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DEVELOPMENT AND EVALUATION OF TWO AND THREE LINE HYBRIDS IN RICE (*Oryza sativa* L.)

PANCHUMARTHI SARVARI

DIVISION OF GENETICS
INDIAN AGRICULTURAL RESEARCH INSTITUTE
NEW DELHI - 110 012

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DEVELOPMENT AND EVALUATION OF TWO AND THREE LINE HYBRIDS IN RICE (*Oryza sativa* L.)

A Thesis

By

PANCHUMARTHI SARVARI

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Approved by :

Chairman : _____
(Dr. M.J. Abraham)

Member(s) : _____ 6.12.99
(Dr. V.P. Singh)

_____
(Dr. T. Mohapatra)

_____
(Dr. Anju Mahendru)

_____
(Dr. A.K. Singh)

Dr. M.J. Abraham
Senior Scientist

Division of Genetics
Indian Agricultural Research Institute
New Delhi - 110 012, India

CERTIFICATE

This is to certify that the thesis entitled "**Development and evaluation of two and three line hybrids in Rice (*Oryza sativa* L.)**" submitted to the Faculty of Post-Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfilment of the requirements, for the award of the degree of **Master of Science** in **Genetics**, by **P. Sarvari** embodies the result of bona-fide research work carried out by her under my guidance and supervision. No part of the thesis has been submitted by her for any other degree or diploma.

I further certify that such help or information, as have been availed of in this thesis, is duly acknowledged.

(**M.J. Abraham**)
Chairman
Advisory Committee

New Delhi
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P. Sarvari

(SARVARI)

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INTRODUCTION

With the introduction of semi-dwarf, high tillering, photo-insensitive, nitrogen responsive, high yielding varieties in mid sixties, there has been more than two fold increase in the rice production. It increased from 257 million tonnes in 1965 to 562 million tonnes in 1995 and helped several chronically food deficient countries in the region not only to become self-sufficient but some of these as exporters.

In India the production level during the same period rose from less than 46 million tonnes to 124 million tonnes. However, if one examines the genetic yield potential obtained through semi dwarf technology it can be seen that there has been a plateauing. Rice scientists around the world now have the challenge to develop technologies to break the yield barriers in order to make a big leap in food production to meet the requirements of post 2000 AD. Of the various short and long term approaches being contemplated, development of hybrid rice technology is considered to be one of the most practicable technology available to meet this immense challenge after China has successfully developed and adopted this technology since the mid seventies. It has been over thirty years since the research on hybrid rice started in 1964 when Prof. Yuan Longping first put forward the idea of utilising the heterosis in rice and initiated the research on hybrid rice in China. Great advances have been achieved both in the theoretical research aspects and practical utilisation.

The discovery of strong and stable source of cytoplasmic male sterility in the early seventies by Chinese scientists enabled them to synthesize commercially viable rice hybrids (Yuan, 1987; Lin and Yuan, 1980).

In China the national average grain yield of rice increased from 4.2 t/ha in 1976, the very beginning of hybrid rice commercialisation, to 6.8 t/ha in 1990. In general, hybrid rice outyields conventional rice by 1.5 t/ha or by more than 20%.

The hybrid rice technology in a strictly self pollinated crop like rice with an yield advantage of 1-2 t/ha over the conventional varieties is a landmark in the history of rice breeding. Among the countries ensuring keen interest to adopt this technology, India is the foremost. Using improved parental lines, especially the male sterile lines which are better adapted to tropical environment, India was able to release hybrids for commercial cultivation in 1994. Within a short span of 6 years (from 1990-1996) of concerted research efforts, 7 three-line hybrids from public sector and may be an equal number from private sector are available for cultivation in the country. By the turn of the century the country is poised to bring over two million hectares under hybrid rice. It is expected that a quantum jump in the rice production in the country may be achieved with the maturation of hybrid rice technology.

