ISOLATION AND CHARACTERIZATION OF MAINTAINERS AND RESTORERS FROM INTER SUB- SPECIFIC (*indica x japonica*) DERIVATIVE LINES OF RICE

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CERTIFICATE

This is to certify that the thesis entitled, ***" Isolation and Characterization of Maintainers and Restorers from Inter Sub-specific (indica x japonica) Derivative Lines of Rice "*** *submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE in Genetics & Plant Breeding,*** *embodies the result of a piece of bona fide research work carried out by* ***ARMIN BHUIYA,*** *Registration No. 27449/ 00656 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated: June, 2008
Place: Dhaka, Bangladesh

....
(Prof. Dr. Md. Sarowar Hossain)
Supervisor

Dedicated To
My
Beloved Parents

SOME COMMONLY USED ABBREVIATIONS

Abbreviation		Full word
%	:	Percent
AEZ	:	Agro-Eecological Zone
A line	:	Male sterile line
BBS	:	Bangladesh Bureau of Statistics
BC	:	Back cross
B line	:	Maintainer line
BRRI	:	Bangladesh Rice Research Institute
cm	:	Centi meter
CMS line	:	Cytoplasmic male sterile line
et al.,	:	et alu = other people
F_1	:	First filial generation of a cross between two genetically dissimilar homozygous parents.
g	:	Gram (s)
I-KI	:	1 % Iodine Potassium Iodide solution
IARI	:	Indian Agricultural Research Institute
i.e	:	ed est (means That is)
IRRI	:	International Rice Research Institute
ml	:	Mili litre
ms	:	Male Sterile
NARS	:	National Agricultural Research System
No	:	Number
OCR	:	Out crossing rate
PER	:	Panicle extertion Rate
PGMS	:	Photoperiod sensitive genetic male sterility
pp	:	Pollen parents
R line	:	Restorer line
SAU	:	Sher-e-Bangla Agricultural University
TC	:	Test cross
TGMS	:	Temperature sensitive genetic male sterility
Viz.	:	Namely
WA	:	wild abortive
wc gene	:	Wide compatibility gene

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(Armin Bhuiya)

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ISOLATION AND CHARACTERIZATION OF MAINTAINERS AND RESTORERS FROM INTER SUB- SPECIFIC (*indica x japonica*) DERIVATIVE LINES OF RICE

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ABSTRACT

The study was conducted at the experimental farm of Sher-e-Bangla Agricultural University, Dhaka-1207, during Kharif 2006, Rabi 2006-2007 and kharif 2007 seasons with the objective of identification of potential restorers and maintainers from *indica/japonica* derivative lines and conversion of selected maintainers possessing desirable plant type into CMS lines. To achieve the objectives, 65 test cross combinations including one wild abortive cytoplasmic male sterile (CMS) lines and 46 pollen parents were used as material of the study. The aspects of the study were development of source nursery and test cross nursery, estimation of pollen fertility, identification of potential restorers and potential maintainers. The study showed that of all the pollen parents in 65 success test crosses, 14 were restorers (21.54%), 27 were partial restorer (41.54%), 14 were partial maintainers (21.54%) and 10 were maintainers (15.38%). Out of 14 restorer lines, 11 were selected on the basis of crop uniformity and all types of observation were recorded only from uniform lines. Based on scale of different characters a combined score was computed and on the basis of pooled approach of ranking, five restorers having the rank I to III will be used for production of experimental hybrid in next season. The 10 lines identified as maintainers were further subjected to screening based on various morphological characters favouring out-crossing, increased seed production, good grain quality as well as high level of heterosis. Following various scoring parameters and overall rankings four maintainer lines identified which were chosen for conversion into new CMS lines in the next crop season.

Chapter 1
Introduction

1. INTRODUCTION

Rice (*Oryza sativa*) is second most important food crop next to wheat as it feeds more than half of world population. The current world population is over 6 billion and will reach to 8 billion in 2030. Meanwhile, the annual loss of land to other use is 10 to 35 million ha, with half of this lost land coming from cropland (Yuan, L.P.2004). Facing such severe situation of population growth pressure plus cropland reduction, it is obvious that the only way to solve food shortage problem is to greatly enhance the yield level of food crops per unit land area through advance of science and technology.

It has been estimated that the world will have to produce 60% more rice by 2030 than what it produced in 1995. Therefore, increase production of rice plays a very important role in food security and poverty alleviation. Theoretically, rice still has great yield potential to be tapped and there are many ways to raise rice yield, such as building of irrigation works, improvement of soil conditions, cultural techniques and breeding of high yielding varieties. Among them, it seems at present that the most effective and economic way available is to develop hybrid varieties.

In Bangladesh, rice is grown in 10.579 million hectares and production is 27.318 million metric tons with average yield is 2.58 tons per hectare (BBS 2006-2007). Rice is a major source of livelihood in terms of providing food, income and employment in Bangladesh. Increasing population growth rate of Bangladesh and continuous shrinking land area with expanding urbanization and industrialization, on the other hand plateauing of yield level of high yielding variety- increase production must be achieved with less land, less labour, less water and fewer chemicals. Cultivation of semi dwarf varieties, the achievement of the required yield target is an uphill task.

Of the various short and long-term approaches contemplated for raising further the yield threshold of rice (*Oryza sativa* L.), exploitation of hybrid vigour is considered the most feasible and readily practicable approach. China pioneered the hybrid rice research as early as 1970s and demonstrated 20-30% yield advantage of hybrids over conventional varieties. Out side of China, India is the first country to develop and commercially exploit the hybrid rice technology for the last eight years and in a short span of ten years time, 17 hybrids have been released for commercial cultivation. About 20 countries including Bangladesh in the world have started strong research program on hybrid rice technology and in the mean time Bangladesh has released two hybrids by BRRI and 8 to 10 hybrids by private seed companies for commercial cultivation. But there has been a massive international exchange of *indica* germplasm among the rice growing countries during the last 34 years through international nurseries; it has led to the narrowing of genetic diversity among the improved *indica* germplasm of various breeding programs, hence the lack of heterosis in many *indica/indica* hybrid combinations (Khush and Aquino, 1994).

The magnitude of heterosis depends upon the genetic diversity between two parents. In general, the more distantly the parents are related, the higher the heterosis. Rice hybrids grown in China, India, Bangladesh, Vietnam and the experimental hybrid under tests in other countries are based on *indica* rice germplasm and it has been already proved by many scientists that *indica/indica* hybrids presently started to show their yield plateauing.

Indica and *japonica* germplasm, however, have remained distinct and there has been very little gene flow between these two varietals group. With the availability of wide compatibility genes (WC) allowing normal spikelet fertility in *indica/japonica* crosses, exploitation of heterosis between two sub specific groups has become possible. Hybrids from *indica* and *japonica* parents show 30-40% yield advantage over the best existing *indica/indica* hybrids (Yuan,1994). Though *indica/japonica* hybrids give 30-40% heterosis but hybrids developed directly from *indica/japonica* varieties suffer from serious problems of grain quality, as both of them are very diverse in quality traits. In order to overcome the problems of grain quality of *indica/japonica* hybrids, it has been proposed to use *indica/japonica* derived lines with diverse grain quality for development of such inters sub-specific hybrids, without losing of heterosis. Therefore, it requires large inter sub specific derived population to identify right kind of parental lines.

For the development of three-line rice hybrid using *indica/japonica* derived lines, it is necessary to have heterotic three lines from such derivatives. Three parental lines includes a cytoplasmic male sterile (CMS) line (A line), maintainers (B line) line that is similar to A line with respect to nuclear genome but is male fertile and a restorer (R) line for restoring in the hybrid (F_1). Therefore, the first step in this direction involves development and identification of effective and stable restorer (R lines) and maintainer lines (B lines) in Bangladesh condition. Test crossing (CMS x source line) is the first basic step in hybrid rice breeding program to identify restorers and maintainers on the basis of fertility/sterility of pollen and spikelet in the resulting hybrids. These lines combine new plant type characters like strong culms, moderate tillering, dark green leaves and high grain number per panicle.

The restorer lines must ensure completely spikelet fertility and high degree of standard heterosis. The success of hybrids development would depend upon the availability of good restorer combining ideal rice quality. Therefore, there is a need to have a continuous restorer development, identification and improvement program using genetic material from *indica/japonica* derivatives. Similarly the lines to be converted into new CMS lines (A lines) must combine good agronomic traits and grain quality with complete maintaining ability of the cytoplasmic male sterility. At the same time it must show high level of heterosis when crossed with restorers of *indica* varieties.

Keeping the forgoing problems in view, present study was under taken using *indica/japonica* derived lines with the following objectives:

1. Identification of potential maintainers and restorers from *indica/japonica* derivatives
2. Conversion of selected maintainers possessing desirable plant type into CMS lines

Chapter 2

Review of Literature

2. REVIEW OF LITERATURE

Heterosis refers to the increase or decrease of F_1 value over the mean parental value (relatives heterosis). The term heterosis was coined by Shull in 1908 and is usually referred to relative heterosis. From the view point of plant breeding, increase of F_1 over the better parent and/or the best commercial variety is more relevant. The former is designed as heterobeltiosis (Fouseca and Patterson, 1968) and the latter standard heterosis.

Though the phenomenon of heterosis in rice was known since 1928, its exploitation in rice could become a commercial reality in 1978 after release of first rice hybrids in China under the able leadership of Prof. Yuan Long Ping. After the first report by Jones (1926) who observed that some hybrids (F_1) of rice had more culms and higher yield than their parents, during the next 35 years, several other researchers (Ramiah 1935; Idsumi 1936; Kadam *et al.,*1937; Capinpin and Singh 1938; Ramiah and Ramaswamy 1941; Brown 1953; Oka 1957; Sen and Mitra 1958) confirmed its occurrences in this strictly self pollinated crop.

In the 1970s, Chinese scientists amply demonstrated that the use of hybrid rice could increase rice yields in China by 15-20%. Hybrid rice is now used extensively in China. But hybrid rice from China was neither adapted to tropical conditions nor was it available freely to countries outside China. Therefore, IRRI began research in 1979 to explore the potential of adapting this technology for the tropics. Soon, it was concluded that hybrid rice offered an important option to increase varietals yields in the tropics. This encouraged several tropical rice-growing countries to develop this technology either independently or in collaboration with IRRI and/or China. Currently, 17 national programs are involved in developing hybrid rice: Bangladesh, Brazil, Colombia, Egypt, India, Indonesia, DPR of Korea, the

Republic of Korea, Japan, Malaysia, Myanmar, Pakistan, the Philippines, Sri Lanka, Thailand, the United States, and Vietnam. Hybrid rice research programs in national agricultural research systems (NARS) are in different stages of development. Although China has the strongest national network in hybrid rice, programs in Brazil, India, Japan, and the United States are well established. Other countries may take 3-5 yr to develop such programs. Several private companies in Brazil, India, Japan, and the United States have developed a strong research base in hybrid rice while some are still developing this and others are involved solely in seed production activities.

Hybrid rice technology has been recognized as a viable technology for meeting the increased demand for rice in Bangladesh. Research and development (R&D) of hybrid rice technology began in 1993 with the introduction of rice hybrids and parental materials from IRRI. The technology was initially constrained by (1) a lack of conviction by researchers, policy makers, and administrators about the economic viability of the technology, (2) a lack of trained human resources to develop and use the technology locally, (3) a lack of knowledge about its seed production technology, and (4) the poor performance of introduced hybrids over conventionally bred released varieties. Over the past few years, some of these constraints have been overcome and the government is convinced about the potential of this technology and is committed to giving priority to accelerating hybrid rice R&D.

Several IRRI-developed hybrids were tested in preliminary to advanced yield trials during the last four years at different locations in Bangladesh. The objective was to test the yield potential of the IRRI-developed hybrids under ideal boro conditions and then select the best hybrids for Bangladesh. Two hybrids, IR69690H and IR68877H, were nominated in the National Hybrid Rice Trial in the boro season

(1999-2000) coordinated by the Seed Certification Agency and the Department of Agriculture Extension. After extensive evaluation, the National Seed Board recommended IR69690H as BRRI hybrid dhan-1, primarily in the Jessore and Barisal region, with a view to subsequent cultivation in farmers' fields in other regions of the country. Seed production is an important component of the development and use of hybrid rice technology. Results of the past two years show the possibility of raising the yield of F_1 hybrid seed on average to 2.0 t ha^{-1}.

The Bangladesh Rice Research Institute (BRRI) maintains close collaboration with IRRI and receives technical support, including seed materials and training, to establish an effective hybrid rice program in Bangladesh. Nucleus and breeders' seeds are being produced under the supervision of the plant breeder at BRRI and those are being supplied to public and private seed-producing agencies for commercial seed production. BRRI is sharing its parental materials of promising hybrids with NGOs to multiply seed of parental lines and produce F_1 hybrid seeds.

The level of heterosis for yield and/or any other agronomic traits in hybrids depends to some extent on genetic distance between parents. Most of the hybrids grown in China and else where are based on *indica* rice germplasm which has adequate genetic diversity. Heterosis in *japonica* rice hybrids developed in China, Japan, and DPR Korea, is relatively lower (usually less than 10%). However, there are reports that *indica/japonica* hybrids display remarkable heterosis in yield-effecting traits such as spikelet number. Yuan, (1994) has reported that the magnitude of heterosis in Asian cultivated rice is in the order of *indica/japonica* > *indica/javonica* > *javonica/javonica* > *indica/indica* > *japonica/japonica* , where, *indica /japonica* hybrids exhibit stronger heterosis for both sink source, including total biomass, spikelet number, 1000-grain weight etc. Their theoretical yield may be 30% more than the existing highest yield of *indica/indica* hybrid varieties.

