)	13 (26.53)	7 (14.29)	3 (6.12)
D.ShanA	7 (14.29)	4 (8.16)	12 (24.49)	12 (24.49)	6 (12.24)	8 (16.33)
BRR19A	3 (6.12)	7 (14.29)	9 (18.37)	19 (38.78)	6 (12.24)	5 (10.20)
IR73328A	4 (8.16)	6 (12.24)	11 (22.45)	17 (34.69)	10 (20.41)	1 (2.04)

*Figure in parentheses expressing percentage of total genotypes

Table 9 Identified maintainers of five alien CMS lines among 49 local and exotic rice elite breeding lines

CMS lines	Genotypes identified as maintainers
IR58025A	BAU572, BAU581 and Purbachi
BRR11A	BAU553, BAU581, SWEON-287, AOKAZE, AKI HI KARI, KOGAHIKARI and Purbachi
D.ShanA	BAU553, HARE BARE, KOSHINIHSINI, YAMABIKO, AOKAZE, AKI HI KARI and KOGAHIKARI
BRR19A	BAU581, Chinese Variety 5 and Purbachi
IR73328A	BAU553, BAU572, BAU581 and Chinese Variety 5

Table 10 Identified restorers of five alien CMS lines among 49 local and exotic rice elite breeding lines

CMS lines	Genotypes identified as restorers
IR58025A	BR6592-4-6-4, BR6839-41-5-1, IR74052-184-3-3 and BKNLR-75091-GNT-B3-RST-48-2-2
BRR11A	BR5563-3-3-4-1, IR72593-B-3-2-2-2 and IR9708-5-1-2
D.ShanA	BR368-15-2-2-3, BR6592-4-6-4, BR6723-1-1-2, BR6839-41-5-1, BR7011-37-1-2, IR9202-36-3-2, IR72049-B-R-22-3-1-1 and IR72593-B-3-2-2-2
BRR19A	IR25924-51-2-3, IR72049-B-R-22-3-1-1, IR74052-184-3-3, OM1490 and MOROBEREKAN
IR73328A	BR7011-37-1-2

Jayamani et al. (1998) found F_1 of V20A \times Dular as completely sterile and used for subsequent backcrossing up to BC_6 generation. Considering pollen sterility and spikelet fertility pattern of the crosses, it appeared that Boro season possessed lower pollen sterility and higher spikelet fertility than those of T. Aman season. Out of forty nine local and exotic rice elite breeding lines, three were identified as maintainers for IR58025A, seven for BRR11A, seven for D.ShanA, three for BRR19A and four for IR73328A (Table 9). Among the maintainers, eight genotypes (BAU553, BAU572, BAU581, Purbachi, Aokaze, Aki Hi Kari, Kogahikari, and Chinese Variety 5) were common for these five CMS lines. Ultimately, 12 genotypes were found as maintainer against five CMS lines. The identified complete sterile crosses could be transferred as new CMS line through substitution backcrossing with their respective pollen parents as recurrent parent. Ali and Khan (1995) found 47456 and PK4112 as potential maintainer whereas, 4029-2 and 4029-3 as effective restorers for all the three CMS lines of IR58025A, IR62829A, and IR64608A in 15 non-aromatic rice varieties. Among 49 local and exotic rice elite breeding lines, four were found as restorers for IR58025A, three for BRR11A, eight for D.ShanA, five for BRR19A, and one for IR73328A (Table 10). Among the restorers, six genotypes (BR6592-4-6-4, BR6839-41-5-1, IR74052-184-3-3, IR72593-B-3-2-2-2, BR7011-37-1-2, and IR72049-B-R-22-3-1-1) were common for five CMS lines. So, actually 15 genotypes were identified as restorers against five CMS lines. In case of maintainer and restorer identification, same picture appeared that number of maintainers and restorers against D.ShanA was higher than the rest of the four alien CMS lines. Out of 49 genotypes 12 genotypes (24%) were identified as maintainers and 15 (31%) were identified as restorers. The findings of this investigation were somewhat different from the results of GobindaRaj and Virmani (1989) who found 40% maintainer and 24% restorer when tested 37 *indica*, *japonica* and *indica/japonica* derivatives against CMS-GA. This difference might be due to genetic background of the test genotypes. Jayasudha and Sharma (2010) identified 10 potential restorers based on pollen and spikelet fertility percent. Out of 245 crosses only 45 crosses contributed directly to identify maintainer and

restorer. Other crosses were more or, less of intermediate types which indicated neither maintainer nor restorer. It is well known that pollen fertility is controlled by one dominant gene (Rf/Rf). If pollen fertility is governed by only one gene the product would be either restorer or, maintainer, no existence of intermediate types. So, there might be modifier genes in different genotypes which interacted with male sterile nuclear genes and resulted intermediate male sterility in the crosses. Ganesan and Rangaswamy (1998) concluded that minor genes present in the pollinator might have reacted with CMS lines used and resulted in this type of variation. On the contrary, GobindaRaj and Virmani (1988) reported that restoration is governed by two genes. Epistasis or, epistasis with incomplete dominance plays a vital role for restoration. Teng and Shen (1994) also supported the opinions of GobindaRaj and Virmani (1988). Kumari et al. (1998) suggested that the presence of multiple nuclear genes (minor) for fertility in CMS lines whereas, Ali and Khan (1995) concluded that expression of restorer genes varied with the genetic background of female parent. To overcome this problem Singh et al. (2000) proposed that incomplete restoration might be converted to complete restorer or maintainer by special breeding programme.

Conclusion

Six tester's viz., BR6592-4-6-4, BR6839-41-5-1, IR74052-184-3-3, IR72593-B-3-2-2-2, BR7011-37-1-2, and IR72049-B-R-22-3-1-1 were identified as restorers for highest 2 lines of the five CMS lines, differently. None of them were found to be restorer for all the five CMS lines. BAU581 was found to be maintainer for four lines except for D.ShanA and Purbachi was found to be maintainer for three lines except for D.ShanA and IR73328A out of the five CMS lines. Out of 245 crosses 200 crosses were intermediate type and only 45 crosses contributed directly to identify maintainer and restorer.

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