But about 95 per cent of the commercial hybrids in China and elsewhere are based on one and only cytosterility system viz., WA (wild

abortive) (Virmani, 1990). Any technology based on such narrow genetic base can lead to 2 kinds of problems viz., (i) Restricted adaptability and (ii) Genetic vulnerability to sudden outbreaks of diseases and insect-pests. Besides these the other disadvantages associated with the cytoplasmic male sterility system are (iii) long and cumbersome process of developing parental lines with desired agronomic features (iv) Need of maintainers for multiplication of A-line (v) Care to maintain the genetic purity of all three lines (vi) higher seed cost and (vii) the three-line system is only capable of using only less than 10% of the available elite lines developed in rice as only full maintainers and restorers, whose frequency is extremely low.

These limitations have prompted scientists to look for alternative approaches to exploit hybrid vigour. Early efforts much before the development of cytoplasmic genetic male sterility - fertility restorer system, like identification and use of selective male sterilising chemicals such as arsenic compounds gave unsatisfactory efficacy in inducing male sterility and also resulted in phytotoxicity. Sodium and zinc methyl arsenates are the two gametocides that are being commercially used in China. They too unfortunately are not in wide use due to their reported carcinogenicity.

Yet another approach of two-line breeding emerged through the chance discovery of a photoperiod sensitive genic male sterile (PGMS) plant, Nongken 58S, from the japonica variety Nongken 58 by Prof. Shimingshong

in Hubei province of China (Shi, 1981, 1985; Shi and Deng, 1985, 1986). Interest aroused by this important discovery had led to the identification of several additional source of photoperiod sensitive and temperature sensitive genic male sterility (TGMS) of spontaneous and induced origin (Lu and Wang, 1986; Tan *et al.*, 1990; Maruyama *et al.*, 1990a, 1990b). They remain male sterile under long days (>14h/d) or / and high temperature (>30°C) and will become male fertile under reduced day length (<13.75 h/d) or / and temperature (<24°C) at panicle initiation stage, and this situation has been taken as an advantage for hybrid seed production and seed increase of PGMS / TGMS. Hybrids such as W61548 / Taquing and W6154 S/312 of indica background and W 50478 / R9-1 and N5088 S / R9-1 of japonica background had become popular in parts of China.

There are several advantages of two line approach to heterosis breeding:

1. There is no need of maintainer line, hence procedure for development of hybrids is simpler.
2. Any genotype can be utilised as male parent; hence, the chances of development of heterotic hybrids are greatly increased.
3. Negative effects associated with sterile cytoplasm are not encountered.
4. Seed production programme is simple and more efficient. The field area ratio between TGMS multiplication, hybrid seed production and

commercial cultivation of F_1 hybrid is 1:100:10000 as against 1:50:5000 in three-line system.

In a country like India, since the required day length differences do not exist, photoperiod sensitive male sterility system (PGMS) would not be suitable. However, taking advantage of temperature ranges available, temperature sensitive genic male sterility system (TGMS) can be effectively used. The TGMS are well adapted to both tropical and subtropical conditions. Unlike PGMS where in fertility - sterility transformation process is greatly influenced by day length, TGMS is less affected by day length.

Besides ANNONG-1S of China, induced mutants Norin PL-12 from Japan and IR 32364-20-1-3-2 from IRRI, there are not many TGMS sources for study and use. The TGMS gene of Norin PL-12 has been transferred to tropical indica varieties at IRRI, Philippines and they are available freely to the countries wanting to pursue research on these lines.

Keeping in view the advantage of TGMS based two line hybrid system and the limitations of CMS based three-line hybrid system it was felt worth while to undertake studies with a set of CMS and TGMS lines and hybrids based on them. For a comparative assessment of the two types of hybrids, the experiment was started with the following objectives:

1. Screening of TGMS lines for stability of sterility and their morphological characterisation.
2. Screening of CMS lines for stability of sterility and their morphological characterisation.
3. Production and comparative evaluation of two and three-line hybrids.

REVIEW OF LITERATURE

Utilisation of heterosis in agricultural crops is one of the most attractive achievements in plant breeding in this century. Popularisation of hybrid maize, which contributed 60% of the total yield increase, promoted the per unit area yield of maize to go up to 7.5 t/ha on an average in 1980s from about 2.0 t/ha in 1930 in USA. With per unit area yield rise by about 30% than conventional rice varieties, hybrid rice has helped China to increase food production by over 300 million tonnes since 1970s when the hybrid rice began to be expanded in the country. According to the statistical data by FAO (1990) hybrid rice contributes about 20% of the total rice output in the world with only 10% of the total rice planted area.