Exploring the strong heterosis in *indica/japonica* hybrids has been the major goal of the three line hybrid breeding program. However, in order to achieve it, four barriers normally found in such hybrids (F_1) must be overcome; these are: low seed setting rate , too tall plant height, very long growth duration and poorly filled grains. Ikehashi and Araki (1984) discovered a genetic tool, designated as wide compatibility (WC) gene(s). By using this genetic tool (WC genes), the low seed setting rate caused by semi-sterility due to incompatibility between *indica* and *japonica* can be raised to nearly normal levels. They showed that gamete abortion by an allelic interaction at a locus (designated as S_5) on chromosome 6 caused hybrid sterility and that incorporation of neutral allele into one of the parents overcomes sterility in F_1\`s. This neutral allele is now popularly known as "Wide Compatibility" (WC) gene. At S_5 locus on chromosome 6, *indica* and *japonica* have $S_5{}^i$ and $S_5{}^j$ alleles, respectively and some *javonica* (topical *javonica*) have a neutral allele $S_5{}^n$. The SS genotype shows gamete abortion, while $S_5{}^n$ / $S_5{}^i$ and $S_5{}^n$ /$S_5{}^j$ or $S_5{}^i$ /$S_5{}^n$ and $S_5{}^j$ /$S_5{}^n$ do not.

Ikehashi and Araki (1984) screened 74 rice varieties and identified some WCV_S which originated from Indonesia or Bengal. Subsequently, the WC ($S_5{}^n$) locus was closely linked with marker gene C (chromosome for pigmentation) and wx (waxy endosperm) which are located on chromosome 6 (Ikehashi and Araki, 1986, 1987). \`WC' gene has been incorporated in to *japonicas* and successfully used to obtain fertile *indica/japonica* hybrids (Araki *et al.,* 1988, Ikehashi, 1991). Recently, large number *indica/ japonica* derivative lines possessing WC genes have been developed IRRI and IARI.

Transferring an allelic dwarf gene (Sd-1) into male as well as female parent can lower the plant height of *indica/jponica* hybrids to a semidwarf level, yet they still express strong heterosis. By crossing parental lines of different growth duration, except the photosensitive late varieties, *indica/jponica* hybrids with medium and even shorter growth duration can be obtained.

Commercial production of hybrid rice depends on three-line breeding, male sterile (A line), maintainer (B line) and restorer (R line). Development of new parental lines and their production is essential for the sustainability of hybrids. About 95% of the commercial hybrids in China and elsewhere are based on one and only one wild abortive (WA) cyto-sterility system. This excessive reliance on a single source of sterility may lead to genetic vulnerability of hybrids to sudden outbreak of diseases and insect pests. Other drawbacks of the WA system are the poor panicle exertion and undesirable flowering behavior leading to low seed yield in seed production plots. To overcome these disadvantages diversification of the CMS sources should be carried out for developing inter- varietal hybrids as well as inter-sub specific hybrids.

Development of effective restorers which ensures complete spikelet fertility and high degree of standard heterosis in traits of economic importance is yet another pre-requisite for hybrid breeding. However, the restorer gene is very rare in *japonocas.* Ikehashi *et al.,* (1984) reported that advantage of *indica /japonica* crosses is that the *indica*-resotring gene can be used directly for *japonica* cytoplasmic male sterile lines.

An attempt has been made here to review the available literature covering the areas of basic importance relevant to development and evaluation of three-line rice hybrids using inter-sub specific *(indica/japonica)* derivatives.

These are given head- wise below:

2.1 IDENTIFICATION OF POTENTIAL RESTORERS AND MAINTAINERS FOR EXPLOITATION OF HETEROSIS IN RICE

The availability of effective restorers and promising maintainer lines is an essential prerequisite for exploitation of heterosis in grain crops using cytoplasmic sterility system. Test crossing (CMS x source line) is the first basic step in hybrid breeding program to identify restorers and maintainers on the basis of, fertility / sterility of pollen and spikelets in the resulting hybrids. Accordingly the respective male parents are designated as maintainers or restorers and only promising maintainer are converted into new CMS and restorer lines for use of developing experimental hybrids. Therefore, identification and/or development of large number of genetically diverse CMS and restorer lines are the key to success of hybrid breeding programme.

2.1.1 IDENTIFICATION AND DEVELOPMENT OF RESTORERS FROM DIFFERENT SOURCES

The effective restorer lines for WA, BT, HL, Dian1and GAM-CMS lines used in China in commercial hybrid rice production were identified or developed through screening, hybridization and mutation breeding (Li and Zhu, 1988). Frequency of restorer lines was higher among rice verities originating in lower latitudes as compared to those originating in higher latitudes. Fertility restorers were found more frequently among wild, photoperiod sensitive and temperature sensitive *indica* rices than in *japonica* or *javonica* rices (Virmani, 1996). Among *indica* rices, restorer lines have been more frequent in late maturing cultivars (Lin and yuan, 1980), perhaps because late maturing *indica* rices are primitive and relatively closer to the wild rice (Yuan, 1985). However, this correlation does not hold true for improved rice varieties and breeding lines (Yuan and Virmani, 1988). Li and Zhu (1988) observed that among three ecotypic rice cultivars viz aman, aus and boro (*indica* rices cultivated in the eastern region of Indian Subcontinent), aman and boro cultivars had a higher frequency of restorers as compared to aus cultivars.

Chinese rice breeders have developed new elite restorers lines through hybridization between restorers/restorers, restorers/maintainers, maintainers /restorers and male sterile lines/restorers besides screening and isolation of restorers on the basis of fertility restoration in test cross progenies. Teng and Shen (1995) suggested four methods for developing wide compatible restore lines (Yang, 1997; Xie *et al.,* 1997) using *japonica* rice as pollen parent. Bharaj and Virmani (1997) suggested random mating of composite populations of restorers developed by male sterility facilitated recurrent selection for improvement of restorer line (Shen *et al.,* 1994)

In the Jiangi province in China, all 16 accessions of wild rice species (O. rufipogon) collected from the Hainan Island completely restored the male fertility of the WA-rice. Similarly, Boro II restored the male fertility of the BT-CMS lines. This indicated that the nuclear genome of the rice germplasm that provides the male sterile cytoplasm is an important source of restorer gene(s). A basic way to obtain restorers is to screen by making many test cross between CMS line and other varieties, including native and new varieties. Cultivars closely related to the male sterile cytoplasm donor germplasm may have restorer gene(s). Most restorer lines of the WA-type CMS lines are cultivars from IRRI-bred materials or with origin close to wild rice, or late season *indica* cultivars found at low altitudes. Mutation induction by chemicals or by irradiation may create new restorer line with the lower plant height, early maturity along with a stronger fertility restoration capacity (Shen *et al.,*1994). The evolution sequence was considered to be from wild rice to late *indica,* to early *indica* to late *japonica*, and to early *japonica*. It appears that the rice cultivars closer to wild rice are likely to have the restorer gene(s) for the WA-type A line (Li and Yuan, 2000).

A large number of effective restorers for WA-CMS lines have been identified among cultivated rice varieties and elite breeding line in China and outside. Restorers have also been identified for the CMS-ARC system at IRRI (Virmani et *al.*, 1994) and CMS-DI, CMS-DA, CMS-IP, CMS-HL and CMS-GAM system in China (Virmani, 1996). In India, identification of restorers/maintainers for CMS-WA and some other system have been reported by Hassan and Siddiq (1983), Mohanty and Sharma (1983), Govinda Raj *et al.,* (1984), Ratho and Pande (1985), Sahai *et al.,* (1986), Rangaswamy *et al.,* (1987), Singh and Sinha (1987), Sharan and Mandal (1988), Bijral *et al.,* (1989), Sharma and Mani (1989), Pande *et al.,* (1990), Tomar and Virmani (1990), Bharaj and Sidhu (1991), Bijral *et al.,* (1991), Manuel *et al.,* (1991), Pradhan and Jachuck (1991), Pradhan *et al.,* (1992), Bijral *et al.,* (1993), Manuel and Rangaswamy (1993), Maurya *et al.,* (1993), Oniam and Kaushik (1993), Jayamani *et al.,* (1994), Leenakumari *et al.,* (1994), Siddiq *et al.,* (1994). Mahadevappa (1994), Singh *et al.,* (1991), Leenakumari *et al.,* (1997), Sarial and Singh (2000), Hariprasnna *et al* (2001) and Prasad and Mani (2001)

Japonica rices are mostly non-restorer, Casal and Virmani (1998) reported that for identification of restorer lines among improved tropical *japonicas,* 239 test crosses were made at IRRI. Almost all the tropical *japonica* lines were found to be maintainers or at least partial maintainers; none of the 128 lines tested so far could restore the fertility of WA, ARC and mutagenized IR62829B CMS lines. Tai (1995) reported that for searching fertility restorers among traditional tropical *japonica* varieties, about 225 testcrosses were made at IRRI with *indica* CMS lines carrying CMS-WA and CMS-O. *perennis* cytoplasm. None of the lines was found to be effective restorer. The appreant lack of restoration ability in *japonica* germplasm indicated that direct *japonica* lines can not be used as a pollen parent of *indica*/tropical *japonica* rice hybrids.

In breeding for *indica/japonica* hybrids suited for tropical conditions, using *japonicas (*temperate/tropical*)* as female parents is more appropriate as they are poor pollinators when used as male parent. It is reported that natural out crossing is more in *japonica* compared to *indica* (Oka, unpubl). Additionally the occurrence of fertility restoring gone is more common in *indica* genotypes unlike in their counterpart *japonicas,* it is suggested that *japonicas (*temperate/tropical*)* should be used as female parents and *indica/japonica* derivatives lines as a male paerent in the development of *japonica/indica* inter sub-specific hybrid (Vijayakumar, 2003).

There are two possible approaches to develop *indica/japonica* derivative lines, one to use *japonica* to transfer 'WC' gene into *indica* to develop male parents and the second is to use *indica* source transfer 'WC' gene. In the first approach by using *japonica*, it is likely that male parents finally developed would contains genes from *japonica* by which their relatedness to *japonica* female parent will be closer and thereby the magnitude of heterosis may be reduced, and it is also expected that the desirable floral characteristics of pollen parent may get affected in the male parents. Therefore, the second approach seems to be more appropriate, where WCVs that are most related to *indicas* should be used (Vijayakumar, 2003). It has been suggested that use of WCVs such as 'Dular' having been confirmed by many workers including Ikehashi as good sources of 'WC' gene should be used for developing *indica/japonica* derivatives male parents carrying WC gene.

2.1.1.1 Breeding for restorer lines

The low frequency of effective restorers in the natural population necessitates breeding for restorer lines with strong restoring ability (> 80% seed set in the F_1 hybrid), good combing ability and other traits such as good adaptability, resistance to pests and diseases, high grain quality etc., and good outcrossing characteristics like large quantity of pollen, long blooming duration, good pollen shedding, strong tillerilng capacity, slightly longer growth duration, and more height than A lines. Restorer breeding is also essential to further enhance the yield levels of hybrid rice. The common breeding procedure for R lines involves transfer of restorer gene(s) to a new rice cultivar through single crossing. The segregating progeny that have restoring ability are selected for the improved traits using the pedigree method. The single crossing can consist of RxR, BxR or RxB and AxR (iso-cytoplasmic R lines) combinations. Multiple cross breeding methods can combine advantages, such as good resistance, good grain quality and early maturity from each parent into a new restorer line (Li and Yuan, 2000). Isolation of restorers through mutation breeding following gamma ray irradiation of existing restorers. IR 24 and IR 26, for WA cyto-sterility was reported by Li and Yiao (1982).

Development of restorers that can restore fertility in CMS lines of different cyto-sterile sources of both *indica* and *japonica* origin will be helpful in diversifying the parents of both hybrid combinations. Yan *et al.* (1996) proposed androgenesis as a quick and effective way of developing widely compatible restorers in rice and that is applicable to the direct use of inter sub-specific heterosis with the three-line method. They selected 20 doubled haploid lines from F_1 plant that showed normal fertility with both *indica* and *japonica* CMS lines.

Yang (1997) reported breeding of widely compatible restorer lines in rice through anther culture of various hybrids (F_1) of *indica* restorers lines x wide compatible varieties and *japonica* restorer lines x wide compatible varieties. The lines developed had shown fertility restoration of 36.7 to 64.7% and seed setting of more than 80%. The doubled haploid lines showed wide compatibility and fertility restoration ability to both WA and BT CMS lines. Strong standard heterosis was observed for yield in F_1 of CMS x widely compatible restorers. Araki *et al* (1986) reported that incorporating the S_5^n allele in to; *indica* or *japonica* breeding lines is expected to overcome the sterility problem in *japonica/indica* crosses. Specifically, those lines with S_5^n can be used in *japonica/indica* crosses for hybrid seed production, because such lines show high heterosis without F_1 sterility. They suggested that hybrid seed production from a *japonica/indica* cross, it is necessary to consider the components of hybrid seed production into which the S_5^n allele is to be incorporated. Many high yielding *indica* varieties, including IR36 and IR50, are known to possess a restorer gene *Rf* for cytoplasmic male sterility (CMS) from a tropical rice variety Chinsurah Boro II. If any *indica* line with S_5^n is developed, that can be used directly as the restorer in hybrid seed production.

Another way to use S_5^n is to breed *japonica* lines by backcrossing the S_5^n line into a CMS donor. That way, it is possible to develop *japonica* CMS lines and *japonica* maintainers, both of which have S_5^n. The Hybrids of those two, fertilized by any *indica* pollen donor, would produce fertile *indica/japonica* hybrids, which could exhibit high heterosis.

