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Hybrid Rice Parental Lines Development Utilizing Different Rice Germplasms

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ABSTRACT

Hybrid rice development is an attractive approach for enhancing productivity of rice. For an efficient hybrid development program through CMS approach, a stable CMS line, its suitable maintainer line and restorer lines for high pollen load and spikelet fertility is needed. With this view, five CMS lines were crossed as lines with 30 genotypes as 'testers' to get 150 test hybrids. The 150 test hybrids were subjected to pollen and spikelet fertility analysis. Among the 150 test hybrids, pollen parents of 25 hybrids were expressed as restorers 78 as partial restorer, 22 as maintainers and 25 as partial maintainers. Four testers viz., BR7166-5B-1, F2277, PR828 and BR1543-1-1-1-1 were identified as restorers for all the five CMS lines and three testers PR52, PR95 and PR185 were identified as complete maintainer for all the tested CMS lines.

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INTRODUCTION

Rice (*Oryza sativa* L.) is the most important staple food crop in the world and used by more than half of the world population [10]. In view of the growing population, the basic objective of the plant breeders would always be towards yield improvement in staple food crops. It has been estimated that the world will have to produce 60% more rice by 2030 than what it produced in 1995 [2]. The dire need of increasing rice productivity and production encouraged rice scientists to develop and disseminate hybrid rice technology in the tropics. Experience in China [12] and outside China, in IRRI [24], India [14], Vietnam [6], the Philippines (Redona *et al*., 2003), Bangladesh (Julfiquar and Virmani 2003), and several other countries clearly indicates that hybrid rice technology offers a viable option to meet this challenge. The use of cytoplasmic male sterility system in developing hybrids in crops is possible only when effective maintainers and restorers are identified. The CMS lines introduced from China are unstable to use as such in developing hybrid rice in Bangladesh. Therefore, it is imperative to identify maintainers and restorers among the lines developed through conventional breeding procedures. Pollen (or) spikelet fertility or both have been used as an index to fix the restoration ability of the lines (Sutaryo, 1989). Use of male sterility system would be appropriate approach for commercial exploitation of heterosis in rice. CMS system is controlled by the interaction of cytoplasm with nuclear genes [9].

Test cross programs help to identify maintainers as well as restorers. Maintainer lines are used for conversion into new CMS lines and restorer lines are subsequently used as male parent in hybrid development program. CMS lines introduced from elsewhere may not be well adapted to a 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. Julfiquar *et al.,* [7] evaluated some Chinese CMS lines V20A and Zhen Shan97A along with their maintainer for adaptability and performance but these were not adapted under Bangladesh condition due to high susceptibility 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. Ali and Khan [1] successfully transferred

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CMS gene(s) from IR58025A and IR62829A into Basmati line 47456 in Pakistan. A little work on identifying CMS in local aromatic rice varieties is done so far in Bangladesh. Roy [17] identified 10 maintainer and four restorers of five exotic CMS lines while working with 33 local aromatic genotypes of Bangladesh.

So, the present investigation was undertaken to identify maintainer and restorer lines from diverged rice genotypes against exotic and local cyto-sources.

MATERIALS AND METHODS

Five cytoplasmic male sterile lines viz., BRRI11A (L1), II32A (L2), Jin23A (L3), IR79156A(L4), BRRI13A(L5), and 30 testers viz., BR8815-1-B (T1), AS996-HR-1 (T2), BR7166-5B-1 (T3), WAB96-1-1 (T4), Super Basmati (T5), Basmati386 (T6), Sugandhi Dhara (T7), IR62 (T8), IRAT267 (T9), F2277 (T10), PR828 (T11), BR7465-1-4-1 (T12), PR52 (T13), BR7166-5B-1 Ran (T14), PR95 (T15), BRRIdhan49 (T16), DSL78-8 (T17), Parija (T18), BR1543-1-1-1-1 (T19), OM4498 (T20), N22 (T21), JH15-1-1-2 (T22), FL478 (T23), IR86960-66-12-2 (T24), FL378 (T25), Wanxian763 (T26), Wanxian77 (T27), PR185 (T28), Cau-2 (T29) and RC-8 (T30) were raised in the LXT (5X30) mating design to get 150 test hybrids during T. Aman season of 2010 at the experimental plot of Bangladesh Rice Research Institute (BRRI), Gazipur. Three staggered sowing of both male and female parents were taken to synchronize flowering. 25 days old seedlings were transplanted in main field with three separate blocks for all entries according to seeding dates. The plots were 3.5 meter in length containing five rows. Adequate soil fertility was ensured by applying Urea-TSP-MP-Gypsum-$ZnSO_4$@ 150:100:70:60:10 kg/ha, respectively. Total TSP, MP, Gypsum and $ZnSO_4$ were applied during final land preparation. The urea was applied in three installments, at 15 days after transplanting (DAT), 30 DAT and 45 DAT. Necessary intercultural operations was carried out during cropping period for proper growth and development of the plants.