The most effective way to make use of crop heterosis is to produce hybrids by taking advantages of the male sterility, with the utilisation of the three-line system as the classical method. Although the three line system is effective and is playing and will still play an important role in exploiting heterosis at present and for some long period in future, the system has shown some inherent characteristics unfavourable to the further development of crop hybrids as explained in introduction chapter.

Considering from the strategy for the long lasting development in plant heterosis utilisation, quite a few breeders are probing into the new technological methods which are easily operated and in higher efficiency

to replace the three-line system. In view of the developmental level and the superiority, exploiting crop heterosis by two-line system hybrids should be firstly recommended. With the above background and with the objective of producing and evaluating better heterotic hybrids the present study was undertaken and it is reviewed under the following heads:

1. Heterosis
2. Cytoplasmic male sterility
3. Environment sensitive genetic male sterility
4. Thermosensitive genetic male sterility

Heterosis

Heterosis refers to the superiority of the F_1 hybrid over the mean parental value (relative heterosis) or the better parent (heterobeltiosis)or the best commercial variety (standard heterosis). The term "Heterosis" was coined by Shull in 1908. The term "Hybrid vigour" is essentially synonymous with heterosis, the difference lies in that hybrid vigour is the visible manifestation effects of heterosis, whilst heterosis is the increased size and vigour of the crossbred organism in comparison to its inbred parents. In general heterobeltiosis and standard heterosis are more relevant from the plant breeder's point of view.

Heterosis for yield and yield components in rice

Jones (1926) first reported heterosis in rice, for culm number and grain yield. Since then several rice researchers have reported the occurrence of heterosis for yield and yield contributing characters. Kim

and Rutger (1988) and Virmani (1994) reviewed literature on heterosis in rice until1990 and reported the presence of significant standard heterosis for yield and its components.

Commercial rice hybrids show 20-30% standard heterosis for grain yield (Lin and Yuan,1980; Yuan *et al.*, 1989). Yuan *et al.* (1994) also showed a 29 to 45% yield advantage of hybrids over conventional rice varieties in China. Outside China, significant standard heterosis for yield in rice has been observed in trials conducted at IRRI and in several national programmes, *viz.* Philippines, India, USA, Korea and Egypt. At IRRI the best rice hybrid outyielded the best inbred variety by about 17% (Virmani,1996).

In India, reports by Patnaik *et al.* (1990), Murty *et al.* (1991), Reddy and Nerkar (1991), Chandra *et al.* (1992), Ram (1992), Singh *et al.* (1992), Bobby and Nadarajan (1993), Deosarkar and Nerkar (1994), Patel *et al.* (1994),Ramalingam *et al.* (1994), Pandey *et al.* (1995), Reddy and Nerkar (1995), Singh *et al.* (1995), Yolanda and Das (1995), Padmavathi *et al.* (1996), Rao *et al.* (1996a), Singh *et al.* (1996b), Yolanda and Das (1996), Singh and Maurya (1997), Singh *et al.* (1996b), Yolanda and Das (1996), Singh and Maurya (1997) have confirmed the presence of significant heterosis for yield and yield contributing characters.

The extent of standard heterosis and heterobeltiosis observed for various traits in rice as reported by different rice researchers have been presented in tabular form below:

Extent of standard heterosis (Sc) and Heterobeltiosis (Hb) observed for various traits in rice since 1961