Tsuchiya *et al.* (2002) reveals that breeding of improved restorer or maintainer lines with more or less genetic background of *indica* is more practical than a direct hybrid of *indica* and *japonica*.

The hybrids of *indica/ japonica* have shown high heterosis but their grain quality seems to be serious impediment. This has promoted to the use of *indica/ japonica* – derived lines to overcome quality problems. In this direction, IARI scientist, using locally developed improved varieties/lines viz. Pusa 2-21 and PB-1, two IRRI varieties IR72, two land races (N-22 and Gharbharan) and two *japonica* varieties (Tainan 3 and Xiangnuo 4) have successfully developed *indica /japonica* derived lines combining long slender grain and new plant type characteristics following a breeding scheme.

2.1.2 IDENTIFICATION AND DEVELOPMENT OF MAINTAINERS FROM DIFFERENT SOURCES

Male sterility is a reproductive deficiency of some plants where male organs in hermaphrodite flowers are rendered defunct. It relates particularly to non-viable pollen grains which are followed through a chain of vital process during microsporogenesis. These process are so delicately balanced under genetic control of many loci that mutation at any one locus may throw the entire process of microsporogenesis astray resulting in the formation of non-functional microspores, and hence male sterility. Male sterility may be conditioned due to cytoplasmic or genetic factor, or due to interaction of both. Depending on these factors, it is classified into four types:

1. Genetic male sterility
    a) Temperature-sensitive genetic male sterility (TGMS)
    b) Photoperiod sensitive genetic male sterility (PGMS)
    c) Transgenic genetic male sterility
2. Cytoplamsmic male Sterility (CMS)
3. Cytoplasmic genetic male sterility (CGMS)
4. Chemically induced male sterility

Among these sterility systems, cytoplasmic male sterility (CGMS) system which is also referred as three line system is in maximum use for exploitation of heterosis in rice.

2.1.2.1 Cytoplasmic-genetic male sterility (CGMS)

The phenomenon where sterile pollen grains are produced on a plant by interaction of cytoplasmic factors and nuclear gene(s) is known as cytoplasmic genetic male sterility (CGMS). The CGMS is controlled by a homozygous recessive gene(s) for sterility in sterile cytoplasm, but not in the fertile cytoplasm i.e., the sterility reaction is under the control of interaction between cytoplasm and nuclear genes. The corresponding dominant allele (s) when present either in sterile or in normal cytoplasm, makes the male fertile. This type of male sterility has been found to be the most effective genetic emasculation tool for developing hybrid (F_1) seeds. The system for hybrid seed production includes a cytoplasmic male sterile (CMS) line (A line), maintainers (B line) that is similar to A line with respect to nuclear genome but is male fertile, and a restorer (R) line for restoring the male fertility in the hybrid (F_1) seed. The " cytoplasmic genetic male sterility " is essentially " cytoplasmic male sterility" with a provision for fertility restoration by nuclear gene(s) and hence also referred as CMS in plant breeding.

2.1.2.2 Source of sterile cytoplasms

The earliest reports of cytoplasmically induced male sterility in rice were made by weerarante (1954) and Sampath and Mohanty (1954), as cited by Virmani *et al.* (1986). The first CMS line was developed by Shinjyo and Omura (1966) in Japan using 'Chinsurah Boro II' cytoplasm in the nuclear background of 'Taichung 65'. Some other male sterile lines from the *japonica* subspecies were also developed in the 1960s (Watanabe *et al.,* 1968).

In China, the research on male sterility in the rice was initiated in 1964 when Yuan Long Ping found several male sterile plants in a field of Dong-Ting-Wan-Xian, a late-maturing *indica* rice. And a breakthrough was made in 1970 when Li Bihu found a natural male sterile wild plant (designated WA) on Hainan Island. Then in 1973, some elite *indica* MS lines of WA type (Er-jiu-Nan 1A, Zhen-Shan 97A, and V41A), their maintainers and restorers were successfully developed by the cooperative research of Hunan, Jianxi, Fujian and other provinces (Yuan, 1977).

Also, in 1965 Li Zhengyou (1980) found some MS plants in the field of Taibei 8 and later developed the *japonica* MS lines of Dian 1 type, such as Hong-Mao-Ying A. in 1972, Zhang and Zhu developed *indica* MS lines of Hong-Lien type, in which the sterile cytoplasm was formed red-awned rice (Wuhan University, 1977). Zhou *et al.* developed Gam type sterile lines by using Gambiaca, an *indica* variety of Western Africa as the source of sterile cytoplasm (Sichuan Agricultural college, 1973).

After then a large number (more than 600) of CMS lines have been developed in China, IRRI, and else where from various wild and cultivated accession. It is believed that there are about 64 cytoplasmic sources, among which 22 sterile cytoplasms are from wild rice (Li and Yuan 2000).

Virmani and Edwards (1983) and Virmani and Wan (1988) listed some of the CMS sources identified in and out side China. These CMS sources are designated, in principle, according to the name of the cultivar from which the cytoplasmic factor including male sterility is derived. Some of the cytoplasmic sources for including male sterility in rice are listed in Table 1.

2.1.2.3 Breeding for cytoplasmic genetic male sterile lines

Elite lines are first testcrossed with the CMS line of a desired cytoterlity source to test their maintaining ability. Those elite lines which are identified as maintainers are repeatedly backcrossed up to six to eight generations for complete transfer of cytosterility source.

The basic methods for breeding cytoplasmic male sterile lines are of two types:

a) Intervarietal nucleus substitution backcross

The parents of most crosses either have relatively distant genetic relations, or originated in distant areas or belong to different ecotype. More than 10 types of MS lines with sterile cytoplasm have been developed by nucleus substitution between distantly related *indicas*. In addition, MS lines have been developed through nucleus substitution between distantly related *japonicas*, inducing MS lines of Dian, Dian 4, and Dian 6 types, etc.(Zebing and Yingguo. 1986.)

Table 1. Cytoplasmic sources for including male sterility in rice

Designation	Cytoplasm	Strain	Nuclear donor
CMS-WA	*Oryza Sativa f. Spontanea*	Wild abortive	Several
CMS-DA	Do	Dwarf abortive	Xue-Qin-Zhao
CMS-IP	Do	Indonesian paddy	11-32
CMS-DI	Do	Dissi type	297
CMS-HL	Do	Hong Lien	Lien – Tong
CMS-YC	Do	Ya Cheng	Guang-Xuan 3
CMS-TD	Do	Tiandong	Zhen-Shan 97
CMS-LZ	Do	Liuzhou	Zhen-Shan 97
CMS-IN	Do	Indian	Jing-nan-Te 43
CMS-DP	Do	Dong Pu	,,
CMS-JNY	Do	Jun Niya	Chao-Yang 1
CMS-HP	Do	He Pu	Li-Ming
CMS-TQ	Do	Teng Qiao	Er-Jiu-Quing
CMS-SY	Do	San Ya	Jing Ying 1
CMS-RP	Do	Rao Ping	No. 6964
CMS-GZ	Do	Guangzhou	No. 6964
CMS-CW	Do	Chinese	Fujisaka 5
CMS-OF	*Oryza fatue*	-	Fujisaka 5
CMS-KR	*Oryza rufipogon*	KR 7	Taichung 65
CMS-W18	*Oryza perennis*	W 1080 (India)	Taichung 65
CMS-W19	Do	W 1090 (India)	Taichung 65
CMS-O	Do	-	-
CMS-IR	Do	Acc. 104823	IR 64
CMS-OG	*Oryza glaberrima*	-	Colusa
CMS-DBT	Do	Danboto	Hua-Ai 15
CMS-bo	*Oryza sativa*	Chinsurah Boro II	Taichung 65
CMS-Id	Do	Lead Rice	Fujisaka 5
CMS-Bi	Do	Birco	Calrose Caloro
CMS-TN	Do	Taichung Native 1	Pankhari 203
CMS-ak	Do	Akebono	O. glaberrima
CMS-STB	Do	E-Shan-Ta-Bai-Gu	Hong-Mao-Ying
CMS-TJD	Do	Tian-Ji-Du	Fujisaka 5
CMS-IR 24	Do	IR 24	Xiu-Ling
CMS-JCN	Do	Jing-Chuan-Nuo	Nan-Tai-Geng
CMS-SQ	Do	Shenqi	Nong-Ken 8
CMS-LU	Do	Li-Up	Jing-Ying 83
CMS-ZJF	Do	Zao-Jin-Feng	Lanbery
CMS-GAM	Do	Gambiaca	Chao-Yang 1
CMS-ARC	Do	ARC-IRRI Acc. 13829	IR 54755
CMS-TA	Do	Tadukan	Norin 8
CMS-ZTB	Do	Zao-Tong-Bei	Ke-Qing 3
CMS-sp	Do	MS 577A	IR 42

Source: Virmani and Edwards (1983), Virmani and Wan (1988), Kinoshita (1997), Virmani *et al.* (1997)

b) Distant nucleus substitution backcross : Two types:-

i) **Interspecies nucleus-substitution backcross:** Crossing between wild species and cultivated varieties can help to identify new CMS sources.

Yuan (1985) reported that when wild rices were used as female parents to cross with cultivar rices, as recurrent male parents, the sterile plant percentage was rather high in the progeny of such nucleus-substitution crosses particularly in wild rice/*japonica* rice, all crosses produced sterile progeny. In the crosses of wild rice/*indica* rice, sterile progeny rate was about 85%. The MS lines developed through the nucleus-substitution between wild rice and cultivar rice is most abundant in China. However, with few exceptions, such as Hong-Lien, Tianye, and Dian 9 types, most MS lines have similar relationships between maintainers and restorers as do the MS lines of WA type. Most progeny are fertile in adverse nucleus-substitution crosses i.e., cultivars as non recurrent female parents and wild rice as recurrent male parents. Exceptions include four types of sterile lines.

ii) Inter subspecies nucleus substitution backcross

Inter subspecies nucleus substitution backcross between *indica* and *japonica*, usually *indica*s are used as female parents and *japonica* as recurrent male parents. More than 20 types of *indica* cytoplasmic MS line have been developed in China (table2). Most cytoplasms came from relatively primitive native *indica* varieties or geographically distant *indica* varieties. Almost all *japonica* MS lines developed this way are gametophytic. Also, *indica* MS line Taichung Native 1A (Dian 8 type) was developed by substitution backcrossing of the genome of *indica* variety Taichung Native 1 into the cytoplasm of *japonica* variety Ke-Qing 3. However, rarely are MS lines developed by nucleus substitution backcross of *japonica* (zebing and Yingguo, 1986).

2.1.2.4 Classification of male sterile lines

On the basis of morphology of sterile pollen, genetic properties, stainability with I-KI as well as relations between restores and maintainers, Chinese have categorized MS lines into the following three groups:

i) **WA type**: The MS lines of WA type were developed by crossing the wild abortive rice (WA) as the female parent with recurrent male parents, mainly early maturing *indicas* such as Er-Jiu-Nan 1, Zhe-Shan 97, V 20, and V 41. Their sterility was controlled by two pairs of recessive nuclear genes and sterile cytoplasm,. (Gao 1981, hu and Li 1985, Lei 1984, Li 1985, Liang 1980, Liu et al 1982, Lu 1983, yang et al, 1984, Zhou 1983). Most pollen was characterized by irregular shape and unstable with I-KI solution (table- 2). Abortion occurred possibly at or around uninucleate stage or between uninucleate and early Binucleate stages and hence typical sporophytic type (Jiangxi University 1977; Xu 1980, 1982).

In addition to WA type, it also includes *indica* MS lines derived from crosses of wild rices, such as Ai-Bai, Teng-Ye, He-Ye, Lie-Ye, and Yin-Ye types, and some MS lines derived from crosses between *indicas* such as Gam, D, Gu, Yinni and No. 228 types, All these are saprophytic; possess typical aborted pollens, and have maintainers restorer relationships similar to those of WA types (Zebing and Yingguo 1986)

ii) Hong-Lien type: The MS lines of Hong-Lien type were developed by backcrossing red-awned wild rice as the female parent with the recurrent male parent Lian-Tang-Zao, an early matuiing tall *indica*. Their sterility was controlled by some modifying genes as well as sterile cytoplasm (Hu and Li 1985). The pollens are characterized by spherical and sustainable or lightly stainable with I-KI solution. The pollen abortion mainly occurred during the binucleate stage and in spite of partial pollen sterility (fertile pollen is 50 percent in hybrids) seed set is normal. No fertility segregation occurred in the F_2 generation. It is of gametophytic sterility. The restoration spectrum of this type is wider than that of the WA type.

iii) BT type: The MS lines of BT were developed by crossing an Indian spring *indica* Chinsurah Boro II as the female parent with a Chinese *Japonica* variety Taichung 65 as the recruitment male parent. Their sterility was controlled by a pair of recessive nuclear genes and sterile cytoplasm (Hu and Li 1985, Shinjyo 1975). The pollens are characterized by spherical and unstable or stainable with I-KI solution. Normally anthers do not dehisce. The pollen abortion mainly occurred during binucleate or tri nuclear stage i.e. relatively late stage. MS lines of Dian 1 and Dian 3 types come under this group. The hybrids derived from them have normal seed set but with only about 50% pollen fertility. No sterile plants occur in F_2 generation. Maintenance restoration behavior is similar to Hon-Lien type (Xu 1980, Shinjyo 1975).

