Determination of Potential Restorers and Maintainers by Evaluation of Test Crosses of Rice Hybrids

WS Priyantha[1]*, RPDH Hemachandra[1] and DMOKB Dissanayaka[1]
[1]Rice Research and Development Institute, Batalagoda, Ibbagamuwa, Sri Lanka.

Abstract
Experiment was conducted to identify the new restores and maintainers for hybrid rice programme in Rice Research and Development Institute Batalagoda. Seventy-six crosses were produced by using 24 CMS lines crossing with 24 elite inbred lines in *Maha* 2015/16. F_1 hybrids were field evaluated in test cross nursery in *Yala* 2016. All F_1 hybrids and respective male parents were planted progeny basis in test cross nursery side by side. Other agronomical practices were conducted according to the DOA recommendation. Pollen sterility and fertility of F_1 hybrids were studied via light microscope after staining them with I-KI solution. Four pollen fertile F_1 crosses were identified having >81% pollen fertility (Bg CMS4A/ SN 307, Bg CMS4A/ SN 309, Bg CMS4A/ SN 313 and IR68280A/SN 394) and male parents of such crosses were selected as restorers (SN 307, SN 309, SN 313and SN 394). Meanwhile four pollen sterile F_1combinations were identified and they showed >98.6% pollen sterility. The pollen parents of such hybrid combinations were selected as maintainers (SN395, SN405 and SN308) and they were advanced to back cross breeding programme in order to develop new CMS lines.

Key words: F_1 hybrids, Restorers, Pollen sterility
Corresponding author: keenawalapriyantha@yahoo.com

Introduction
Rice (*Oryza sativa* L.) is the staple food for nearly half of the global population (FAO, 2004). Enhancement of rice production is important to increase the 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 show the higher degree of maintainance 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 programme 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, a research and development (R&D) programme on hybrid rice was able to identify several hybrids 1.0-1.5 t ha^{-1} with yield advantage over the best inbred rice, grown under similar environments (Iqbal, 2009). However, limited genetic resources of the parental lines (CMS, Maintainers and Restorers) of hybrid rice programme was one of the constrains at present and it directly affected the development of high heterotic hybrid combinations. Therefore, objective of this study was to identify the restorers and potential maintainers to develop CMS line to use them as parents for future hybrid rice programme.

Materials and Methods
The experiment was conducted at Rice Research and Development Institute (RRDI) Batalagoda in two consecutive seasons (*Maha* 2015/2016 and *Yala* 2016). For this experiment, 24 elite inbred and 24 CMS lines were established in the field in order to synchronize the flowering and 76 crosses were done following the emasculation and pollination techniques in *Maha* season. Three weeks after pollination F_1 seeds were harvested and used to field evaluation in testcross nursery in *Yala* 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 nursery and 18 days old seedlings were transplanted in the field. F_1 seedlings and respective pollen parents were planted in progeny following one plant per hill basis. Spacing of two plants was 20cm 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. Pollens fertility and

sterility of F1 plants were observed through light microscope. For this observation, youngest panicles were randomly selected from five plants and spick which were having 15-20 spikelets were collected and put into 70 percent ethanol container. In laboratory, anthers were taken out from at least six spikelets using needle and placed on glass slides with a drop of 1 percent Potassium Iodide 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 by the microscope to identify the spikelet fertility/sterility. Simultaneously, five panicles of F1 plants of different crosses were covered with the paper bags to avoid foreign pollen contamination and confirm the sterility of respective F1 plants. The following criteria was used for classifying the parental lines as maintainers and restorers (Virmani *et al.*, 1997) (Table 1). Morphological characters of fertile crosses (F_1) and respective pollen parents were also measured in order to identify heterosis.

Table 1: 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

Four pollen fertile F_1 crosses were identified such as Bg CMS4A/ SN 307, Bg CMS4A/ SN 309, Bg CMS4A/ SN 313 and IR68280A/SN 394 and these crosses displayed pollen fertilities of 89.8%, 94.6%, 81% and 93.8% respectively (more than 80% pollen fertility). Therefore, these pollen parents SN307, SN 309, SN 313 and SN 394 have restoration ability and can be selected as restores to produce hybrids. However, their restoration ability should be confirmed by doing re-test cross in next season. F_1 combination (IR68280A/SN 394) showed significantly higher productive tillers (18.6) than its pollen parent (SN394) so that it has the ability to produce higher yield than pollen parent (Table 2).

Cross combination Bg CMS4A/ SN 309 and Bg CMS4A/ SN 313 produced significantly higher spicklets per panicle (178.2 and 130.2) than their pollen parents respectively so that it helped to produced high grain yield than pollen parents. Meanwhile cross combination BgCMS4A/SN307 showed significantly lower spicklets per plant than its pollen parent while it did not show significantly higher values for NPT, PL and PH.

Out of 76 crosses, four cross combinations such as IR69625/ SN395, IR73794/ SN395, IR69625/ SN405, IR73794/ SN308 were identified as pollen sterile crosses with the pollen sterility of 99.2%, 98.6%, 99.2% and 99.6% respectively and it indicated that the male parents used to produce cross combinations have an ability to maintain the respective CMS lines so that such male parents can be used to developed new CMS lines by following back cross breeding method. All these male parents SN395, SN405 and SN308 recorded >98.6% pollen sterility and it was selected to advance the back-cross breeding programme. Meanwhile, 42 partially maintainers and 26 partially restorers could be found and it indicated that such male parents, could not have restoring or maintaining ability.

Table 2: Comparison of morphological characters of F1 hybrid with respective selected restorers

F1 hybrids	NPT		PL(cm)		PH(cm)		NUPT		NS/P	
BgCMS4A/SN307	12.2ns	±1.2	24.8ns	±0.37	94.8ns	±2.7	3.6ns	±0.93	73.4*	±8.1
SN307	12	±1	24.8	±0.37	101.4	±4.3	4.6	±0.75	119	±14
BgCMS4A/SN309	9.4ns	±1.2	25.8ns	±0.86	95.4	±2.6	1.4ns	±0.51	112	±13
SN309	11.6	±1.3	25.2	1.2	96ns	2.3	1	0.32	178.2*	11
BgCMS4A/SN313	13.2	±1	26ns	±0.71	93ns	±3.6	1.4ns	±0.24	66.8	±13
SN313	13.6ns	±1.2	27.8	±0.86	99.4	±1.2	1.4	±0.51	130.2*	±10
IR68280A/SN394	18.6*	±0.98	26ns	±1.4	84.6ns	±4.6	2.8ns	±0.37	201.6ns	±25
SN394	12.4	±1.6	25	±0.45	91.2	±3.1	1.6	±0.51	224.8	±13

NPT- *No. of productive tillers, PL-Panicle length, PH-Plant height, NUPT-No. of unproductive tillers, NS/P-No. of speckles per panicle*