Character		Heterosis range %		Reference for	
		Min.	Max.	Min.	Max.
Grain yield per plant	Sc:	-98.00	284.55	Ravikumar (1983)	Sarial (1994)
	Hb:	-99.00	368.60	-do-	Rao (1965)
Number of tillers per plant	Sc:	-43.90	242.85	-do-	Rangaswamy & Natarajamurthy (1988)
	Hb:	-30.00	242.85	-do-	-do-
Number of panicles per plant	Sc:	-31.84	246.80	Manueal and Palanisamy (1989)	Paramasivan (1985)
	Hb:	-94.00	424.4	Amrithadevarathinam (1984)	-do-
Panicle length	Sc:	-31.80	73.01	Paramasivan (1985)	Anand Kumar and Sree Rangasamy (1984)
	Hb:	-42.01	25.41	Nijaguna and Mahadevappa (1983)	Rangaswamy and Natarajamurthy (1988)
Number of spikelets per panicle	Sc:	-65.85	131.29	Rangaswamy and Natarajamurthy (1988)	Srivastava and Seshu (1982)
	Hb:	-67.81	101.30	-do-	Vishwanath (1989)
Number of grains per panicle	Sc:	-100.00	160.00	Sarial (1994)	Amrithadevara thinam (1984)
	Hb:	-100.00	127.00	Balan (1987)	Pillai (1961)
	Sc:	-100.00	70.19	Sarial (1994)	Sarial (1994)
	Hb:	-100.00	63.40	-do-	Singh and Maurya (1997)
Panicle density	Sc:	-53.93	238.50	Manuel and Palanisamy (1989)	Rangaswamy and Natarajamurthy (1988)
Spikelet sterility	Hb:	-51.06	908.90	Nijaguna and Mahadevappa (1983)	-do-
	Sc:	-81.77	78.00	Rangaswamy and Natarajamurthy (1988)	Sivasubramanian *et al.* (1989)
1000 grain weight	Hb:	-83.13	505.1	Paramasivan *and* Sree Rangasamy (1988)	Rao (1965)
	Sc:	-53.35	219.81	Sarial (1994)	Sarial (1994)
	Hb:	-56.51	151.36	-do-	-do-
Biological yield per plant	Sc:	-87.23	105.88	-do-	-do-
	Hb:	-87.88	21.74	-do-	-do-
Harvest index	Sc:	-46.22	82.27	Manuel and Palanisamy (1989)	Srivastava and Seshu (1982)
Plant height	Hb:	-63.35	99.80	Balan (1987)	Pillai(1961)
	Sc:	-30.85	31.90	Panwar *et al.* (1983)	Karunakaran (1968)
Days to 50% flowering	Hb:	-37.00	83.60	Singh and Richharia (1980)	Balan (1987)
	Sc:	-11.67	6.43	Sarial (1994)	Sarial (1994)
	Hb:	-14.83	28.45	-do-	-do-
Days to maturity	Sc:	-52.38	104.76	-do-	-do-
	Hb:	-60.00	104.76	-do-	-do-
Flag leaf area					

Increased yield in rice hybrids has been attributed to their increased dry matter, resulting from higher leaf area index (LAI) and higher crop growth rate (CGR); and increased harvest index, resulting from their increased spikelet number and to some extent, increased grain weight (Ponnuthurai *et al.*, 1984; Akita *et al.*, 1986; Blanco *et al.*, 1986; Agata, 1990). Virmani *et al.* (1982), Ponnuthurai *et al.* (1984) and Patnaik *et al.* (1990) reported that increased yield in heterotic hybrids in rice was mainly due to heterosis in spikelet number and, to some extent, heterosis for 1000 grain weight. Pandey *et al.* (1995), Reddy and Nerkar (1995), Padmavathi *et al.* (1996) and Rao *et al.* (1996a) pointed out increased number of tillers per plant as the main factor for increased grain yield of hybrids. Also, increased number of panicles per plant (Yolanda and Das, 1995; Padmavathi *et al.*, 1996)and 1000 grain weight (Padmavathi *et al.*, 1996) were found to be directly related with increased grain yield.

Lin *et al.* (1996a) reported that the high number of productive tillers per plant had the largest direct effect on grain yield, resulting in increased sink capacity. The higher tiller number and number of grains per panicle were attributable to higher leaf areas, higher net photosynthesis in individual leaves and favourable partitioning of photosynthate to plant organs.

Variation in heterosis and heterobeltiosis over different environments and higher heterosis in wet season than in dry season was reported by Young and Virmani (1990). They also concluded that for days to flowering, the overall mean heterosis, heterobeltiosis and standard heterosis, all had negative values.