Crossing was done by adopting clipping method. Before crossing, CMS lines individual plants with complete pollen sterility were identified by observing the pollen grains under the microscope using one percent Iodine potassium iodide stain. The spikelets were clipped off one third from the top without damaging the stigma, between 7.00 and 9.30 AM and immediately after clipping; the panicles were covered with glossing paper covers. At the time of anthesis, panicles with fully opened spikelets were collected from the male parents and the pollen grains were dusted over the clipped panicles between 10.30 and 11.30 AM. Parents in Line x Tester fashion and a total of 150 cross combinations were obtained. After 25 days, the matured panicles were harvested.

The F_1 generations of all the crosses were raised during Boro season of 2010-11 in a test cross nursery. Thirty days old seedlings were transplanted in the well prepared main field with a spacing of 20x20 cm with two replications. Standard doses of fertilizer and irrigation were maintained. Identification of maintainers and restorers was carried out by observing pollen and spikelet fertility under bagged condition. Potential maintainers were identified as having >90% pollen sterility and <10% spikelet fertility and effective restorers as having <10% pollen sterility and >90% spikelet fertility [23].

Results:

The pollen fertility percent of test hybrids was varying from 0.00 (L4 x T 28) to 93.3% (L2 x T19). A very low magnitude of pollen and spikelet fertility was observed for test hybrids (Table 1). The lines identified as effective maintainers can be further back crossed with their respective F_1's to look for completely sterile back cross progenies so that these can be developed as new CMS lines in the background of the corresponding male parent. In some cases, the same genotype behaved as a restorer for one CMS line and as a maintainer for the other CMS line. Tester IR86960-66-12-2 behaved as an effective restorer for CMS line II32A but behaved as effective maintainer with CMS line Jin23A. Same tester acted as a partial restorer with BR11A, IR79156A and BRRI13A. Testers BR7166-5B-1 (T3), F2277 (T10), PR828 (T11) and BR1543-1-1-1-1 (T19) were found as effective restorers for all the five CMS lines.

Table 1: Pollen fertility percent of test hybrids in test cross nursery

Lines/ Tester	BRR111A (L1)	II32A (L2)	Jin23A (L3)	IR79156A (L4)	BRRI13A (L5)
BR8815-1-B (T1)	59.3 (PR)	73.2 (R)	75.5 (R)	82.3(R)	57.6 (PR)
AS996-HR-1 (T2)	36.3 (PR)	55.7 (PR)	48.2 (PR)	42.5 (PR)	33.7 (PR)
BR7166-5B-1 (T3)	76.5 (R)	82.7 (R)	80.3 (R)	82.6 (R)	87.1 (R)
WAB96-1-1 (T4)	0.85 (M)	31.6 (PR)	0.95 (M)	0.50 (M)	0.73 (M)
Super Basmati (T5)	47.2 (PR)	43.1 (PR)	52.0 (PR)	37.6 (PR)	33.1(PR)
Basmati386 (T6)	51.6 (PR)	52.1 (PR)	37.0 (PR)	33.5 (PR)	50.8 (PR)
Sugandhi Dhara (T7)	58.3 (PR)	35.2 (PR)	33.6 (PR)	57.0 (PR)	54.9 (PR)
IR62 (T8)	55.6 (PR)	68.3 (R)	59.1 (PR)	48.3 (PR)	42.5 (PR)
IRAT267(T9)	53.2 (PR)	35.8 (PR)	33.1 (PR)	50.4 (PR)	36.4 (PR)
F2277 (T10)	86.1(R)	78.2 (R)	89.6 (R)	88.2 (R)	92.3 (R)

