Short Communication

Development of Locally Adaptable Cytoplasmic Male Sterile Rice Lines via Backcross Breeding

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Introduction

Rice (*Oryza sativa* L.), being the staple food crop of Sri Lanka, is cultivated in almost all agro-ecological regions of the country. Yield potential of rice varieties grown in Sri Lanka is 10-11 t ha^{-1}, while the current average yield is about 4.3 t ha^{-1}. Productivity of rice has to be increased in major rice producing areas to meet future demand. However, the yield improvement achieved with semi-dwarf inbred varieties may not be sufficient to feed the nation. Furthermore, an increase in yield potential is expected to pull the realized yield at farmer level (Abeysiriwardena, 2000). Hybrid Rice (HR) technology is one of the options to overcome yield stagnation of rice. Cytoplasmic Male Sterility (CMS) system is an essential component of hybrid seed rice paddy production. Research and Development (R & D) programme on hybrid rice production began in *Yala* 1983 at the Central Rice Breeding Station, Batalagoda, in order to transfer the new Cytoplasmic Male Sterility (CMS) systems to local inbred rice varieties (Pathinayake and Dhanapala, 1985). Later, few hybrid varieties were identified with yield advantages of 1.0 - 1.5 t ha^{-1} over the best inbred varieties grown under similar environments (Abeysekara and Abeysriwardane, 2000). Successful use of CMS lines (A line) for breeding depends on the stability and adaptability of the A lines across different environments, ability for restoration, genetic diversity of the restorer parent and out-crossing potential and combining ability of A line with restorer (Virmani *et al*., 1997). Under the three line system, three breeding lines; A line, B line (responsible for maintaining the A line) and R line (used to restore the fertility of A line and to produce hybrid seeds) are required for seed production of hybrid rice. Identification of promising parents (CMS and Restorer) with better general combining ability (GCA) is important to develop best hybrid combinations and enhance rice production (Hariprasad, 2011). Lack of better parents (A, B and R lines) are one of the major constraints of the hybrid rice R & D programme. Hence, identification of superior parental lines possessing comparatively better characteristics with high GCA is important for successful hybrid rice programmes. Therefore, the objective of the present study was to identify newly developed promising CMS lines through back cross breeding that are well adapted to local conditions.

Materials and Methods

Six CMS lines and six locally adapted inbred lines were selected based on their morphological and grain quality characteristics to produce F_1 hybrids (Table 1). CMS characteristics were transferred to locally adapt inbred lines through backcross breeding using five backcrosses (Fig. 1).

Thirty six crosses were made during 2014/15 *Maha* season as in Table 2. Three weeks after crossing F_1 seeds were harvested. These F_1 seeds and their respective pollen parents were evaluated to identify the performances of F_1 hybrids in 2015 *Yala* season. F_1 seedlings (18 days old) and pollen parents were established on adjoining rows in a well prepared field. Each F_1 and respective pollen parents were planted at a spacing of 40 cm between rows and 15 cm within plants.

Table 1. Parental rice lines selected to develop F_1 hybrids

CMS lines	Morphological & grain characters	Inbred lines	Morphological & grain characters
IR 68886A	Age 3 ½ months, steady clump, big panicle, long medium grain	SN273	Age 3 ½ months, high yield, steady clump, big panicle, short round grain
Bg CMS4A	Age 3 ½ months, steady clump, big panicle, long medium grain	SN357	Age 3 ½ months, high yield, steady clump, big panicle, long medium grain
IR71563A	Age 3 ½ months, steady clump, big panicle, long medium grain	SN269	Age 3 ½ months, high yield, steady clump, big panicle, short round grain
IR71564A	Age 3 ½ months, steady clump, big panicle,	SN271	Age 3 ½ months, high yield, steady clump, big panicle, long medium grain
CH1	Age 4 months, steady clump, big panicle, long slender grain	Bg 10-2654	Age 3 ½ months, high yield, steady clump, big panicle, long medium grain
IR77803A	Age 3 ½ months, steady clump, big panicle,	BgCMS1B	Age 4 months, high yield, steady clump, big panicle, long medium grain