Heterotic rice hybrids were observed to possess varying growth duration ranging from 105 to 136 days (Virmani,1987), indicating that growth duration did not correlate with expression of heterosis. Yield advantage of a hybrid over inbred check was higher at high yielding environments than low yielding environments, although percentage heterosis may reduce due to the higher mean yield of the check variety at high yielding environments (Virmani, 1996). Akita (1988) reported that heterosis in panicles per plant, spikelets per panicle and spikelet fertility varied highly among crosses and cultivation conditions due to yield component compensation.

Comparison of Heterosis in two and three line hybrids:

In test crosses made at IRRI, the frequency of heterotic rice hybrids derived from the TGMS system was higher than from the CMS system which is represented in tabular form below. Some two-line rice hybrids yielding 1t/ha higher than inbred check varieties were identified in preliminary yield trials at IRRI. Bulk quantities of seed of these hybrids

are now produced to enable multilocational evaluation in tropical rice growing countries (Lu Xinggui *et al.*, 1996).

Relative frequency of heterotic hybrids derived from CMS and TGMS systems in a test-cross nursery, IRRI, 1993-94

Male sterile system	No. of crosses		Number and frequency (%) of heterotic hybids	
	1993	1994	1993	1994
CMS	103	106	17(16)	64(31)
TGMS	131	115	47(36)	77(67)

To develop two line rice hybrids, there are many types of PGMS and TGMS lines available, which pose no restrictions for the restorer - maintainer relationship as in the case of CMS lines. It is therefore relatively easier to develop desirable new two-line rice hybrids than three line rice hybrids. In early work in breeding two-line rice hybrids, Chinese breeders usually used the newest restorers, such as Minghui 63, Wanhui 9, and 6078, from the three - line breeding programme and the newest cultivars such as Teqing, Shanqing 11, Te-San-ai and 77 Zhan, from the conventional breeding programme as two-line system restorers. A number of two-line hybrid rice combinations with a yield increase of 5-10% over the three-line combinations have been developed and commercialised. (Wang and Li 1992; Luo *et al.*, 1994; Wang *et al.*, 1995; Chen *et al.*, 1996).

Cytoplasmic Male Sterility

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 processes during microsporogenesis. These processes 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 factors, or due to interaction of both. Depending on these factors, it is classified into three types:

1. Cytoplasmic male sterility
2. Genetic male sterility
3. Cytoplasmic - genetic male sterility (Sears, 1947)

1. **Cytoplasmic male sterility** : It occurs due to the mutation of mitochondria or to some other cytoplasmic factors outside the nucleus, resulting in the transformation of the fertile cytoplasm into a sterile one. Nuclear genes are not involved with two types of cytoplasm (i.e. sterile and fertile). At the most only two kinds of genotypes are possible one of them is sterile (B line) and the other fertile (A line).

2. **Genetic male sterility** : In this case the cytoplasm is not involved. If we designate the fertile gene (dominant) as `R' and the sterile (recessive) as `r' there could be then three possible genotypes for this locus and only one of them is male sterile viz., RR- Fertile (R line), Rr - Fertile (B line) and rr - Sterile (A line).

   By crossing A x B lines sterile and fertile progenies are produced in equal proportions. The fertile plants need to be promptly removed before they shed pollen grains, thus leaving only sterile progenies. The removal of fertile plants may become easier if fertility is associated with some marker genes in plants, expressed preferably at seedling stage. By crossing A x R line hybrid seed can be produced. However taking advantage of pleiotropic effects of male sterility gene(s) phenotypic expression of closely linked seedling marker gene to genetic male sterility has been successfully exploited through simple and innovative means in self pollinated crops like tomato (Rick, 1945; Philouze 1969, 1974), Barley (Suneson, 1951; Wiebe, 1964; Falk *et al.*, 1987; Ramage, 1965, 1975), wheat (Driscoll, 1972, 1985) and pigeon pea (Wallis, 1980).

