SHORT COMMUNICATION

Evaluation of test cross combinations of rice hybrids to identify the potential restorers and maintainers

W.S. Priyantha[1]*, R.P.D.H. Hemachandra[1], D.D. Witharana[1], D.M.N.D. Dasanayaka[1]

[a]*Rice Research and Development Institute, Batalagoda, Ibbagamuwa, 60 500, Sri Lanka*

Submitted: January 12, 2018; Revised: October 13, 2018; Accepted: November 26, 2018
*Correspondence: rrdihybridrice@gmail.com

ABSTRACT

Hybrid rice technology is one of the good options to enhance the productivity of rice. However, identification of locally adaptable maintainers and restorers is important to develop well adoptive hybrid rice varieties. Therefore, an experiment was conducted with the objective to identify the restorers and potential maintainers to develop (CMS) lines in order to use them as parents for future hybrid rice programme. The experiment was conducted at the Rice Research and Development Institute, Batalagoda, Sri Lanka in Maha 2016/17 and Yala 2017 season.147 crosses were produced by using 29 CMS lines crossing with 58 elite inbred lines in Maha 2016/17. F_1 hybrids were field evaluated in test cross nursery in Yala 2017. All F_1 hybrids and respective male parents were planted on progeny basis in a test cross nursery. Other agronomical practices were conducted according to the Department of Agriculture (DOA) recommendation. Pollen fertility/sterility of the F_1 hybrids was observed via light microscope after staining them with I-KI solution. 56 pollen fertile F_1 crosses were identified having >81% pollen fertility and 31 male parents were selected for restoration ability. Four pollen sterile F_1 combinations were identified and they showed >98.6% pollen sterility and the pollen parents in these crosses were selected for maintaining ability (RES 256) and advanced to backcross breeding programme to develop new CMS lines.

***Keywords**: Restorers, hybrid rice, pollen sterility*

INTRODUCTION

Rice (*Oryza sativa L.*) is the staple food for near half of the global population (FAO, 2004). Enhancement of rice production is important to reduce increasing demand for rice in future. Therefore, hybrid rice technology is one of the good options to enhance the productivity of rice. Hybrid rice is the product of a cross between genetically distinct male and female parents. In hybrid rice production, the use of cytoplasmic genetic male sterility (CMS) system is possible only when effective maintainers and restorers are identified. The CMS lines, which were introduced from China, are unstable to use in developing hybrid rice in Asian countries (Sutaryo, 1989). Therefore, identification of locally adaptable maintainers and restorers from local germplasm, which shows higher degree of maintaining and restoration ability for CMS lines, are very important. Identification of maintainers and restorers from elite breeding lines and landraces through test crossing and their use in further breeding programmes are the initial steps in three line heterosis breeding (Siddiq, 1996). Successful use of hybrid vigor in rice largely depends on the availability of locally developed CMS and restorer lines (Kumar *et al.,* 1996). In Sri Lanka, the research and development programme on hybrid rice breeding technology was initiated at the Rice Research and Development Institute (RRDI) and have been able to identify several hybrids with 1.0 to 1.5 t ha^{-1} yield advantage over the best inbred grown under similar environments (Iqbal, 2009). However, limited genetic resources of parental lines (CMS, Maintainers and Restorers) of hybrid rice programmes were one of the constraints at present and it directly affects on development of high heterotic hybrid combinations. Therefore, the objective of this study was to identify the restorers and potential maintainers to develop CMS lines, which could be used as parents for future hybrid rice programmes.