2.1.2.5 New CMS lines with diversified CMS sources

The first CMS lines used to develop commercial hybrid (F_1) was developed from a single male sterile plant (*Orzya sativa f spontanea*) designated as wild abortive (WA). The WA cytoplasm is the most stable source of male sterility for which a high frequency of restorer is conveniently found, and hene has been most extensively used in China and several other countries. Zhou (1994) observed that most of the rice hybrid in China are based on CMS-WA cytoplasm (87.9%) followed by CMS-DI (7.8%), CMS-IP (0.5%) and other viz., CMS-bo, CMS-HL and CMS-GA (1.2%). According to Brar *et al.*, (1996) about 95 percent of the total area planted to commercial hybrid in China and elsewhere has a single CMS sources, the WA cytoplasm. However, it has been reported that area under combinations involving other male sterility sources is growing gradually year by year (Zeng *et al., 2000*). All the hybrids released in India so far based on the WA cytoplasm alone.

Cytoplasmic diversity among CMS lines is desirable to reduce the potential risk of genetic vulnerability associated with rice hybrids (F_1) developed by using a single cytoplasmic source. To address this problem Virmani *et al.*,(1986) considered identification and used of additional sources of cyto-sterility. To study the cytoplasmic diversity, Virmani *et al.,* (1989) used for *indica* CMS lines viz, IR 54753A (WA), IT 54755A (ARC), Yar Ai Zhao A (GAM) and MS 577A (sp) and two *japonica* CMS lines, viz., Wu 10A and Reimei A (KR), for testcrossing with 28 rice cultivars from IRRI, India, Indonesia and Korea. Based on the spiklet fertility in F_1 plants they concluded that the cytoplasmic factors controlling male sterility in four *indica* CMS lines are different. However, the two *japonica* CMS lines Wu 10A and Reimei A showed similar F_1 sterility reaction and hence were regarded as having the same CMS factors, which are different from those in CMS-WA.

In a study by Rao (1990) two new CMS line; CRMS1 and CRMS2 were identifying following inter varietal crosses and reciprocal between varieties of diverse origins. CRMS 1 was isolated from the F_2 generation of the cross Ratna x Rajai, while CRMS 2 was isolated from the segregation population of the cross Dugansail x IR8/ Waikoku. Variation in fertility restoration in the two cyto-sterile stocks *indica*ted that the cytoplasms conferring male sterility in these two was different. Crosses with maintainers of WA cytoplasm resulted in fertile F_1s showing that male sterility causing cytoplasm in these two lines were different from WA. Pradhan and Ratho (1990) attemted inter-subspecefic and inter-varietal crosses to develop new CMS lines of different sources, using five *indica* varities and four *japonica* varieties. From their study, they could isolated two new CMS lines, Zhunghua 1A and Krishna A of diverse cytoplasm. The male sterile cytoplasm (Kalinga 1) of *indica* CMS line Krishna A was completely different from the WA, GAM, TN and sp male sterile cytoplasms.

At IRRI some new cytoplasmic sources for male sterility have been identified, such as V20B (an A line with different male sterile cytoplasm from WA-type A lines), CMS-ARC, IR 66707A from O. *perennis, O. glumepetlta* and IR 62829B (IRRI, 1995). In addition Blackhall *et al., (*1998*)* reported the development of two novel CMS lines from BT-type CMS source using the asymmetric protoplast fusion technique. In India three new and diverse CMS sources-one from *O. rufipogon* and two from *O. nivara* were identified using substitution backcrossing and embryo rescue technique.

Hoan *et al.,* (1998) studies 132 inter specific-crosses involving four wild *(O. rufipogon, O. nivara, O. barthi and O. longistaminta)* and two cultivated species (*O. sativa and O. glaberrima*) to identify new sources of CMS within A-genome of genus *Oryza.* The newly developed CMS lines were grouped into four types based on pollen morphology and staining pattern, and type of interaction with a set of maintainers and restorers of WA cyto-sterile stock. For the two stable CMS lines MS 577A and IR 66707A, no restorer could be identified from the cultivated rice germplasm. Among the wild accession of A-genome species, three accessions of *O. rufipogon* and one each from *O. sativa f. spontanea* and *O. glaberrima* restored fertility in MS 577A. Rangaswamy and Jayamani (1998) identified some diversified CMS sources for crosses *o. nivara* (105343)/Co 45, *O. barthii (100934)/*IR 50 and *O. nivara* (101508) / IR 64.

2.1.3 Pollen fertility

Male sterility in rice results due to abnormality at any stage in between microsporogenesis and pollen maturation. Failure of the mechanism to form microspores from sporogenous cells results in 'pollen free' types; while failure of the mechanism during pollen development and maturation results in pollen abortion. Chinese scientists classified aborted pollen as typical aborted and binucleate as untypical aborted type.(Devaraj, 1993.)

Chaudhary *et al.,* (1981) studied the pattern of pollen abortion in five cytoplasmic-genetic male sterile (CMS) lines of rice. Pollen grains from six anthers collected from six randomly selected spikelets from each CMS plant was stained in 1% Iodine-Potassium Iodide (I-KI) solution. More than 1000 pollen grains from each CMS line were examined. Based on shape and staining pattern of pollen grains, they classified them into four classes and elucidated the features of different classes (Table 2).

Table 2. Pollen sterility-fertility based on shape and staining pattern

Category of pollen	Nuclear Stage	Development Stage	Shape and Staining behavior
Unstained withered sterile (UWS)	1	Early, middle & late microspore phase	withered and underdeveloped, Unstained
Unstained spherical sterile (USS)	2	Vacuolated pollen phase	Spherical and smaller, Unstained
Stained round sterile (SRS)	3	Engorged pollen phase	Round, Small and Lightly Stained
Stained round fertile (SRF)	3	Engorged pollen phase	Round and large, darkly stained, smooth surfaced

Several authors have attempted to classify restorer and maintainers based on pollen fertility. Saran and Mandal (1988) designated male parents of the F_1 that showed 70-80 percent pollen fertility as restorers and those showing 5-7 percent pollen fertility as maintainers,. Govinda Raj and Virmani (1988) classified the genotypes into restorers/maintainers after testcrossed with cyto-sterile lines based on pollen fertilities of F_1 plants. The classification is as given below (Table 3).

Table 3. Classification of pollen parents based on pollen fertility percent

Class	Pollen fertility (%)
Effective restorer	60-100
Partial restorer	30-60
Partial maintainer	1-30
Effective maintainer	<1

Manoz and Lasso (1991) classified pollen parents of the F_1 with 90-100 percent pollen sterility as maintainers, those with 21-89 percent as partial restorers and those with 0-20 percent as restorers. Manuel *et al.*, (1991) evaluated pollen sterility in the F_1s of 24 crosses involving six CMS lines and 11 varieties. They identified potential maintainers as having 90% pollen sterility and effective restorers as having 10% pollen sterility. Chandra *et al.* 1993 designated male parents of hybrids that showed cent percent pollen sterility as maintainers and restorers where those showing more than 90 percent pollen fertility and the rest were partial restorers. But, according to Prasad *et al.*, (1993), cultivars with more than 95 percent sterile pollen were potential maintainers; those with less than 5 percent were effective restorers. Oinam and Kaushik (1993) categorized zero percent pollen fertility as maintainers, 10-69 percent fertility as partial restorers and 70 percent as effective restorers. Leenakumari and Mahadevappa (1997) identified restorers, partials restorers and maintainers for eight CMS lines of two cyto serile sources (WA and MS 577A) following a criterion based on the observed fertility of CMS lines (0%) as well as the pollen parents and standard check varieties (75-90%). The test entries were calssified into effective maintainers (pollen and spikelets <1%) , partial restorers (pollen and spikelets fertility 1-75%) and effective restorers (pollen and spikelet fertility >75%). They identified 20 completed restorers out of 77 genotypes testcrossed with MS 577A. IRRI (1997) classified the pollen grains based on their shape, size and extent of staining. The classification is as follows (Table 4).

Table 4. Classification of pollen based on stainability and pollen shape

Category	Classification
Unstained withered sterile (UWS)	Sterile
Unstained spherical sterile (USS)	Sterile
Stained round sterile light(SRS)	Sterile
Stained round fertile (SRF)	Sterile

They also classified the CMS lines as follows (Table 5) based on the extent of pollen sterility

Table 5. Classification of CMS lines based on percent pollen sterility

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

The result of the studies conducted at IRRI (IRRI, Annual Report 1982) showed that the stainabilty reaction and shape of pollen grains in male sterile lines is determined by the stage at which the pollen abort and they observed partial to complete stained round pollen grains in MS 577A and Wu10A. But they were found to be inviable later. It suggested that the pollen grains aborting at the uninucleate stage would be unstained, withered and spherical, but those aborting at binucleate stage or even later would be partial to completely stained and round. So, sole dependence on pollen fertility will likely mislead the identification of restorers or maintainers (Sharma 2000, unpublished)

2.1.4 Spikelet fertility

Spikelet fertility has been used as the basis for classification of restorers and maintainers by many researchers. Virmani *et al.*, (1996) revealed that spikelets fertility alone can not be used as a reliable index for studying fertility restoration as the environmental influence on spikelet fertility is greater than on pollen fertility and a combination of the two will be more reliable way to classify restorers. Hariprasanna (1998) observed that low temperature, late maturity and other environmental conditions during reproductive phase might have influenced the spikelet fertility. Sarker (2000) reported that some plants with lower fertility grades based on pollen fertility studies had higher fertility scores based on spikelet fertility analysis. Therefore, studies based on both pollen and spiklet fertility counts are more precise and reliable than those based on spikelet fertility alone.

The spikelet fertility studies for identification of restorers and maintainers by various authors are presented in Table No. 6

Table No 6. Classification of restorers and maintainers based on spikelet fertility

Effective restorers	Partial or weak restorers	Partial or weak maintainers	True maintainers	References
80-100	30-80	1-30	0	Chaudhuryn *et a.l* (1981)
>75	10-75	-	<10	Singh and Sinha (1987)
>80	5-80	-	<5	Watanesk and Sa-nguansaj (1987)
>75	1-75	-	<1	Bijral *et al.*, (1991)
>80	30-79	1-29	0-1	Govinda Raj and Virmani (1989) Bijiral *et al.*, (1991)
>80	10-79	-	<10	Sharma and Man (1989) and Phen Cong Voc *et al.*,(1990)
>80	20-80	5-20	0-5	Tomar and Virmani (1990)
>80	40-79	1-39	<1	Pande *et al.*, (1990)
>80	25-79	<25	<1	Pradhan and Jachuck (1991) and Pande *et al* (1992)
>80	20-79	-	<20	Singh *et al.*, (1991a)
>80	1-79	-	<1	Bijral *et al.*, (1993)
80-100	21-79	2-20	10-1	Mauyra *et al.*, (1993)

Source: Sarkar (2000)

Chapter 3
Materials And Methods

3. MATERIALS AND METHODS

The experiment was conducted at the experimental farm of Sher-e-Bangla Agricultural University, Dhaka-1207, during June 2006 to December 2007 (Kharif *2006,*Rabi *2006- 2007 and* Kharif 2007 season).

The location of the site is situated at 23° 41'N latitude 90° 22' E, longitude and 8.6 m above from the sea level. It falls under sub-tropical climate. The soil of experimental site lies in Agro ecological region of "Madhupur Tract" (AEZ No.28). Its top soil is clay loam in texture and olive gray with common fine to medium distinct dark yellowish brown mottles.

The experimental site has sub-tropical climate. It is characterized by high temperature accompanied by moderate high rainfall during Kharif season (April to September) and low temperature in the Rabi season (October to March).

3.1 OBJECTIVE I: IDENTIFICATION OF POTENTIAL RESTORERS AND MAINTAINERS FROM *indica/japonica* DERIVATIVE LINES

3.1.1 Materials

The experiment materials consisted of 71 testcross combination including one wild abortive cytoplasmic male sterile (CMS) lines and 46 pollen parents (Appendix I). These 71 testcrosses were effective during Kharif-2006 at Sher-e-Bangla Agricultural University Farm, Dhaka. The parents comprised of new plant type lines derived from *indica/japonica* crosses collected from Genetics division, IARI, India and Hybrid Rice Project, BRRI, Bangladesh.

3.1.2 Methods

3.1.2.1 Development of source nursery

It included one CMS line (IR 58025A) and 46 prospective parental lines mainly comprised of *indica/japonica* derivative lines. CMS lines with most stable pollen sterility were used to identify maintainers and restores. The male parent's seed and CMS line's seed were sown in the nursery bed at SAU farm, on 2nd week of June 2006 for this experiment. All the parental lines were grown using single plant per hill with 12 plants per row. Spacing used were 20 x 20 cm. CMS lines were planted over 3 staggered dates at 10 days intervals to synchronize flowering within the lines.

At flowering stage just before panicle emergence completely male sterile plants of the desired CMS line were shifted in pots without disturbing their root system in the afternoon and were placed in net house for 24 hours for proper establishment. In the afternoon of next day spikelet clippings were done with a slant cut for easy pollination.

Next day morning appropriate panicle of pollen parent were collected from source nursery on single plant basis between 9.00 to 10.00 AM and were placed in conical flask with water and kept under sunlight to allow proper exertion of the anthers. Sometimes florets were given slant cut before placing under sun light for effective dusting of the pollens. Panicles with exerted anthers were carefully shaken over the clipped male sterile spikelets and rebagged. Each pollinated panicle was tagged carefully. The crossed CMS plants were maintained under proper care in the net house. Seeds were harvested after 21 days of pollination from CMS plant and also from respective pollen parents from field on single plant basis and stored carefully after proper drying for use in the next season in test cross nursery. For individual test cross 30-100 seeds were produced.