3. **Cytoplasmic - genetic male sterility** : Such sterility arises from the interaction of nuclear gene(s) conditioning sterility with sterile cytoplasm. Actually cytoplasmic - genetic sterility is essentially a cytoplasmic sterility with a provision for restoration of fertility (which is

not possible in the latter case). The fertility is restored by the R gene(s) present in the nucleus. Thus, the combination of both nuclear gene(s) and cytoplasmic factors determines the fertility or sterility in such plants. Based on these combinations, there can be a maximum of six kinds of genotypes; only one of them is sterile. The six genotypes are:

i)	RR	F	RR	genes with	F	cytoplasm	-	Fertile (R line)
ii)	RR	f	RR	genes with	f	cytoplasm	-	Fertile
iii)	Rr	F	Rr	genes with	F	cytoplasm	-	Fertile
iv)	Rr	f	Rr	genes with	f	cytoplasm	-	Fertile
v)	rr	F	rr	genes with	F	cytoplasm	-	Fertile (B line)
vi)	rr	f	rr	genes with	f	cytoplasm	-	Sterile (A line)

As visualised by their genetic compositon and cytoplasm only rr F genotypes can maintain the sterility of A line as follows:

rr	f	x	rr	F	rr f
A line			B line		A line

Fertility restoration of hybrid seed production can be achieved by suitable restorer lines which can give rise to all fertile progenies on crossing with A - line as follows:

rr	f	x	RR	f	Rr f
A line			(or)		Fertile hybrid
			RR	F	
			R line		

Cytoplasmic - genetic male sterility can also be induced as demonstrated by Faveret and Ryan (1964) in Barley, by Burton and Hanna (1976) in pearl millet, Kinoshita and Takashi (1969) in sugar beet, Mallick (1980); Young (1983), Minocha and Gupta (1988) in rice.

Most of the male sterile sources developed in rice are of sporogenous type. On the basis of pollen morphology and stainability with I-KI as well as restoration or maintenance behaviour, Chinese have categorised them into the following three groups:

1. **WA type** : Pollen are characterised by irregular shape and non-stainability with I-KI. Abortion occurs possibly at or around uninucleate stage or between uninucleate and early binucleate stages and hence typical `sporophytic type'. In maintenance and restoration behaviour similar to WA, Gam, Di-Gu Y_{12} and Indonesia belong to this group. Male sterility is governed by two pairs of recessive nuclear sterility genes and sterile cytoplasm (Gao, 1981; Liang, 1980; Hu and Li, 1985).

2. **Honglian type** : Characterised by pollen of spherical shape and non-stainability or light stainability with I-KI. Pollen abortion occurs possibly at binucleate stage; fertile pollen is 50 per cent in F_1 hybrids involving this type based male sterile lines and no sterile plants in F_2, inspite of

partial pollen sterility. It is of gametophytic sterility. Maintenance restoration behaviour is similar to 'WA type'. Sterility is governed by a pair of recessive nuclear sterility genes along with a set of modifier genes in interaction with sterile cytoplasm.

3. **BT type** : It is of gametophytic type like Honglian type (F_1 involving CMS of this type is having 50 per cent pollen fertile but with no seed set and no sterile plants in F_2 though pollen are partially sterile). Pollen stainable with I-KI abort just before reaching trinucleate stage. Maintenance - restoration behaviour is similar to Honglian type. Dian 1, Dian 3 etc., come under this group.

Cytoplasmic male sterility in Rice

Weeraratne (1954) and Sampath and Mohanty (1954) reported on cytoplasmically induced male sterility in rice for the first time. Shinjyo and Omura (1966) developed the first CMS line in cultivated rice by substituting the nuclear genes of the Ponalai variety of Taichung 65 into the cytoplasm of the indica variety Chinasurah Boro II and designated it as CMS-boro. Following this many CMS lines were developed by several workers using distant nuclear substitution backcrosses involving wild, semi wild, indica and japonica rices.

The first CMS line used to develop commercial F_1 rice hybrid was developed in China in 1973 from a naturally occuring male sterile plant in a

wild rice population of *Oryza sativa* f spontanea on Hainan island (Yuan, 1977). This plant was designated as wild rice with aborted pollen (WA) since then, a number of CMS lines have been developed from various wild and cultivated rice accessions in China and outside. Virmani and Edwards (1983) and Virmani and Wan (1988) listed some of the CMS sources identified in and outside China. These CMS sources designated in principle, according to the name of the cultivar from which the cytoplasmic factor inducing male sterility is derived. Some of the important, designated CMS lines include WA, HL, DA, bo, TN, Gam, ARC, UP, KA, OF, OR etc. Important cytoplasmic donor species include *Oryza sativa* f spontanea, *O. sativa* (strains - Chinasurah Boro II, Taichung Native-I, Gambiaca, Kalinga 1 etc.), *O. fatua*, *O. rufipogon*, *O. perennis*, *O. nivara* and *O. glumaepatula*. Inspite of large number of CMS lines identified, only few reported to have been identified for commercial use in hybrid rice. It has been reported that about 95% of the total area planted with commercial hybrids in China and elsewhere has a single CMS source, the WA cytoplasm (Brar *et al.*, 1996).