PR828 (T11)	75.3 (R)	80.0 (R)	83.2 (R)	86.3 (R)	90.6 (R)
BR7465-1-4-1(T12)	13.8 (PM)	33.7 (PR)	54.2 (PR)	18.6 (PM)	15.4 (PM)
PR52 (T13)	0.45 (M)	0.62 (M)	0.30 (M)	0.73 (M)	0.62 (M)
BR7166-5B-1 Ran (T14)	85.3 (R)	56.2 (PR)	78.6 (R)	82.8 (R)	58.1(PR)
PR95 (T15)	0.35 (M)	0.85 (M)	0.55 (M)	0.45 (M)	0.25 (M)
BRRIdhan49 (T16)	33.2 (PR)	28.5 (PR)	17.3 (PM)	38.2 (PR)	15.2 (PM)
DSL78-8 (T17)	65.2 (R)	57.2 (PR)	55.2 (PR)	69.3 (R)	71.0 (R)
Parija (T18)	13.2 (PM)	26.3 (PR)	18.2 (PM)	33.9 (PR)	14.0 (PM)
BR1543-1-1-1-1 (T19)	80.3 (R)	93.3 (R)	87.0 (R)	78.2 (R)	86.5 (R)
OM4498 (T20)	23.0 (PR)	66.0 (R)	35.2 (PR)	44.1 (PR)	15.3 (PM)
N22 (T21)	45.0 (PR)	55.2 (PR)	35.6 (PR)	23.6 (PR)	47.0 (PR)
JH15-1-1-2(T22)	11.3 (PM)	33.0 (PR)	18.2 (PM)	28.1 (PR)	13.0 (PM)
FL478 (T23)	26.0 (PR)	33.5 (PR)	45.2 (PR)	21.0 (PR)	37.3 (PR)
IR86960-66-12-2 (T24)	44.2 (PR)	85.0 (R)	0.85 (M)	33.2 (PR)	27.6 (PR)
FL378 (T25)	37.2 (PR)	47.1 (PR)	53.0 (PR)	30.5 (PR)	33.0 (PR)
Wanxian763(T26)	36.2 (PR)	27.1 (PR)	33.0 (PR)	38.5 (PR)	43.0 (PR)
Wanxian77 (T27)	46.2 (PR)	57.3 (PR)	45.0 (PR)	32.5 (PR)	47.2 (PR)
PR185 (T28)	0.10 (M)	0.23 (M)	0.60 (M)	0.00 (M)	0.20 (M)
Cau-2 (T29)	56.4 (PR)	77.3 (R)	35.0 (PR)	52.5 (PR)	48.0 (PR)
RC-8 (T30)	36.2 (PR)	67.3 (R)	35.0 (PR)	65.5 (R)	43.7 (PR)

M = Maintainer; PM= Partial maintainer; R= Restorer and PR = Partial restorer

The tester PR52 (T13), PR95 (T15) and PR185 (T28) were found to be effective maintainers all the CMS lines. Tester WAB96-1-1 (T4) were found as effective maintainers for CMS lines BRRI11A, Jin23A, IR79156A and BRRI13A but partial restorer for II32A. The variations in behavior of fertility restoration indicate that either the fertility-restoring genes are different or that their penetrance and expressivity varied with the genotypes of the parents or the modifiers of female background. This kind of the differential reaction of the same genotype in restoring the fertility of different CMS lines of same cytoplasmic source was reported by Gannamani [4], Sao [19], Hariprasanna *et al.*, [5] and Murugan and Ganesan [15]. This could be due to differential nuclear cytoplasmic interactions between the testers and CMS lines. The spikelet fertility of F_1's ranged from 0.00 to 95.5% (L5 x T19) (Table 2). The tester WAB96-1-1 (T4) exhibited complete sterility with BRRI11A (L1), Jin23A (L3), IR97156A (L4) and BRRI13A (L5) but showed partial sterility (25.6) with L2 (II32A).

The tester PR52 (T13), PR95 (T15) and PR185 (T28) behaved as complete maintainer with all the CMS lines. While the tester BR7166-5B-1 (T3), F2277 (T10), PR828 (T11) and BR1543-1-1-1-1 (T19) showed above 80% spikelet fertility and regarded as potential restorer with all CMS lines. The tester IR86960-66-12-2 (T24) showed differential spikelet fertility level with different CMS lines. It showed high fertility with II32A (83.0) but showed sterility with Jin23A (8.7). This might be due to differential penetrance and expressivity of fertility restoring gene with different CMS lines.