3.1.2.2 Development of Test cross nursery

It included the testcross F_1s, and their corresponding male parents planted side by side. The test cross hybrids' seeds along with their pollen parent seeds were sown in the nursery beds on 1st week of December. Twenty eight day old seedlings were transplanted in the main field at spacing of 30 x 30 cm. Each hybrid and the corresponding pollen parent were grown in single row each of 2 m length (Plate 1). Standard agronomic practices were followed for raising crop. The observation on pollen sterility and fertility of the hybrids were recorded and used as indices to identify maintainers and restorers of different CMS lines.

3.1.2.3 Estimation of Pollen fertility

At the time of flowering, panicles from randomly chosen plants in each of the testcross hybrids were collected and taken to the laboratory and fixed in 1 : 3 acetic acid : alcohol solution. The anthers were collected from two randomly chosen spikelet from top and middle portion of the panicles and all the six anthers of each spikelets were smeared in a drop of solution containing 0.05 percent iodine in 2 percent potassium iodide (2 gm KI + 0.2 gm I_2 in 100 ml distilled water and then few minutes warmed in water bath until I_2 is dissolved in solution) on a glass slide. The pollen grains were teezed out of the anthers and the slide were placed under the binuclear microscope for counting the number of fertile and sterile pollen in three microscopic fields. The pollen grains that were completely round and deeply stained were counted as fertile and those that were unstained withered, unstained spherical, partially stained round or lightly stained round were considered sterile.

Plate 1: (A) Field view of test cross nursery for identification of maintainers and restorers

(B) Close view of preliminary selected *I/J* derived hybrids (F_1 plants)

Pollen fertility in per cent was then calculated as:

$$\text{Pollen fertility} = \frac{\text{No. of fertile pollen grains (stained round)}}{\text{Total no. of pollen grains in the microscopic field (fertile + sterile)}} \times 100$$

Based on the pollen fertility, the pollen parents of testcross progenies were classified into following categories as produced by Chaudhury *et al.*, 1981 and supported by Govinda Raj and Virmani, 1989.

Category	Pollen fertility
Probable restorers	> 60
Partial restorers	31-60
Partial Maintainers	1-30
Maintainers	<1

3.1.2.4 Identification of Potential restorers

The male parents of hybrids having more than 60% pollen fertility were categorized as restorers (R line) (Appendix II). These were further subjected to screening based on various morphological characters favoring high level of heterosis. Thus, as a second step the following characters were considered to identify potential restorers:

i. **Days to 50% flowering:** Number of days required for 50% of the plant show panicle emergence, from the date of sowing both the test cross hybrid and respective pollen parents were recorded.

ii. **Crop uniformity:** The extent of similarity among the individuals for a given testcross progeny were evaluated morphologically and only uniform lines were selected.

iii. **Pollen fertility (%):** The test cross hybrids having more than 60% pollen fertility were again scored on 1-9 scale.

Scale	Pollen fertility (%)	Description
1	>90	Highly effective restorer
3	81-90	effective restorer
5	71-80	Good restorer
7	60-70	Fair restorer
9	<60	Partial restorer

iv. **Plant height:** Height of the plant from ground level to the tip of the main panicle was measured in cm. Plant height was scored with a 1-9 scale proposed by IRRI, 1980.

Scale	Plant height (cm)	Description
1	<110	Semi dwarf
5	111-130	Intermediate
9	>130	Tall

v. **Number of productive tillers/plant:** Total number of panicle-bearing tillers in a plant was counted at the time of harvesting.

This character was scored with a 1-9 scale proposed by IRRI, 1980.

Scale	No. of tillers/plant	Description
1	>25	Very prolific tillering
3	20-25	Good prolific tillering
5	10-19	Medium prolific tillering
7	5-9	Poor prolific tillering
9	<5	Very Poor prolific tillering

vi. **Panicle length (cm):** Length of panicles from the base to the tip of panicle excluding awans was measured in cm. Panicle length was scored on 1-9 scale.

Scale	Panicle length (cm)	Description
1	>34	Excellent length
3	30-34	Very good length
5	25-29	Good length
7	20-24	Fair length
9	<20	poor length

vii. Panicle density (cm^{-1}): The ratio of the number of filled spikelets to total panicle length was calculated. It gives the number of grains per unit of panicle length.

$$\text{Panicle density} = \frac{\text{No. of filled spikelets}}{\text{Length of panicle}}$$

Panicle density was scored on 1-9 scale

Scale	Panicle density(cm)	Description
1	>11	Excellent density
3	9-11	Very good density
5	6-8	Good density
7	3-5	Fair density
9	<3	poor density

viii. Number of filled grains/panicle: Number of filled grains per cent on the main panicle was recorded and scored on 1-9 scale

Scale	No. of filled grains/panicle	Description
1	>250	Excellent Number of filled grains
3	201-250	Very good Number of filled grains
5	151-200	Good Number of filled grains
7	100-150	Fair Number of filled grains
9	<100	poor Number of filled grains

ix. Spikelet fertility (%): At the time of harvesting, five panicles were harvested at maturity from five randomly chosen plants in each of the testcross and the number of filed, unfilled and total spikelets was counted. Spikelet fertility percentage was then computed as:

$$\text{Spikelet fertility} = \frac{\text{No. of filled spikelets in the panicle}}{\text{Total nc. of spikelets in the panicle}} \times 100$$

Based on spikelet fertility, pollen parents of the test cross hybrids were scored on 1-9 scale.

Scale	Spikelet fertility (%)	Description
1	>80	Highly effective restorer
3	61-80	Effective restorer
5	46-60	Partial restorer
7	30-45	Weak restorer
9	<30	Maintainers

x. Grain yield per plant (g): Grain yield in gm per plant was taken after harvesting, threshing, cleaning and drying the produce to 14 per cent moisture content. Grain yield per plant was scored on 1-9 scale.

Scale	Grain yield per plant (g)	Description
1	>30	Excellent yield
3	26-30	Very good yield
5	21-25	Good yield
7	15-20	Fair yield
9	<15	Poor yield

xi. Phenotypic acceptability: Phenotypic acceptability of pollen parents and restorer F_1 were scored on a scale proposed by IRRI, 1997:

Scale	Description
1	Excellent plant type and absence of disease
3	Very good appearance
5	Fair appearance but has a few essential short coming
7	Poor appearance
9	Unacceptable appearance (discard)

xii. Scoring, ranking and selection of potential restorers

A combined score was computed based on scale of different characters mentioned above. The restorer genotypes having the lowest score of scale were given the top rank (I) and the restorer genotypes having the highest score was given the lowest rank (VI). Following this procedure, genotypes having the top rank(s), could be used as potential restorers for producing experimental hybrids in the next season.

3.1.2.5 Identification of potential maintainers

The male parents of hybrids having 100% pollen sterility were identified as effective maintainer lines (B line). These were further subjected to screening based on various morphological characters favoring out crossing, increased seed production as well as high level of heterosis. The following characters were considered to identify potential maintainers for conversion into new CMS lines.

i. Days to 50% flowering

ii. Crop uniformity

iii. Plant height

iv. Productive tillers/plant

v. Phenotypic acceptability

The descriptions of above listed are as described earlier.

vi. Leaf characteristic: Leaves were classified on the basis of their morphological characters. This character was scored on 1-3 scale.

Scale	Leaf appearance	Description	Scale	No. of leaves	Description
1	Erect	Desirable	1	More leafy	Desirable
3	Droopy	Undesirable	3	Less leafy	Undesirable

vii. Panicle exertion rate (PER) (%): Panicle exertion rate refers to the proportion of the panicle that is exerted from the flag leaf of the total panicle length after the full blooming, which is expressed in percentage:

$$\text{PER (\%)} = \frac{\text{Length (cm) of exerted panicle}}{\text{Total length (cm) of panicle}} \times 100$$

These characters were scored using modified IRRI scale (1997)

Scale	Panicle exertion rate (%)	Description
1	100	Well exerted
3	90-99	Moderately Well exerted
5	75-89	Good exerted
7	60-74	Partially exerted
9	<60	Poorly exerted (Unacceptable)

viii. Out crossing rate (OCR) : Out-crossing rate refers to the extent of seed set on open pollinated panicles which is expressed in percentage.

$$\text{OCR (\%)} = \frac{\text{Total no. of filled spikelets}}{\text{Total no. of spikelets}} \times 100$$

But in this study, out crossing rate was scored on a 1-9 scale proposed by IRRI, 1997b on the basis of number of seed set on open pollinated panicle.

Scale	No of seed set on open pollinated panicle	Description
1	>35	Excellent OCR
3	26-35	Good OCR
5	16-25	Fair OCR
7	5-15	Poor OCR
9	<5	Very Poor OCR (Unacceptable)

ix. Scoring and selection of potential maintainers

A combined score was also computed based on scale of different characters. The maintainer genotype having the lowest score of scale were given the top rank (I) and the maintainer genotype having the highest score were given the lowest rank (IV). Following the procedure, genotype having the top rank, could be used as potential maintainers for conversion into new CMS lines.

The above system of combine scoring/ranking of maintainer lines and restorer lines were developed and used for the second time in this study.

3.2 OBJECTIVE II: CONVERSION OF SELECTED MAINTAINERS' POSSESSING DESIRABLE PLANT TYPE INTO CMS LINES

3.2.1 Materials

In all 71 test crosses using CMS lines IR 58025A were screened during Rabi *2006-2007* to identify effective new plant type maintainers (Appendix II). Among these test crosses, four maintainer lines (F_1) were preliminary chosen on the basis of pollen sterility, floral traits in flowering, out crossing, panicle exertion, leaf characteristics and overall plant type (Table 12). The seeds of selected pollen parents and corresponding CMS lines were collected for retest crossing. The seeds of IR 58025A were used as standard check.

Cross combination of maintainer lines used in objective II

Sl. No.	Test cross No.	Test Cross
1.	TC-32	IR 58025A / P-5B
2.	TC-33	IR 58025A / P-5B
3.	TC-39	IR 58025A / P-25B
4.	TC-40	IR 58025A / P-25B

3.2.2 Methods

Seeds of selected maintainer lines (B-line) were raised for retest crossing as male parents during Kharif- 2007 at SAU Farm. Corresponding CMS lines of the F_1s showing complete pollen sterility were raised as female parents. Retest crosses of identified lines were effected on single plant basis to confirm their perfect maintaining ability.

F_1 seeds were sown in nursery beds at SAU Farm on December 2007 along with their pollen parent and check variety seeds. Twenty eight day old seedlings were transplanted in the main field at a spacing of 20 x 20 cm. Each F_1 and the corresponding pollen parent were grown in single row side by side each of 2m length using a single seedlings/hill. CMS line IR 58025A was raised as standard check. Standard agronomic practice was followed for raising the crops. The observation on pollen sterility of the hybrids was recorded under microscope as early mentioned method. The F_1 maintainers were selected for back crossing with respective pollinator to produce BC_1F_1 seed.

Chapter 4
Results And Discussion

4. RESULTS AND DISCUSSION

4.1 OBJECTIVE I: IDENTIFICATION OF POTENTIAL RESTORERS AND MAINTAINERS FROM *indica/japonica* DERIVATIVES

The availability of complete restorer and good maintainer lines is an essential prerequisite for exploitation of heterosis in grain crops using cytoplasmic male sterility system. On the basis of pollen and spikelet fertility/sterility, the respective male parents are designated as restorers or maintainers, subsequently, only promising maintainers are converted into new CMS lines and restorer are used to develop experimental hybrids. Therefore, identification and/or development of genotypes for sterility maintenance and fertility restoration in CMS lines have become important considerations in the hybrid breeding programme.

Identification of restorers and maintainers, their further improvement through hybridization (maintainer/maintainer, maintainer/restorer or restorer/restorer) and finally their utilization in the development of three-line hybrids appear to be long term breeding approach for exploitation of heterosis in rice (Teng and Shen, 1995; Yang, 1997, Bharaj and Virmani, 1997). In the short term, screening locally adapted or available breeding lines for isolation of restorers, evaluation of restorers for combining ability and heterosis and their utilization in the development of three-line hybrids are the principal steps commonly followed in hybrid rice breeding programme. In the present study, the second method was followed for identification of parental restorers and maintainers.

The experimental materials consisted of 71 test cross progenies derived from *indica/japonica* lines and 46 pollen parents (Appendix I). Out of 71 test crosses, 6 test cross hybrids did not germinate or number of plants were insufficient, therefore the data relating to present study were recorded on 65 crosses. Pollen sterility/fertility was used as the indices to classify pollen parents of test crosses into different categories following the method of Govinda Raj and Virmani (1988). The study showed that all the pollen parents used in 65 success test crosses, 14 were restorers (21.54%), 27 were partial restorer (41.54%), 14 were partial maintainers (21.54%) and 10 were maintainers (15.38%) (Appendix II).

Hossain (2004) reported that all the parents included in 274 test crosses, 58% were restorer, 13% were partial restorer, 5% were partial maintainers and 24% were complete maintainers. Virmani (1994) reported the frequency of restorer and maintainer lines to be low in India (using *indica* lines) and high in Korea (using *japonica* lines). In India, the frequency of restorer was 21% and maintainer was 14.9%. On the contrary in Korea, it was 53% and 44.4%, respectively. Results of a joint study between IRRI and China aimed at identifying effective restorer lines among elite breeding materials developed at IRRI and other national programs indicated that about 15 and 24% of these lines were effective restorers in China and at IRRI, respectively, but only 6% were effective restorers at both the places. Restoration ability of remaining restorer lines was site-specific; the frequency of restorers genotype was higher in a tropical than in a subtropical or temperature environment (Virmani and Edwards, 1983). According to IRRI report (1997c) the frequency of restorers may vary between ecotypes and geographic regions. The frequency of restorers in *indicas* being high they can be utilized for development of *indica / japonica* hybrids by transferring WC gene in them. Frequency of restorer lines is generally higher in rice varieties from lower altitudes as compared to those from high altitudes. Virmani (1994) reported that, in temperate regions where *japonica* rices are grown, the frequency of maintainer lines is very high, therefore, a large number of CMS lines can be developed, but restorer lines are rather rare. Therefore restore genes from *indica* rices have to be transferred to *japonica* rice hybrids. This has been done successfully in China and Japan. These are indications that several pairs of restorer genes are present in the elite *indica* cultivars which make breeding of restorer lines easier. Xiao *et al.*(1996) concluded that the frequency maintainers and restorers has been significantly increased after initiating specific maintainer (B X B) and (R X R) breeding programme.