Development of CMS lines in India

Development of new CMS lines in India has been reported by several rice researchers (Parmar *et al.*, 1981; Virmani and Edwards, 1983; Chaudhary and Sahai, 1984; Hassan and Siddiq, 1983; Pradhan *et al.*, 1990a; Siddiq *et al.*, 1994; Abraham *et al.*, 1996; Jayamani *et al.*, 1996; Kumar *et al.*, 1996; Ramesha *et al.*, 1996; Hoan *et al.*, 1998; Kumar *et al.*, 1998; Pradhan and Jachuck, 1998; Zaman *et al.*, 1998b).

Research has emphasised evolving CMS lines that are agronomically superior and better adapted to local agro climatic conditions than the Chinese lines. More than thirty improved CMS lines evolved through conversion at IRRI and in India have been evaluated for their relative stability for male sterility over locations / seasons, out crossing potential and combining ability (Siddiq *et al.*, 1994). Some of the CMS lines developed in India are given below:

CMS lines developed in India

CMS line	Source	CMS line	Source
PMS 2A	WA	Krishna A	Kalinga-1
PMS 3A	WA	Henna A	WA
PMS 4A	WA	Krishna A	WA
PMS 5A	WA	Rajeshwari A	WA
PMS 7A	WA	Bharati A	WA
PMS 8A	WA	Kiran A	WA
PMS 10A	WA	Deepa A	WA
Sarasa-1A	WA	Pusa-4-1-11A	WA
Sarasa-11A	WA	Adt. 34A	WA
Pusa 33A	WA	Suphala A	WA
Pragathi A	WA	Pusa 3A	WA
MW 10A	WA	Pusa 5A	WA
CRMS 1	Ratna	Madhu A	WA
CRMS 2	Dunghansali	Madhuri A	WA

Stability of sterility of CMS lines

Stability of CMS lines over environments is an important consideration in developing commercial rice hybrids. Male sterility induced by the interaction of nuclear and cytoplasmic genes are also influenced by environment. Differences in the level of stability among cytoplasmic genetic male sterility lines possessing common sterile cytoplasm tend to suggest nuclear background to be largely responsible for the variation. Although, in many of the CMS lines the precise environmental factor(s) influencing nuclear sterility genes is not known, yet the potent environmental factors are temperature, photoperiod and light intensity.

Pollen grains of CMS lines derived from sporophytic CMS systems abort at the uninucleate stage and hence are more stable than those derived from gametophytic systems in which pollen grains abort at the bi- or trinucleate stage. Among the various sources of cytosterility in rice, CMS lines derived from the WA system have been found to be the most stable ones for their complete pollen sterility (Lin and Yuan, 1980; Chaudhary *et al.*, 1981).

Studies in China indicated that wider crosses between a primitive female line and an advanced male sterile line likely resulted into sporophytic and stable CMS system. Cross involving narrow genetic base

between cytoplasmic and nuclear donor parents produced gametophytic unstable male sterile lines (Virmani and Edwards, 1983).

A large number of CMS lines have been developed in China, IRRI and elsewhere and evaluated in different agro-climatic conditions for their stability of pollen sterility. In India, studies were carried out for the evaluation of stability of CMS lines, using pollen sterility / spikelet sterility as a parameter by Mahadevappa (1985), Pradhan *et al.* (1990b), Manueal *et al.* (1991), Yogeesha (1991), Pandey *et al.* (1992), Mishra and Pandey (1993), Pradhan and Jachuck (1993), Leena Kumari (1994), Seetharamaiah *et al.* (1994), Yogeesha and Mahadevappa (1996) and Pandya *et al.* (1996). The results varied depending upon the CMS line and location / environment.