Table 2: Spikelet fertility percent of test hybrids in test cross nursery

Lines/ Tester	BRRI11A (L1)	II32A (L2)	Jin23A (L3)	IR79156A (L4)	BRRI13A (L5)
BR8815-1-B (T1)	59.3 (PF)	75.3 (PF)	72.5 (PF)	81.3(F)	53.0 (PF)
AS996-HR-1 (T2)	40.7 (PF)	63.2 (PF)	47.0 (PF)	28.5 (PS)	40.2 (PF)
BR7166-5B-1 (T3)	80.2 (F)	83.2 (F)	86.3 (F)	85.0 (F)	83.6 (F)
WAB96-1-1 (T4)	0.00 (CS)	25.6 (PS)	5.6 (S)	3.2 (S)	0.00 (CS)
Super Basmati (T5)	42.0 (PF)	48.5 (PF)	53.5 (PF)	27.3 (PS)	41.6(PF)
Basmati386 (T6)	63.0 (PF)	56.2 (PF)	43.50 (PF)	27.9 (PS)	60.8 (PF)
Sugandhi Dhara (T7)	61.2 (PF)	41.3 (PF)	49.2 (PF)	62.8 (PF)	55.3 (PF)
IR62 (T8)	58.2 (PF)	70.0 (PF)	58.2 (PF)	50.1 (PF)	46.0 (PF)
IRAT267(T9)	47.5 (PF)	26.8 (PS)	38.7 (PF)	55.2 (PF)	29.0 (PS)
F2277 (T10)	85.2 (F)	83.0 (F)	90.5 (F)	87.2 (F)	93.5 (F)
PR828 (T11)	80.0 (F)	82.6 (F)	85.1 (F)	81.5 (F)	87.9 (F)
BR7465-1-4-1(T12)	18.0 (PS)	41.0 (PF)	53.2 (PF)	23.8 (PS)	20.1 (PS)
PR52 (T13)	0.00 (CS)	0.00 (CS)	0.00 (CS)	0.00 (CS)	0.00 (CS)
BR7166-5B-1 Ran (T14)	81.2 (F)	59.8 (PF)	77.2 (PF)	83.2 (F)	53.6(PF)
PR95 (T15)	0.00 (CS)	0.00 (CS)	0.00 (CS)	0.00 (CS)	0.00 (CS)
BRRIdhan49 (T16)	41.2 (PF)	27.5 (PS)	13.2 (PS)	44.0 (PF)	21.8 (PS)
DSL78-8 (T17)	70.0 (PF)	61.3 (PF)	58.6 (PF)	72.7 (PF)	76.4 (PF)
Parija (T18)	9.5 (S)	24.0 (PS)	13.8 (PS)	41.2 (PF)	12.5 (PS)
BR1543-1-1-1-1 (T19)	82.47 (F)	89.3 (F)	89.0 (F)	81.5 (F)	95.5 (F)
OM4498 (T20)	28.2 (PS)	68.7 (PF)	45.2 (PF)	51.8 (PF)	21.6 (PS)
N22 (T21)	48.3 (PF)	60.0 (PF)	41.6 (PF)	28.2 (PS)	55.8 (PF)
JH15-1-1-2(T22)	8.6 (S)	40.0 (PF)	23.5 (PS)	28.7 (PS)	21.5 (PS)
FL478 (T23)	33.8 (PF)	43.2 (PF)	48.6 (PF)	18.0 (PS)	45.0 (PF)
IR86960-66-12-2 (T24)	51.6 (PF)	83.0 (F)	8.7 (S)	40.5 (PF)	33.8 (PF)
FL378 (T25)	43.2 (PF)	53.8 (PF)	55.6 (PF)	47.3 (PF)	40.0 (PF)
Wanxian763(T26)	47.2 (PF)	29.0 (PS)	41.5 (PF)	43.6 (PF)	44.8 (PF)

Wanxian77 (T27)	60.5 (PF)	70.2 (PF)	28.8 (PS)	40.5 (PF)	55.7 (PF)
PR185 (T28)	0.00 (CS)	0.00 (CS)	0.00 (CS)	0.00 (CS)	0.00 (CS)
Cau-2 (T29)	57.8 (PF)	81.2 (F)	28.0 (PS)	60.3 (PF)	49.9 (PF)
RC-8 (T30)	42.0 (PF)	74.2 (PF)	48.1 (PF)	66.0 (PF)	48.3 (PF)

CS = Complete sterile; S = Sterile, PS= Partial sterile; F= Fertile and PF= Partial fertile
[CS +S = Maintainer lines, PS = Partial maintainer lines, F= Restorer lines and PF= Partial restorer lines]

The potential restorers and maintainers were identified and presented in Table 3. The potential maintainers for all the CMS lines are being used in the back cross program to develop new CMS line. There were instances which the classification of tester based on the pollen fertility did not correlate with the classification based on the spikelet fertility. For example OM4498 (T20) categorized as restorer based on pollen fertility and as partial fertile by spikelet fertility analysis. Tester IR86960-66-12-2 (T24) behaved as an effective maintainer for Jin23A and found to be an effective restorer with II32A. Such non-correlation between pollen fertility and spikelet fertility was reported by Murugan and Ganesan (2006). The testers BR7166-5B-1 (T3), F2277 (T10), PR828 (T11) and BR1543-1-1-1-1 (T19) behaved as complete restorers with all the CMS lines.