4.1.1 Identification of potential restorers

A total of 14 pollen parents of hybrids showing pollen fertility >60% were categorized as restorer (R line) (Table 7). These were further subjected to screening based on morphological characters and mean performance of test cross hybrids (Table 8). On the basis of over all score, restorer lines were grouped into different ranks for selection of potential restorers which is presented in Table 9.

4.1.1.1 Mean performance and scoring of restorers

A good restorer line should posse's good agronomic characters, grain quality, crop uniformity and overall phenotypic acceptability besides showing perfect pollen fertility and good hybrid vigour for use as male parent of the hybrid combination. The salient findings of mean of performance (Table 8) as the characters which were considered for screening of different scale of score and ranking (Table 9) to identify potential restorers are briefly described below under the following sub-heads:

i. Days to 50% flowering: Crop duration and days to 50% flowering besides being major agronomic trait are important for deciding seeding interval of parental lines for proper synchronization of flowering. In the present study days to 50% flowering of

Table 7. Restorer lines with their pollen fertility percentage

Sl. No.	Test cross	Pollen fertility %	Category
1.	TC-6	64.06	R
2.	TC-10	68.33	R
3.	TC-35	80.54	R
4.	TC-41	64.28	R
5.	TC-42	61.76	R
6.	TC-52	68.89	R
7.	TC-54	60.69	R
8.	TC-58	60.95	R
9.	TC-59	60.79	R
10.	TC-60	66.27	R
11.	TC-63	66.19	R
12.	TC-68	60.98	R
13.	TC-69	73.29	R
14.	TC-71	62.86	R

R = Restorer

Table 8. Mean performance of restorers for various morphological traits

Sl No.	Test cross No.	Days to 50% flowering		Crop Uniformity	Pollen fertility %	Plant height (cm)	No. of effective tillers/plant	Panicle length (cm)	Panicle density / cm	No. of grains/panicle		Spikelet fertility (%)	1000 grain weight (g)	Yield/ plant (g)
		F_1	PP							Filled	Total			
1	TC-6	117	119	NU	64.06	-	-	-	-	-	-	-	-	-
2	TC-10	126	121	U	68.33	92.80	30.00	29.00	6.21	180.00	202.00	89.11	23.80	62.92
3	TC-35	132	137	NU	80.54	-	-	-	-	-	-	-	-	-
4	TC-41	128	142	U	64.28	97.60	22.00	28.84	6.91	199.40	224.80	88.70	22.20	42.90
5	TC-42	130	128	NU	61.76	-	-	-	-	-	-	-	-	-
6	TC-52	137	147	U	68.89	113.86	19.20	28.00	6.34	177.40	216.60	81.90	23.50	57.22
7	TC-54	131	134	U	60.69	108.17	23.67	26.90	8.00	215.33	264.00	81.56	22.70	55.74
8	TC-58	134	140	U	60.95	112.26	21.00	30.70	6.15	188.80	228.80	82.52	19.90	56.01
9	TC-59	126	130	U	60.79	87.50	29.40	24.78	8.82	218.60	266.40	82.06	20.80	52.77
10	TC-60	134	144	U	66.27	104.28	29.40	27.28	7.84	214.00	280.80	76.21	20.30	71.00
11	TC-63	131	129	U	66.19	112.75	24.50	30.00	7.67	230.00	296.50	77.57	21.00	62.65
12	TC-68	128	129	U	60.98	119.64	25.20	27.48	7.17	197.00	229.00	86.03	21.03	60.21
13	TC-69	137	134	U	73.29	119.27	27.00	26.80	8.02	215.00	302.00	71.19	20.50	51.94
14	TC-71	128	131	U	62.86	103.40	24.80	28.30	6.80	192.00	253.20	75.83	17.00	53.45
Mean		**129.93**	**133.21**	-	**65.71**	**106.50**	**25.11**	**28.01**	**7.27**	**202.50**	**251.28**	**81.15**	**21.16**	**56.98**
Range		**117-137**	**119-147**	-	**60.69-80.54**	**87.50-119.64**	**19.20-30.0**	**24.78-30.70**	**6.15-8.82**	**177.40-230.00**	**202.00-302.00**	**71.19-89.11**	**17.23-23.80**	**42.90-71.00**

Table 9. Scoring and ranking of different restorers over various characters based on mean performance of testcross hybrids

Sl No.	Test cross No.	Crop Uniformity	Pollen fertility (%)	Plant height (cm)	No. of effective tillers/ plant	Panicle length (cm)	Panicle density/ cm	Filled grains/ panicle	Spikelet fertility (%)	Yield/ plant (g)	Phenotypic acceptability	Total score of scale	Rank (on the basis of over all score)
1	TC-10	U	7	1	1	5	5	5	1	1	3	29	III
2	TC-41	U	7	1	3	5	5	5	1	1	3	31	IV
3	TC-52	U	3	5	5	5	5	5	1	1	3	33	V
4	TC-54	U	7	1	3	5	5	3	1	1	1	27	II
5	TC-58	U	7	5	3	3	5	5	1	1	3	33	V
6	TC-59	U	7	1	1	5	5	3	1	1	1	23	I
7	TC-60	U	7	1	1	5	5	3	3	1	1	27	II
8	TC-63	U	7	5	3	3	5	3	3	1	1	31	IV
9	TC-68	U	7	5	3	5	5	5	1	1	3	35	VI
10	TC-69	U	5	5	1	5	5	3	3	1	1	29	III
11	TC-71	U	7	1	3	5	5	5	3	1	3	33	V

TC = Testcross, U = Uniform, I = Top Rank, VI = Least Rank test cross hybrids widely ranged from 117 to 137 days, with a mean of 129.93 days (Table 8). Majority of the test cross hybrids showed their 50% flowering time earlier than their corresponding pollen parents. Ikehashi (1994) reported that some *indica/japonica* hybrids showed their 50% flowering later than their parents due to photoperiod sensitivity of the hybrids though their parents were photoperiod non-sensitive. For instance, both 02428 and Nante Hao parents flower early, but their hybrids flower later, due to sensitivity to short day length.

ii. Crop uniformity: Uniformity and homogeneity of parental lines is important for their use in development of hybrids. In the present study, out of 14 restorer lines, 11 lines were selected on the basis of crop uniformity of the respective hybrids for agronomic traits for further evaluation (Table 8). Though the material used for test crossing have passed 10-15 generations of selfing yet appearance of variability could possibly be due to presence of suppressor or modifier genes which is known to appear in *indica/japonica* progenies.

iii. Pollen fertility: Pollen fertility of test cross hybrids ranged from 60.69% to 80.54% with a mean of 65.71%. The hybrids having more than 60% pollen fertility were again categorized into 1-9 scale. Among these 11 hybrids, 1 hybrid (TC-52) got score 3 (81-90% pollen fertility), 1 hybrids (TC-69) had score 5 (71-80% pollen fertility) and rest 9 hybrids received score 7 (60-70% pollen fertility) (Table 9).

iv. Plant height (cm): Ikehashi (1994) reported that even when both the parents of *indica/japonica* hybrids are semidwarf, their hybrid often shows tall stature and lodging. It seems desirable to select parental lines with shorter height. Futsuhara and Kikuchi (1984) reported that shorter plants are often accompanied by lodging resistance and thereby adapts well to heavy fertilizer application. Pillai (1961) and Singh (1978) also reported negative heterosis for plant height, where Virmani *et al* (1982) observed the hybrids almost equal to or slightly taller than the parents. Akita

(1989) reported that a plant height of 90-100 cm is considered ideal for maximum yield. Vergara (1988) suggested that without strong thick culm, however, increase biomass result in lodging, increased disease incidence and lower yield. In this study, plant height of test cross hybrids ranged from 87.50 to 119.64 cm, with a mean of 106.50 cm. The restores lines were categorized in three distinct groups on the basis of plant height proposed by IRRI. Out of 11 uniform restorer lines, a maximum of 6 lines were categorized as semi dwarf (<110 cm) and 5 as intermediate (111-130 cm). Semi dwarf plant height range had been regarded as desirable for restorer selection and was ranked in scale 1 and intermediate height was scored 5 (Table-8 & 9). However restorer of tall or medium tall plant height combining strong culm and lodging resistance are likely to suit for development of hybrids under lowland condition.

v. Number of effective tillers/plant: Effective tillers number per plant ranged from 19.20 to 30.00 with a mean of 25.11 (Table 8). Incase of number of productive tillers per plant, restorers were categorized in different groups using 1-9 scale (Table 9). Out of 11 test cross hybrids, 4 hybrids showed very prolific tillering (>25 tillers/plant), 6 hybrids showed good prolific tillering (20-25 tillers/plant) and 1 hybrid showed medium prolific tillering (10-19 tillers/plant). Maximum hybrids showed good prolific tillering probably due to new plant type pollen parents most of which possessed good prolific tillering habit. Very prolific tillering lines were scored in scale 1, good prolific lines were scored in scale 3 and medium prolific tillering lines were scored in sale 5. Many workers (Pillai, 1961; Nomboodiri, 1963; Govinda Raj, 1983; Kumar *et al.*, 1994) have reported positive heterosis for number of productive tillers. Khush (1999) reported that NPT lines produced 6-10 tillers as compared to 25-27 tillers for modern high yielding varieties under fovourable growth condition. Only 14-15 tillers in modern varieties produce panicles, which are small and the rest remain unproductive. He concluded that the approach increases the number of grain per panicle rather than the number of panicles per unit area. The hybrids having moderate number of tillering are being developed by rice breeders especially for developing inter-subspecific hybrids. Genotypes with lower tiller number are also reported to produce heavy panicles with higher grain yield (Padmaja, 1987).

vi. Panicle length: Ramesha (2003) suggested that long panicle with large number of spikelets are desirable traits of restorer lines. Kato and Namai (1987a) observed that for hybrid seed production long panicle with large number of spikelets constantly bloom every day producing numerous residual pollen grains per exerted anther. In the present study, the length of main panicle among the test cross hybrids ranged from 24.78 to 30.70 cm, with a mean of 28.01 cm (Table 8). For proper selection of restorer lines panicle length was scored in 1-9 scale. None of the test cross hybrids showed excellent length (> 34 cm), 2 hybrids showed very good length (30-34 cm) and maximum 9 hybrids exhibited good length (25-29 cm). Very good panicle length were scored in scale 3 and the hybrids showed panicles length 25 to 29 cm had been scored in scale 5 (Table 9). Panicle of some promising hybrids with their respective parents is presented in Plate no. 2

vii. Panicle density: During restorer selection both panicle length and panicle density should be considered at a time. Some times large panicles with higher total spikelet numbers give low panicle density due to lower number of filled spikelets. In some cases, lower panicle length, lower number of total spikelets but high numbers of filled grains show high panicle density. In the present study, the panicle density ranged from lowest 6.15 to highest 8.82, with a mean of 7.27 (Table 8). Out of 11 test cross hybrids, all hybrids showed good panicle density (6-8) and were scored in scale 5 (Table 9).