MATERIALS AND METHODS

The experiment was conducted at Rice Research and Development Institute (RRDI) Batalagoda in two consecutive seasons (*Maha* 2016/2017 and *Yala* 2017). For this experiment 58 elite inbred and 29 CMS lines (Table 1) were established in the field in order to synchronise the flowering and 147 crosses were carried out following the emasculation (one third of the spikelet was removed to expose the stigma without removing the anthers) and pollination techniques in *Maha* 2016/2017 season. Three weeks after pollination F_1 seeds were harvested and used to field evaluate in testcross nursery in *Yala* 2017 season. F_1 seeds were germinated in Petri-dishes and germinated seeds were transferred to pots. In the meantime, pollen parents of respective crosses were also established in upland nursery and 18 d old seedlings were transplanted in the field. F_1 seedlings and respective pollen parents were planted in a single line progeny following one plant per hill basis. Spacing of two plants was 20 cm and spacing between two different progenies was 40 cm. All other agronomical practices were followed according to the Department of Agriculture (DOA) recommendation.

Data collection was initiated in flowering period. Pollen fertility and sterility of F_1 plants were observed through light microscope. For pollen fertility and sterility observation, newly emerged panicles were randomly selected from five plants of a single line progeny and spicklets were collected from the panicles having 15 – 20 spikelets. Selected spikelets were individually put into a container having 70% ethanol. In the laboratory, anthers were taken out from at least six spikelets using needle and placed them on glass slide with a drop of 1% potassium iodine (I-KI) solution to stain the anthers. The anthers were gently crushed by using a needle to release the pollen grains. After removing the debris, a cover slip was placed and the slide was observed under the microscope to identify the pollen fertility/sterility. Mean time five panicles of F_1 plants of different crosses were covered with the paper bags to avoid the foreign pollen contamination and to confirm the sterility of respective F_1 plants. The following (Table 2.) criteria was used for the classifying the parental lines as maintainers and restorers (Virmani *et al*., 1997). Morphological and yield characters of fertile crosses (F_1) and respective pollen parents were also measures in order to identify the heterosis (Heterobeltiosis).

Table 1: Pollen and recurrent parents lines used for crossing.

Recurrent parents		Pollen parents		
BG CMS 5A	IR 75601A	RES 147	RES256	RES 251
IR 69616A	IR 80156A	RES277	RES287	RES281
IR 73318A	IR 71564A	RES156	RES269	RES 264
IR 70369A	IR 80154A	RES279	RES298	RES293
IR 77803A	IR 64608A	RES 160	RES316	RES271
IR 58025A	IR 73323A	RES280	RES313	RES310
IR 67684A	IR 79156A	RES 263	RES257	RES 266
IR 78359A	IR 73318A	RES291	RES288	RES295
IR 78364A	BG CMS 7A	RES270	RES261	RES275
BG CMS 1A	BG CMS 8A	RES299	RES289	RES312
IR 71563A	BG CMS 9A	RES286	RES283	RES320
IR 79175A	BG CMS 10A	RES311	RES 265	RES 262
IR 79125A	CH 3A	RES 253	RES294	RES290
IR 69626A	CH 5A	RES273	RES252	
IR 69625A				

Table 2: Classification of rice lines to identify as the restorers and maintainers.

Pollen fertility (%)	Category	Spikelet fertility (%)
0 – 1	Maintainers	0
1.1 – 50	Partial maintainers	0.1 – 50
50.1 – 80	Partial restorers	50.1 – 75
> 80	Restorers	>75

RESULTS AND DISCUSSION

Fifty-six pollen fertile F_1 crosses were identified and these crosses showed more than 80% pollen fertility (Table 3). Therefore, 31 pollen parents from the 56 fertile F_1 crosses; RES 147, RES156, RES 160, RES 252, RES257, RES261, RES 265, RES269, RES 271, RES 275, RES 277, RES 279, RES 280, RES 281, RES283, RES 290, RES 294, RES 295, RES 298, RES 299, RES 313, RES 316, SN 290, SN324, SN327, SN340, SN 342, SN384, SN385, RES 258 and HRSP 668 have identified for their restoration ability and they can be selected as restores to produce hybrids. However, their restoration ability should be further confirmed by conducting re-test cross method.

Table 3: Pollen fertility levels of selected 147 cross combinations.