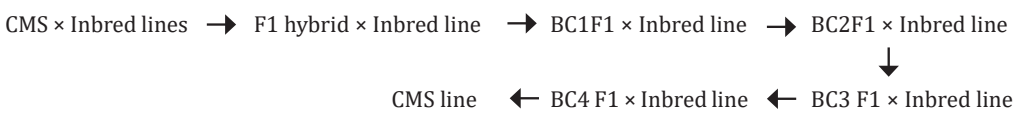

Figure 1. Stepwise backcross breeding scheme adopted to transfer CMS characteristic to inbred lines

Table 2. Thirty six crosses made in 2014/15 *Maha* season

CMS lines	Inbred lines					
	SN273	**SN357**	**SN269**	**SN271**	**Bg 10-2654**	**BgCMS1B**
IR 68886A	1	2	3	4	5	6
Bg CMS4A	7	8	9	10	11	12
IR71563A	13	14	15	16	17	18
IR71564A	19	20	21	22	23	24
CH1	25	26	27	28	29	30
IR77803A	31	32	33	34	35	36

All other agronomic practices were made according to the Department of Agriculture recommendations. Data recording was initiated at flowering. Several spikes which were close to flowering were collected into small bottles containing 70% ethanol, and brought in to laboratory. Anthers taken out from 3 - 4 spikes were crushed and placed on a glass slide. A few drops of I-KI solution was added onto crushed anthers and was covered with a cover slip. The slide was observed through a light microscope and sterile pollens were counted. F_1 plants with more than 95% pollen sterility were back crossed with their respective pollen parents. Meanwhile, selected panicles of F_1 hybrids were covered with paper bags to determine their panicle sterility. Each panicle was harvested after three weeks and filled grain percentage of a panicle was recorded. F_1 crosses that produced fertile panicles were not backcrossed since such crosses lack 100% sterility. Five back crosses were made until the characteristics of improved pollen parent were shown in the final backcross as indicated in the following chart.

Results and Discussion

Pollen sterility percentage and filled grain percentage of panicles of F_1 hybrids evaluated during 2015 *Yala* season are presented in Table 3. Accordingly, 13 F_1 hybrid lines with more than 95% pollen sterility and zero seed setting were identified as male sterile in 2015 *Yala* season. It indicates that the male sterility characteristic has been well incorporated into F_1 generation (*i.e.* selected inbred varieties did not have restoring genetic back ground related to selected CMS sources).

Twenty three partially sterile crosses were identified and those crosses were not used for back crossing. Pollen sterility percentage and filled grain percentage of panicles of BC_1F_1 and BC_2F_1 generations during 2015/16 *Maha* and 2016 *Yala* seasons are presented in Table 4. F_1 hybrids; 26, 33, 7 and 16 showed 94 and 95, 97 and 97 pollen sterility percentages, and 5, 2, 1 and 1 filled grain percentages of panicle (FGPP), respectively. Those lines showed poor pollen sterility in BC_2F_1 generation too.

Eight BC_2F_1 lines showed high pollen sterility and zero seed setting. All these lines were used to do the 3rd backcross with their respective pollen parents and results indicated that all these backcrosses have stable male sterility character.

Table 3. Percentage of pollen sterility (PPS) and filled grain percentage of panicle (FGPP) in F_1 hybrids evaluated during 2015 *Yala* season

F_1 Hybrid No.	PPS	FGPP	F_1 Hybrid No.	PPS	FGPP
1	98	0	19	91	10
2	97	0	20	89	30
3	85	13	21	100	0
4	52	73	22	100	0
5	63	52	23	90	12
6	78	50	24	93	11
7	100	0	25	55	45
8	70	56	26	99	0
9	56	40	27	85	20
10	52	53	28	56	50
11	50	50	29	36	86
12	69	42	30	100	0
13	98	0	31	59	63
14	100	0	32	78	11
15	88	36	33	98	0
16	100	0	34	56	56
17	56	46	35	100	0
18	98	0	36	86	9