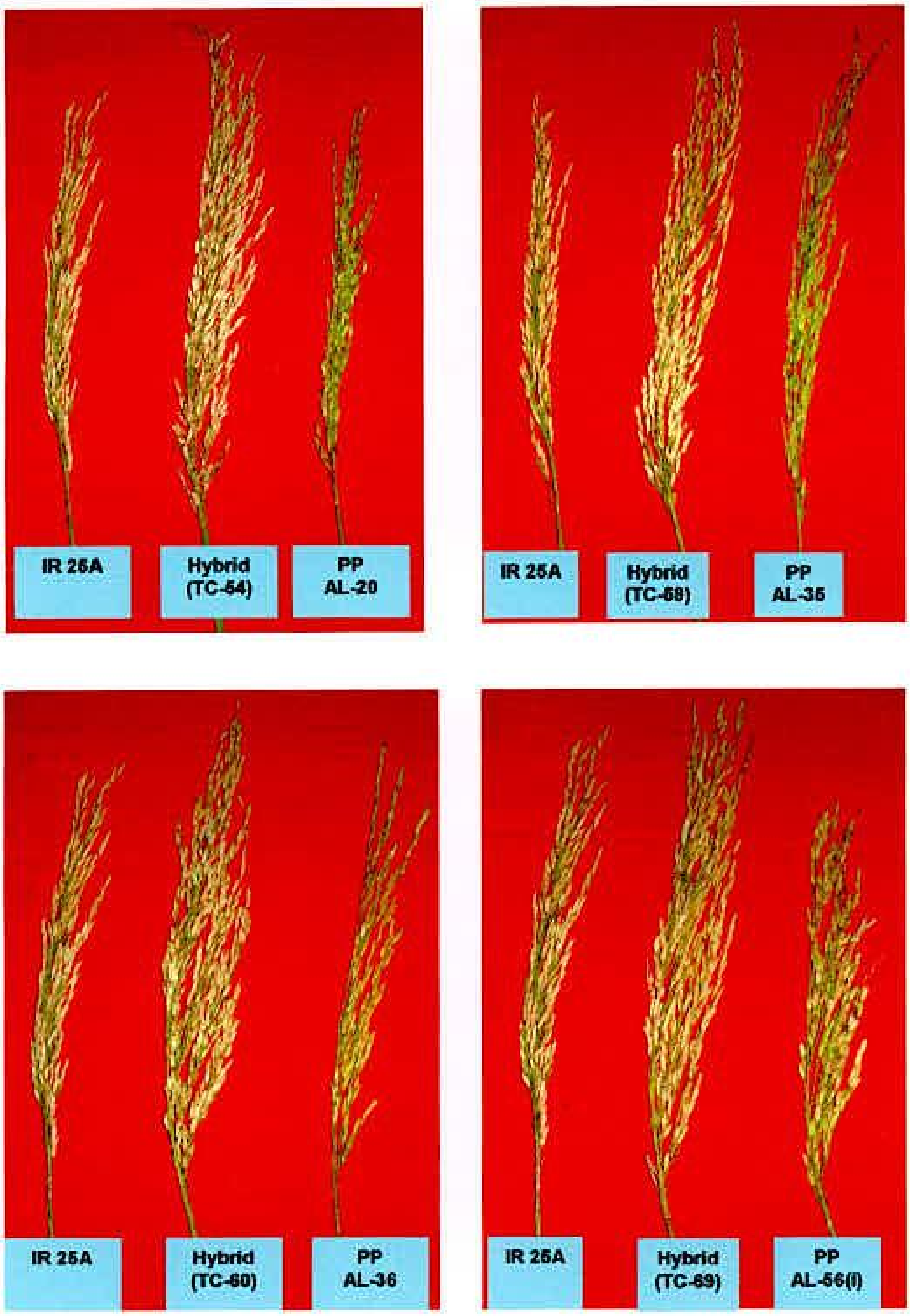

Plate 2. Comparison of panicle of some hybrids developed from *i/j* derived lines with their respective parents

viii. Filled grains/panicle: The problem of poor grain filling per panicle in *indica/japonica* hybrids has been regarded as major impediment in *indica/japonica* heterosis. By using wide compatibility (WC) genes, the seed-setting rate caused by semi sterility from incompatibility between *indica* and *japonica* lines can be raised to nearly normal levels (Yuan, 1998). In the present study, the filled grains per panicle ranged widely from 177.40 to 230.00, with a mean of 202.50 (Table 8). Hybrids with excellent numbers of filled grains (> 250) were scored in scale 1 and poor numbers of filled grains (<100) were scored in scale 9. Out of 11 test cross hybrids, 5 hybrids showed very good number of filled grains and were scored in scale 3 and rest 6 hybrids showed good number of filled grains and were scored in scale 5 (Table 9).

ix. Spikelet fertility: Spikelet fertility is an important character for consideration of potential restorer along with high pollen fertility. Kim and Rutger (1998) reported in some studies, increased yield in most heterotic hybrids was due to heterosis in spikelet fertility and panicle numbers. Conversely, Ramesha *et al.* (1999) found negative heterosis for spikelet fertility. In the present study, spikelet fertility varied from 71.19% to 89.11 %, with a mean of 81.15% (Table 8). Based on spikelet fertility, pollen parents of the hybrids were scored on a 1–9 scale. As many as 7 hybrids showed spikelet fertility more than 80% and 3 hybrids showed 61 to 80%. More than 80% spikelet fertility was scored in scale 1 and 61-80% spikelet fertility was scored in scale 3 (Table 9).

X. Yield/plant (g): Most important character for selection of potential restorer is yield per plant of the test cross hybrids. Yield per plant ultimately reflects the total yield per plot or yield per unit area. Yuan (1990) reported that *indica/japonica* hybrid gave a yield increase of 47% against an *indica* hybrid at Hunan Hybrid Rice Research Center in 1989. In this study, the yield per plant ranged from 42.90g to 71.00g, with a mean of 56.98g (Table 8). Based on yield of test cross hybrids, pollen parents of the hybrids were scored on 1-9 scale. All of 11 hybrids exhibited yield above 30g per plant and had score 1. The hybrids which had score 1 categorized as excellent in yield performance per plant (Table 9).

xi. Phenotypic acceptability: The phenotypic acceptability depends on breeding objectives of the breeder and location where the hybrid is to be grown. Before final selection, test cross hybrids were scored on a scale on the basis of phenotypic acceptability. The hybrids having desirable plant type (NPT) and being free from major pests and diseases were scored in top scale 1 (Table-9). Those having unacceptable appearance showing incidence of disease and pest problems as well scored in lower scale 9. In the present study, out of 11 test cross hybrids, 5 hybrids showed excellent plant type and absence of disease and were scored in scale 1 and rest 6 hybrids showed very good appearance in plant type and were scored in scale 3.

4.1.1.2 Ranking of restorers on the basis of over all score of mean performance of test cross hybrids

On the basis of overall score for the character studied, all the 11 restorer lines were given rankings. The restorer genotypes having the lowest score of scale were given the top rank (I) and the restorers having the highest score of scale had been given the lowest rank (VI). Following the pooled approach, the restorers having the rank I to III were considered as potential restorers for producing experimental hybrids in the next crop seasons (Table- 10).

Table 10. The list of selected top ranking (score I to VI) restorer lines on the basis of desirable characteristics

Sl No.	Test Cross No.	Total score	Rank
1.	TC-59	23	I
2.	TC-54	27	II
3.	TC-60	27	II
4.	TC-10	29	III
5.	TC-69	29	III
6.	TC-41	31	IV
7.	TC-63	31	IV
8.	TC-52	33	V
9.	TC-58	33	V
10.	TC-71	33	V
11.	TC-68	35	VI

4.1.2 Identification of potential maintainers

A total of 10 pollen parents of hybrids having 100% pollen sterility were categorized as maintainer lines (Appendix II). Keeping in view the fact that, all the lines showing good maintaining ability of male sterility might not be useful for use as female parent. The process of conversion of maintainer line into CMS line being costly, labour intensive and time consuming, the few selected lines must combine maximum desirable morphological traits for use a female parent in hybrid rice breeding. Therefore, 10 lines identified as maintainers were further subjected to screening based on various morphological characters favouring out-crossing, increased seed production, good grain quality as well as high level of heterosis (Table 11). The selected maintainer lines were grouped into different ranks for effective selection of potential maintainers on the basis of overall score and are presented in Table 12.

4.1.2.1 Mean performance and scoring of maintainers

Besides perfect pollen sterility, the maintainers should posse's good agronomic characters with desirable floral traits for enhancing out crossing rate. For achieving these objectives, the mean performance of morphological and agronomic characters of test cross hybrids (CMS lines) were measured and scored during Kharif-2 2007 using different scales proposed by IRRI for identification of potential maintainers. The criterion of scoring for selecting most potential maintainer lines based on mean performance of hybrids for various relevant characters are briefly described below under the following sub-heads:

i. Days to 50% flowering: In the present research, the data on 50% flowering was scored on both hybrids as well as maintainers pollen parents to facilitate deciding seeding interval of two parental lines for proper synchronization of the flowering for good seed production. In the present study, the days to 50% flowering varied from

Table 11. Mean performance of maintainer lines for various morphological traits

Sl No.	Test cross No.	Days to 50% flowering		Crop Uniformity	Leaf characters		Plant height (cm)	No. of effective Tillers/plant	Panicle length (cm)		Panicle exertion rate %	Grain No. on open pollinated panicle			% of out crossing
		TC	PP		Erect/ Droopy	More/Less leafy			Total	Exerted		Filled	Unfilled	Total	
1	TC-30	134	131	U	E	L	95.04	24.06	29.78	25.80	86.64	31.60	196.40	228.00	16.09
2	TC-31	137	137	U	E	L	102.12	25.80	30.56	26.70	87.37	19.60	246.40	266.00	7.95
3	TC-32	146	137	U	E	L	89.48	24.40	25.78	22.00	85.34	54.56	148.16	202.72	36.82
4	TC-33	137	134	U	E	L	95.88	30.40	26.36	21.00	79.67	39.00	156.40	195.40	24.94
5	TC-34	134	134	U	E	L	103.00	30.00	26.50	21.88	82.57	19.00	167.00	186.00	11.38
6	TC-36	137	137	NU	D	M	-	-	-	-	-	-	-	-	-
7	TC-38	137	134	U	E	L	88.55	25.00	28.27	24.13	85.36	29.75	178.00	207.75	16.71
8	TC-39	129	134	U	E	L	93.24	22.20	27.90	23.50	84.23	67.40	160.60	228.00	41.97
9	TC-40	137	137	U	E	L	87.06	21.01	24.80	22.33	90.04	34.40	176.20	201.60	19.52
10	TC-50	134	137	U	E	L	115.65	15.75	27.22	23.60	86.70	59.50	116.00	175.50	51.29
	Mean	**136.20**	**131.00**	-	-	-	**96.67**	**24.29**	**27.46**	**23.44**	**85.32**	**39.42**	**171.66**	**210.11**	**25.19**
	Range	**129.00-146.00**	**131.00-137.00**	-	-	-	**87.06-115.65**	**15.75-30.40**	-	-	**79.67-90.04**	**19.00-67.40**	**116.00-246.40**	**175.50-266.00**	**7.95-51.29**

Table 12. Scoring and ranking of different maintainers over various characters based on mean performance of F_1 hybrids (CMS)

Sl No.	Test cross No.	Crop Uniformity	Leaf characters		Plant height (cm)	No. of effective tillers/plant	Panicle exertion rate %	Out crossing rate (Filled grains/panicle)	Phenotypic acceptability	Total score of scale	Rank (on the basis of over all score)
			Erect/ Droopy	More/Less leafy							
1	TC-30	U	1	1	1	3	5	3	3	17	III
2	TC-31	U	1	1	1	1	5	5	3	17	III
3	TC-32	U	1	1	1	3	5	1	3	15	II
4	TC-33	U	1	1	1	1	5	1	3	13	I
5	TC-34	U	1	1	1	1	5	5	3	17	III
6	TC-38	U	1	1	1	3	5	3	3	17	III
7	TC-39	U	1	1	1	3	5	1	3	15	II
8	TC-40	U	1	1	1	3	3	3	3	15	II
9	TC-50	U	1	1	5	5	5	1	3	21	V

129 to 146 days in test cross hybrids with a mean of 136.20 days, where as, in maintainer pollen parent it ranged from 131 to 137 days, with a mean of 135.20 days (Table 11). Majority of the test cross hybrids showed their 50% flowering time earlier than their corresponding pollen parents, but in some crosses 50% flowering time is significantly later than their corresponding pollen parents (TC-3O, TC-32, TC-38). Similar late flowering of hybrids as compared to their pollen parents was also observed by Ikehashi (1994). He reported that some *indica/japonica* hybrids showed their 50% flowering later than their parents due to photoperiod sensitivity of hybrids though the parents were photo period non sensitive.

ii. Crop uniformity: Uniformity of the maintainer line is very important for conversion and use as CMS line in hybrid breeding. In the present study, 1 test cross hybrids showing complete male sterility lacked in morphological uniformity (Table 11). Therefore 9 uniform lines out of 10 lines were selected for further data recording.

iii. Leaf characteristics: In the present study, leaf of test cross hybrids were classified on the basis of morphological characters. Of the 10 uniform lines, only 1 test cross hybrid was categorized as of droopy leaf and others with erect leaf characteristics. Erect leaf being desired characteristics of maintainer line had been placed scale 1 and undesirable droopy leaf characteristic is placed in scale 3 (Table-12). Yoshida (1976) noted that light is used efficiently if the leaves are erect. Photosynthesis is greater when leaves are exposed to light on both sides, as an erect leaves. Droopy leaves are exposed to light on one side. The erect leaf angle facilitates easy pollen movement for pollination enhancing seed set rate for hybrid seed production. Further, on the basis of overall leaf numbers and its length-breadth, the plant was classified into more leafy and less leafy.