Cross combinations	Sterility (%)	Cross combinations	Sterility (%)	Cross combinations	Sterility (%)
BG CMS 5A/RES 147	9.2	IR80156A/RES253	43	Bg CMS 8A/RES 265	42
IR69616A/RES156	12.2	IR58025A/RES 256	100	Bg CMS 8A/RES 289	94.2
IR73318A/RES 156	17	IR78359A/RES 256	100	Bg CMS 8A/HRSP668	8.6
IR70369A/RES 156	13.8	IR78364A/RES 256	100	Bg CMS 8A/Bg CMS 1B	98
IR77803A/RES 156	5	BG CMS 5A/RES256	93	Bg CMS 9A/RES 252	13.3
IR58025A/RES 160	9.2	BG CMS 5A/RES257	6.4	Bg CMS 9A/RES 256	97.6
IR67684A /RES 160	12	IR70369A/RES261	17	Bg CMS 9A/RES 265	56
IR70369A/RES 251	36	IR80156A /RES261	95.6	Bg CMS 9A/RES 277	67
IR78359A/RES 251	70	BG CMS 5A/RES261	68	Bg CMS 9A/HRSP 668	18
IR78364A/RES 251	53	BG CMS 5A/RES 262	20.2	Bg CMS 9A/Bg CMS 1B	96
BG CMS 5A/RES 251	64	BG CMS 5A/RES 263	10.6	Bg CMS 10A/RES 252	9
IR58025A/RES 252	10	BG CMS 5A/RES 264	68	Bg CMS 10A/RES 289	89.2
IR69616A/RES 253	34	IR67684A/RES 265	13.6	Bg CMS 10A/RES 290	12.5
IR70369A/RES 253	37.6	IR71564A/RES266	61.6	IR71564A/RES 279	18.6
IR71563A /RES253	49.6	IR80154A/RES 266	57	IR78359A/RES 279	15
IR75601A/RES253	40	BG CMS 1A/RES 266	78	IR79156A/RES 279	21
IR77803A/RES 253	40	IR64608A/RES269	14	BG CMS 1A/RES 279	6.6
IR73323A/RES269	16	IR79125A/RES 280	9	IR79175A/RES 280	67
BG CMS 5A/RES 269	43.75	CH 3A/RES 280	9	IR79125A/RES 286	65
IR71564A/RES 270	80	CH 5A/RES 280	29	CH 5A / RES 286	80
IR79156A/RES 270	46.6	BG CMS 5A/RES 280	28	BG CMS 5A/RES 286	55
IR80154A/RES 270	28	BG CMS 5A/RES 281	6.6	IR69625A/RES 287	28.6
BG CMS 1A/RES 270	65	IR73323A/RES283	10	IR69626A / RES 287	44
BG CMS 5A/RES 271	9.2	IR79175A/RES 283	29.2	IR80154A /RES 310	75
BG CMS 5A/RES 273	95.6	IR79125A/RES 283	15.4	BG CMS 5A/RES 310	68
IR 71564A/RES 275	18.6	BG CMS 5A/RES 283	16	IR80154A / RES 311	36.6