Table 3: Potential restorers and maintainers for five CMS lines

CMS lines	Restorers	Maintainers
BRRI11A	BR7166-5B-1, F2277, PR828, BR1543-1-1-1-1, BR7166-5B-1 Ran	WAB96-1-1, PR52, PR95, PR185, Parija, JH15-1-1-2
II32A	BR7166-5B-1, F2277, PR828, BR1543-1-1-1-1, IR86960-66-12-2, Cau-2	PR52, PR95, PR185
Jin23A	BR7166-5B-1, F2277, PR828, BR1543-1-1-1-1, BR7166-5B-1 Ran	WAB96-1-1, PR52, PR95, PR185, IR86960-66-12-2
IR79156A	BR8815-1-B, BR7166-5B-1, F2277, PR828 , BR1543-1-1-1-1	WAB96-1-1, PR52, PR95, PR185
BRRI13A	BR7166-5B-1, F2277, PR828, BR1543-1-1-1-1	WAB96-1-1, PR52, PR95, PR185

Discussion:

For developing high yielding three line rice hybrids, the basic step is to identify maintainers having recessive fertility restorer gene or genes and restorers having dominant fertility restorer genes or gene from local or exotic germplasm sources. Extensive research work on identification of restorers and maintainers and the inheritance of fertility restoration has been done on the WA cytoplasmic source [25,20]. Among the various sources of cyto sterility, CMS lines derived from the WA system have been found to be the most stable in terms of complete pollen sterility [3]. Several studies by Singh and Singh [21] and Malarvizhi *et al.,* [13] have reported higher frequency of restorers than maintainers for WA-CMS lines, while Kumari *et al.,* [14] and Salgotra *et al.,* [18] reported higher percentage of maintainers than restorers in their studies. In addition, most lines identified as restorers were *indica* and maintainer lines were landraces and *japonica.* The frequency of restorers for WA system is relatively high among improved *indica* cultivars than *japonica* rice [26]. In the present study, among 150 test hybrids raised through crossing with five CMS lines, the number of restorers (25) was more than that of maintainers (22). However, when compared as common with CMS lines, the number of restorers (4) was closer to maintainers (3). This clearly indicates that studied lines are closer to maintainers with respect to the fertility restoration genes. Furthermore, the relatively little higher frequency of restorers among the lines indicates that with respect to fertility restoration genes lines are closer to restorer gene pool. Since heterosis is often realized in terms of genetic distance, the distinct gene pools of maintainers and restorers can hasten hybrid rice breeding. Among the lines, BR7166-5B-1, F2277, PR828 and BR1543-1-1-1-1 which were identified as restorers are adaptable to rainfed ecosystem of Bangladesh. We propose diversification of restorer line development by crossing the restorers with landraces and effective selection from advance generations lead site specific restorers development for hybrids. Furthermore, landraces could have different restorer genes, which should be elucidated by genetic and molecular marker studies. Simultaneously, similar breeding strategies could be deployed for maintainer development by crossing identified maintainers PR52, PR95 and PR185 with of landraces. Effective diversified maintainer lines could come out to incorporate specialty traits in hybrids. Specific traits evolved from landraces into restorer and maintainer lines could hasten specific trait related hybrid rice variety development. It was observed that the frequency of maintainers among the elite breeding lines is rather low. Furthermore, even if some of the lines are identified as maintainers, they are susceptible to various biotic and abiotic stresses. Hence, there is a need to combine desirable traits through recombination breeding. Sometimes, even partial maintainers with many desirable traits can be used in the breeding program. Some of the desirable traits of the maintainer lines are relatively dwarf/semi-dwarf stature, good and synchronous tillering, high stigma exsertion, high outcrossing potential, sturdy culm, resistance to insect pests and diseases, complete and stable maintenance of sterility. The identified maintainer lines can easily be converted into new CMS lines through backcross breeding and utilize for potential hybrid rice development using newly identified restorer lines BR7166-5B-1, F2277, PR828 and BR1543-1-1-1-1 which will more adaptable with excellent grain quality and high yield potentiality.

Conclusion:

These testers which showed complete sterility with CMS lines could be converted into new potential CMS lines through backcross breeding method. On the other hand, testers which showed complete sterility with all the CMS lines reflect their diverse maintaining ability and could be used for new maintainer development program. The testers which showed high fertility with CMS lines could be utilized in the heterosis breeding program after testing their combining ability and heterosis with different CMS lines.

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