The less leafy character being desirable was scored as of scale 1 and undesirable more leafy character was given score 3. More leafiness hinders the easy pollen movement during pollination and also raised the relative humidity and reduces the temperature since they lessen light penetration and air movement which is favorable for disease infestation. In the present study, 1 maintainer line which had droopy and more leaf was also not uniform. Therefore this line was not considered for further data recording.

iv. Plant height: Short stature of CMS lines as compared to pollen parents helps to higher pollination and also reduces the susceptibility to lodging. A plant height of less than 110 cm is considered ideal for maximum seed yield of CMS lines. Ikehashi (1994) reported that the hybrid derived from *indica/japonica* crosses often shows tall stature and lodging even when both parents are semidwarf. Therefore, while dwarfness of both the parents is important with slightly more height pollen parent, a combination specific information too has to be taken in to consideration for choosing the parents in case Inter sub-specific hybrids. In the present study, plant height ranged from 87.06 cm to 115.65 cm, with a mean of 96.67 cm (Table-11). On the basis of plant height, maximum 8 test cross hybrids were categorized as semi dwarf and 1 as intermediate. Semi dwarf plant height was desirable for maintainer lines which were placed in scale 1 and intermediate was placed in scale 5 (Table 12).

v. Effective tillers/plant: Incase of number of effective tillers per plant, maintainer lines were categorized in different group using 1-9 scale. In the present study, effective tillers per plant ranged from 15.75 to 30.40 with a mean of 24.29 (Table 11). Out of 9 test cross hybrids, 3 hybrids showed very prolific tillering habit (>25) and scored in scale 1, 5 hybrids showed good prolific tillering (20-25) and scored in scale 3 and rest 1 hybrid showed medium prolific tillering habit and scored in scale 5 (Table 12). Khush (1994) conceptualized a new plant type with lower tillering capacity but no unproductive tillers would give more grain yield. But incase of CMS lines selection, prolific or good tillering capacity plant should be preferable than the less tillering plant. Such plant types have increased number of panicles with extended blooming period.

vi. Panicle exertion rate (%): A good panicle exertion in male sterile parent would expose a higher number of spikelets for out crossing compared to a male-sterile line showing incomplete panicle exertion. Similarly, incomplete panicle exertion in a pollen parent would result in lower pollen release. Therefore, a good panicle exertion in both seed and pollen parent is essential to attain high out-crossing rates for hybrid seed production (Virmani, 1994a). In the present study, the percent panicle exertion ranged from 79.67 to 90.04%, with a mean of 85.32% (Table-11). Present study showed that 1 hybrid fell in moderately well panicle exertion category (score 3) and maximum 8 hybrid in good exertion category (score 5) (Table-12).

vii. Out crossing rate (Filled grains/panicle): The most important floral trail influencing out crossing in rice is male sterility. Male fertile plants show very little out-crossing due to the self-pollinating nature of the rice flower. Extent of out crossing on a male sterile line is influenced by its floral traits (viz., duration of blooming period and angle of spikelet opening and stigma size and exertions) and pollen number per anther of the pollen parent (Virmani, 1994a). Oka (1988) stated that outcrossing in rice depends upon the capacity of stigmas to receive alien pollen before self-pollination and the capacity of anthers to emit much pollen to pollinate other plants in the proximity. In the present study, number of filled grains per panicle was recorded as out crossing rate following the IRRI Method (1997b). Among the test cross progenies, the value of out crossing rate widely ranged from 7.95 to 51.29 with a mean of 25.19 (Table-11). On the basis of filled grains per panicle out crossing rate is also scored on a 1-9 scale. Of the 9 test cross hybrids, 4 hybrids exhibited excellent out-crossing rate receiving score 1 of the scale (> 35 seed set/panicle), 3 showed good out crossing rate receiving with score 3 (26-35 seed set/panicle) and 2 hybrids showed fair out crossing rate receiving score 5 (Table-12).

viii. Phenotypic acceptability: The test cross hybrids were also scored for phenotypic acceptability following IRRI method of scale 1-9 scale of on the basis of plant appearance and disease resistance. Among the test cross hybrids, all the hybrids showed very good appearance in plant type and were scored in scale 3 (Table 12).

4.1.2.2 Ranking of maintainers on the basis of over all score of mean performance of test cross hybrids

All the 9 maintainer lines included in the present study were given ranking based on overall score for the characteristics studied. This is done on the basis of sum of scores. The ranking was done from I to IV (Table 13). The hybrids having the highest score received the lower ranking IV. Following this pooled approach, the maintainer genotypes having the rank I to II were considered for conversion into new CMS lines. On the basis of ranking, 4 maintainer lines were finally selected for conversion into new CMS lines in the next crop season.

4.2 OBJECTIVE II: CONVERSION OF SELECTED MAINTAINERS POSSESSING DESIRABLE PLANT TYPE INTO CMS LINES

The CMS lines to be used in hybrid rice breeding should posses complete pollen sterility and should be stable in pollen behavior over varied environments and seasons to avoid self fertilization in hybrid seed production plots. Presence of fertile pollen, even in traces, may lead to considerable amount of selfed seeds in hybrid seed lot and will lead to reduce performance of the hybrid. Instability of male sterile line defeats the very objective of fixing the lines for hybrid seed production and lessens he economic importance and chance of success of hybrids. CMS lines developed at IRRI and China has been evaluated in many countries for their adoptability and stability over environments. Very few viz. IR58025A have shown stability over different environments. Majority of the lines were found unstable due to lack of stability in pollen sterility. This has been attributed by Virmani and Wan (1988) to the presence of minor genes for fertility restoration in maintainer genotype

Table 13. The list of selected maintainer lines securing the rank I to IV

Sl No.	Test Cross No.	Total score	Rank
1.	TC-33	13	I
2.	TC-32	15	II
3.	TC-39	15	II
4.	TC-40	15	II
5.	TC-30	17	III
6.	TC-31	17	III
7.	TC-34	17	III
8.	TC-38	17	III
9.	TC-50	21	IV

Table 14. Pollen sterility and spikelet fertility study of selected hybrids of maintainer lines

Sl. No.	Test cross No.	Pollen sterility (%)	Spikelet fertility (%)
1.	TC-33	100	0
2.	TC-32	100	0
3.	TC-39	100	0
4.	TC-40	100	0

or due to incomplete sterilization or due to breakdown of male sterility in different environments. Among the various sources of cytosterility in rice, CMS lines derived from the WA system have been found to be most stable ones for their complete sterility (Lin and Yuan, 1980; Chaudhary *et al.*, 1981). In this study, four maintainer lines identified following various scoring parameters and overall ranking were chosen for conversion into CMS lines. These were identified from test cross population at SAU farm in Rabi *2006-2007* and back crossing were done by selected maintainer lines for evaluation in next season. During Kharif 2007 all of four selected maintainer lines were evaluated for pollen sterility and spikelet fertility using bagged panicles and it was found that all maintainer lines showed 100% pollen sterility (Table 14). All of these maintainer lines showing perfect and stable pollen sterility were finally selected for back crossing. It will be continued to convert into CMS line by back crossing through regular breeding program or continuation of this study. A comparative view of selected maintainers (F_1) with their parental lines is presented in Plate 3.

Plate 3. Selected maintainer (F_1) with their parental lines

Chapter 5
Summary And Conclusion

5. SUMMARY AND CONCLUSION

Present investigation " Isolation and Characterization of Maintainers and Restorers from Inter Sub-specific (*indica x japonica*) Derivative Lines of Rice " was carried out at the experimental Farm of SAU, during Kharif-2006,Rabi 2006-2007 and,Kharif-2007. Material for present study for identification of potential maintainers and restorers were comprised of 46 *indica/joponica* derived pollen parents, 1 *indica* CMS lines and 71 test cross combinations. Out of 71 test cross combinations, 6 test cross hybrids did not germinate or number of plants were insufficient. Therefore data were recorded on 65 crosses. Out of 65 test crosses, selected 5 restorer lines were used for development of experimental hybrids for evaluation and 4 maintainer lines were used for conversion into new CMS lines by back crossing. The selection of potential restorers and maintainers besides their pollen fertility and sterility were done on the basis of overall score of different scale proposed by IRRI.

- The pollen fertility-sterility study showed that of all the pollen parents in 65 test crosses, 14 were restorers (21.54%), 27 were partial restorers (41.54%), 14 were partial maintainers (21.54%) and 10 were maintainers (15.38%).
- Out of 14 restorer lines, 11 lines were selected on the basis of crop uniformity and all types of observation were recorded only from uniform lines. Based on scale of different characters a combined score was computed and on the basis of pooled approach of ranking, 5 top ranking (I-III) restorer lines were selected.
- Of the 10 maintainer lines, 9 uniform lines were further screened and all lines showed stable and perfect maintaining ability. Among these lines 4 potential maintainer lines were selected on the basis of pooled approach of ranking. These 4 maintainer lines were considered for conversion into new CMS lines.

- So, the selected five restorer lines will be used for production of experimental hybrid and the selected four maintainer lines were used for back crossing and conversion into new CMS lines will be continued in the next crop season. The BC_1F_1 generation (CMS lines) of the 4 selected NPT maintainer lines will be further evaluated for floral and morphological traits favouring out crossing through regular breeding programme of the department.

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Appendix: I. List of testcross hybrids used for identification of potential maintainers and restorers (Rabi) along with their cross combinations:

SL No	Test cross	Cross Combination
1.	TC-1	25A x PP-1
2.	TC-2	25A x PP-1
3.	TC-3	25A x PP-1
4.	TC-4	25A x PP-1
5.	TC-5	25A x PP-2
6.	TC-6	25A x PP-2
7.	TC-7	25A x PP-2
8.	TC-8	25A x PP-3
9.	TC-9	25A x PP-3
10.	TC-10	25A x PP-4B (yellow)
11.	TC-11	25A x PP-4B (yellow)
12.	TC-12	25A x PP-4B (yellow)
13.	TC-13	25A x PP-4B (normal)
14.	TC-14	25A x PP-4B (normal)
15.	TC-15	25A x PP-5
16.	TC-16	25A x PP-5
17.	TC-17	25A x PP-5
18.	TC-18	25A x PP-6
19.	TC-19	25A x PP-8
20.	TC-20	25A x PP-8
21.	TC-21	25A x PP-8
22.	TC-22	25A x PP-9
23.	TC-23	25A x PP -9
24.	TC-24	25A x PP -9
25.	TC-25	25A x PP -10
26.	TC-26	25A x PP -10
27.	TC-27	25A x PP -11
28.	TC-28	25A x PP -11
29.	TC-29	25A x P-3B
30.	TC-30	25A x P-3B
31.	TC-31	25A x P-3B
32.	TC-32	25A x P-5B
33.	TC-33	25A x P-5B
34.	TC-34	25A x P-5B
35.	TC-35	25A x P-6B
36.	TC-36	25A x P-6B
37.	TC-37	25A x P-6B
38.	TC-38	25A x P-6B
39.	TC-39	25A x IR-25B
40.	TC-40	25A x IR-25B

Appendix: I. (Contd.)

Sl. No.	Test cross	Cross Combination
41.	TC-41	25A x R-1
42.	TC-42	25A x R-4
43.	TC-43	25A x R-5(II)
44.	TC-44	25A x R-12
45.	TC-45	25A x BR-29
46.	TC-46	25A x SPS-151
47.	TC-47	25A x AL-1
48.	TC-48	25A x AL-10
49.	TC-49	25A x AL-11
50.	TC-50	25A x AL-12
51.	TC-51	25A x AL-14 (I)
52.	TC-52	25A x AL-14 (II)
53.	TC-53	25A x AL-18
54.	TC-54	25A x AL-20
55.	TC-55	25A x AL-26
56.	TC-56	25A x AL-27
57.	TC-57	25A x AL-29
58.	TC-58	25A x AL-33 (II)
59.	TC-59	25A x AL-35
60.	TC-60	25A x AL-36
61.	TC-61	25A x AL-42
62.	TC-62	25A x AL-44(I)
63.	TC-63	25A x AL-44(II)
64.	TC-64	25A x AL-47 (I)
65.	TC-65	25A x AL-50
66.	TC-66	25A x AL-51(I)
67.	TC-67	25A x AL-51(II)
68.	TC-68	25A x AL-52
69.	TC-69	25A x AL-56(I)
70.	TC-70	25A x AL-56(II)
71.	TC-71	25A x AL-56(III)

Appendix II: Mean pollen fertility of test cross hybrids and classification of pollen parents (Rabi).

(R: Restorer, PR: Partial Restorer, PM: Partial Maintainer, M: Maintainer)

SL No	Test cross	Pollen Fertility %	Category
1.	TC-1	28.61	PM
2.	TC-2	48.85	PR
3.	TC-3	50.26	PR
4.	TC-4	41.19	PR
5.	TC-5	42.45	PR
6.	TC-6	64.06	R
7.	TC-7	45.02	PR
8.	TC-8	36.35	PR
9.	TC-9	42.09	PR
10.	TC-10	68.33	R
11.	TC-11	48.66	PR
12.	TC-12	55.50	PR
13.	TC-13	18.10	PM
14.	TC-14	20.12	PM
15.	TC-15	-	-
16.	TC-16	50.39	PR
17.	TC-17	47.58	PR
18.	TC-18	13.05	PM
19.	TC-19	15.25	PM
20.	TC-20	57.57	PR
21.	TC-21	57.57	PR
22.	TC-22	39.36	PR
23.	TC-23	21.51	PM
24.	TC-24	12.41	PM
25.	TC-25	51.66	PR
26.	TC-26	73.25	R
27.	TC-27	26.35	PM
28.	TC-28	10.15	PM
29.	TC-29	40.12	PR
30.	TC-30	00.00	M
31.	TC-31	00.00	M
32.	TC-32	00.00	M
33.	TC-33	00.00	M
34.	TC-34	00.00	M
35.	TC-35	80.54	R
36.	TC-36	00.00	M
37.	TC-37	15.13	PM

Appendix II: (Contd.)

SL No	Test cross	Pollen Fertility %	Category
38.	TC-38	00.00	M
39.	TC-39	00.00	M
40.	TC-40	00.00	M
41.	TC-41	64.28	R
42.	TC-42	61.76	R
43.	TC-43	4.03	PM
44.	TC-44	47.11	PR
45.	TC-45	39.02	PR
46.	TC-46	26.74	PM
47.	TC-47	-	-
48.	TC-48	-	-
49.	TC-49	-	-
50.	TC-50	00.00	M
51.	TC-51	20.38	PM
52.	TC-52	68.89	R
53	TC-53	40.24	PR
54.	TC-54	60.69	R
55.	TC-55	50.24	PR
56.	TC-56	41.02	PR
57.	TC-57	21.31	PM
58.	TC-58	60.95	R
59.	TC-59	60.79	R
60.	TC-60	66.27	R
61.	TC-61	49.87	PR
62.	TC-62	30.50	PR
63.	TC-63	66.19	R
64.	TC-64	-	-
65.	TC-65	-	-
66.	TC-66	54.34	PR
67.	TC-67	38.36	PR
68.	TC-68	60.98	R
69.	TC-69	73.29	R
70.	TC-70	42.76	PR
71.	TC-71	62.86	R

(-) plant was not available