IR78359A/RES 275	27.6	IR78359A/RES 286	91	IR78359 / RES 312	84.6
CH 3A/RES 277	15	IR79156/RES 286	75	IR78364A / RES 312	81
CH 5A/RES 277	33	IR80154/RES 286	82.4	BG CMS 5A / RES 312	79
BG CMS 5A/RES277	55	IR79175A/RES 286	95	IR80154A / RES 313	17.4
IR71564A/RES 279	18.6	BG CMS 5A/RES 291	33	IR69616A / RES 316	39
IR78359A/RES 279	15	IR 80156A/RES 293	49.6	IR70369A / SN327	16
IR79156A/RES 279	21	BG CMS 5A/RES 293	29	IR71563A / SN340	8
BG CMS 1A/RES 279	6.6	BG CMS 5A/RES 294	10.4	IR70369A / SN340	16.6
IR79175A/RES 280	67	BG CMS 5A /RES 295	20	BG CMS 5A / SN 342	8.6
IR79125A/RES 286	65	BG CMS 5A/RES 298	11.4	BG CMS 5A / SN 348	54
CH 5A / RES 286	80	IR80154A/RES 299	13.6	BG CMS 5A / SN 363	45
BG CMS 5A/RES 286	55	IR80156A / RES 299	17.6	IR70369A / SN375	26
IR69625A/RES 287	28.6	IR78364A / RES 299	18.2	BG CMS 5A / SN 375	54
IR69626A / RES 287	44	IR78359A / RES 299	6.2	IR70369A / SN384	19
IR80156A/RES 287	62	BG CMS 5A / RES 299	35	IR70369A / SN385	11.6
IR 73323A/RES 288	44	BG CMS7A/RES 258	17	IR67684A / BG310	30
IR79125A/RES 288	91.2	CH 3A / BG310	30	BG CMS7A/Bg CMS 1B	97.2
CH 5A/RES 288	93.6	BG CMS7A/RES 256	99	Bg CMS 8A/RES 252	9
BG CMS 5A /RES 288	76.6	IR73323A / BG310	32	Bg CMS 8A/RES 256	98.8
IR71564A / RES 289	97.6	BG CMS7A/RES 277	42	Bg CMS 8A/RES 263	30.6
IR80154A / RES 289	97	BG CMS7A/RES 290	22.6	IR69616A/SN290	7
BG CMS 1A /RES 289	81.8	BG CMS7A/HRSP 668	6	IR77803A/SN 290	10
BG CMS 5A /RES 289	76.6	BG CMS 5A/RES 318	68.6	BG CMS 5A/SN 290	9.6
IR75601A / RES 290	6.8	BG CMS 5A/RES 320	61	IR71563A/SN324	7.4
IR73318A /RES 316	17	IR 80156A /RES 321	90.6	IR71563A/SN327	3.8
IR75601A/ RES 316	5.6	BG CMS 5A/RES 321	82.4		
IR73318A/SN 290	21	IR70369A/SN 290	13		

Out of 147 crosses, four cross combinations IR58025A/RES 256, IR78359A/RES 256, IR78364A/RES 256 and BG CMS 7A/RES 256 were identified as pollen sterile crosses with the pollen sterility of 100,100,100 and 99%, respectively and it indicated that the RES256 has an ability to maintain the respective CMS lines. This male parent can be used to develop new CMS lines by following back cross breeding method. Meanwhile, 52 partially maintainers and 35 partially restorers were found and it indicated that such male parents could not have restoring or maintaining ability.

CONCLUSIONS

Fifty-six F_1 crosses are identified with >80% pollen fertility and 31 pollen parents contributed to produce above F_1 crosses. Hence, these pollen parents can be selected as restorers for hybrid rice production. IR58025A/RES 256, IR78359A/ RES 256, IR78364A/ RES 256 and BG CMS7A/RES 256 crosses are having with 98.6% pollen sterility. Pollen parent of the above crosses (RES 256) is identified as a line, which has maintaining ability. It can be used to develop new CMS line via back cross breeding method.

REFERENCES

Abeysekara, S.W. and Abeysiriwardena D.S.D.E.Z. (2000). Recent developments in hybrid rice research in Sri Lanka. Annual Symposium of the Department of Agriculture, Sri Lanka. 2, 9–17.

Iqbal Y.B. (2009). Analysis of combining ability to identify suitable parents for heteritic rice breeding. MPhil Thesis. University of Peradeniya, Sri Lanka.

Kumar, R.V., Satyanarayana, P.V. and Rao, M.S. (1996). New cytoplasmic male sterile lines developed in Andhra Pradesh, India. Intl. Rice Res. Notes 21(2–3), 30.

Sutaryo, B. 1989. Evaluation of some F_1 rice hybrids developed using MB 365A as CMS line. Intl. Rice Res. Newsl. 14, 7–8.

Virmani, S.S., Viraktamath, B.C., Casal, C.L., Toledo, R.S., Lopez, M.T. and Manalo, J.O. (1997). Hybrid Rice Breeading Manual. Intl. Rice Res. Inst. Los Banos, Leguna, Philippines. 155.