After completion of BC_5F_1 generation, eight new stable male sterile CMS lines could be identified. Morphological characteristics of selected lines are presented in Table 5. All the selected CMS lines showed stable and high level of pollen sterility which is the very important characteristic of CMS lines. All the CMS lines showed intermediate plant height of 80-95 cm which is non lodging. All these CMS lines showed intermediate panicle length along with stable high levels of pollen sterility, which are very important characters for CMS lines. All the CMS lines selected showed white colour stigma and long medium grains except for lines 1 and 14 which showed short round grain type. These two are the first additions to our CMS germplasm with S/R grain type.

Conclusion

Out of the 36 test crosses, eight crosses were successfully retained as male sterile when back crossing five times with their pollen parents. Hence, eight new stable CMS lines (1-IR68886A/6×SN273, 2-BgCMD4A/6×SN357, 13-IR71563A/6×SN269, 18-IR71564A/6×SN269, 22-IR71564A/6×SN271, 14-IR71563A/6×SN271, 35-IR77803A/6×Bg10-2654 and 30-CH1/6×Bg CMS1B) having stable male sterility were developed. These can be used in future hybrid rice production.

Table 4. Percentage Pollen Sterility (PPS) and filled grains percentage of panicle (FGPP) of BC_1F_1 generation during 2015/16 *Maha* and 2016 *Yala* seasons

Cross number	BC_1F_1, 2015/16 *Maha*		BC_2F_1, 2016 *Yala*	
	PPS	FGPP	PSP	FGPP
1	100	0	100	0
2	100	0	100	0
7	97	1	95	2
13	98	0	98	0
14	100	0	100	0
16	97	1	94	3
18	100	0	100	0
22	100	0	100	0
21	99	0	97	1
26	94	5	90	4
30	100	0	100	0
33	95	2	94	3
35	100	0	100	0

Table 5. Morphological characters of finally selected CMS lines in 2017 *Yala* season

CMS lines	Character*						
	PH (cm)	PL (cm)	FLL (cm)	FLW (mm)	PS (%)	SC	GT
1	94.3	19.6	33.9	1.4	99.9	White	S/R
2	92.8	19.5	29.9	1.5	99.6	White	L/M
13	81.7	20.9	25.8	1.6	99.3	White	L/M
18	83.9	21.3	29.1	2.4	99.4	White	L/M
22	86.1	19.4	29.6	1.4	99.8	White	L/M
14	92.7	18.9	29.7	1.4	99.9	White	S/R
35	98.1	21.3	31.8	1.5	99.8	White	L/M
30	88.1	18.8	27.3	1.6	99.6	White	L/M

**PH-Plant height, PL-Panicle length, FLL-Flag leaf length, FLW-Flag leaf width, PS-Pollen sterility, SC- Stigma colour, GT-Grain type, S/R-Short round, L/M-Long medium*

Acknowledgements

Authors wish to acknowledge Sri Lanka Council of Agriculture Research Policy (SL-CARP) for providing necessary funds. The guidance and encouragement of Dr. Amitha P. Benthota, Former Director, RRDI for the successfully completion of this study was deeply appreciated.

References

Abeysekara SW, Abeysriwardane DS de Z 2000. Recent developments in hybrid rice research in Sri Lanka. Annals of Sri Lanka Department of Agriculture 2: 9-17.

Abeysiriwardena DS de Z 2000. Rice varietal improvement for future challenges. Proceedings of the Rice Congress 2000, Department of Agriculture, Sri Lanka. pp. 87-99.

Hariprasad AS 2011. Principles of Hybrid Rice Seed Production : http://www.rkmp.co.in/sites/default/files/ris/research- (Accessed on 01 January 2017)

Pathinayake BD, Dhanapala MP 1985. Present status of hybrid rice research in Sri Lanka. Tropical Agriculturist 141: 77-80.

Virmani SS, Viraktamath BC, Casal CL, Toledo RS, Lopez MT, Monaldo JO 1997. Hybrid Rice Breeding Manual. International Rice Research Institute, Philippines.