Pradhan *et al.* (1990b) studied pollen grains from 22 CMS lines from five cytoplasmic sources and classified as unstained withered sterile, unstained spherical sterile, stained round sterile and stained round fertile. Based on the relative frequency of different classes of pollen, the CMS lines were classified into five groups. Six CMS lines were designated as stable for pollen sterility.

Gautam *et al.* (1997) classified the CMS lines based on pollen sterility percentage as : completely sterile (100% pollen sterility), highly sterile (99-99.9% sterility), sterile (95-98% sterility), partially sterile (70-94.9% sterility) and partially fertile (<70% pollen sterility).

Environment Sensitive Genetic Male Sterility

Following the discovery of highly stable male sterile source `wild abortive' in the early seventies and development of commercially usable male sterile, maintainer and suitable restorer lines by Chinese, three line hybrid technology became the field of reality since late seventies in China and the technology is gaining attention in other countries since early 90s. Inspite of its attractive technology and spread of hybrids to over 58 per cent of the rice area in China alone, the three-line approach has its own limitations. Dependence on a single source of male sterility, cumbersome conversion process and method of maintenance of CMS lines, difficulties in incorporating desired agronomic characters into the parental lines, low frequency of perfect maintainers and restorers with desired agronomic characteristics are some of the major limitations or weaknesses of the three line approach, which prompted scientists to look for alternate approaches to exploit hybrid vigour.

Environment - influenced male sterility - fertility transformation is an alternate approach, which is receiving serious research attention, since the discovery of induction of photoperiod sensitive and temperature sensitive male steriles in China and Japan.

Fertility - sterility is a delicately balanced system. Its expression is highly prone to the influence of various environmental factors and their

interactions. The underlying principle is that phenotypic expression of a gene is specific to a given range of environment. Its expression in one set of condition, (normal temperature for instance) would be different from another set of conditions (very high or very low temperature). Conditional mutations of bacteria induced and extensively used in genetic investigations are of this kind. These mutations are lethal in restrictive environment but viable in permissive environment. Either at transcription and / or at translated product level the restrictive environment limits the expression of the gene. Temperature sensitivity, for instance results from increased heat or cold lability of the mutant gene product- an enzyme. The enzyme active at low temperature might be partially or totally inactive at higher temperature. Occasionally synthesis of the final product of the gene is sensitive. In such cases once the product is synthesised, the expression of the mutant gene will be as good as that of the wild.

Occurrence of environment - sensitive male steriles is known since long in several self-pollinated crop plants. Earliest report on the influence of environment on fertility - sterility was way back in 1947 and 1951, When Rick (1948) and Martin and Crawford (1951) reported temperature sensitive mutants for fertility - sterility in tomato and pepper. Of two mutants obtained in each of pepper and tomato, which were sensitive to temperature and light intensity, one mutant each was found to be male sterile in field and other under glass house conditions. Transfer of these

from glasshouse to field and vice versa has been found to revoke fertility or sterility in them (Martin and Crawford, 1951). Later Rundfeldt (1960) isolated a cabbage mutant which was male sterile in summer and male fertile in winter. Among cereals instance of temperature sensitive male sterile - fertile mutant was reported first in wheat by Jan (1974). He recovered the mutant from the differential behaviour of early and late formed tillers for fertility. Later, Ahokas and Hockett (1977) showed the barley mutant `MS9' to exhibit sensitivity to photoperiod and physiography. It was completely male sterile in Finland but partially in the USA. Inspite of all such reports on the isolation and study of environment - sensitive mutations, hardly the trait has been exploited on a commercial scale for hybrid seed production in any of the crops. Rice proved however, an exception.

Prof. Min - Shong Shi of Hubei province, China was the first to discover photoperiod sensitive male sterile mutant in rice (Shi, 1981, 1985; Shi and Deng, 1985, 1986). Closely following this report several scientists largely Chinese came out with both photoperiod and thermosensitive mutants in rice (Lu and Wang, 1986; Yuan *et al.*, 1988; Wang *et al.*, 1990, Maruyama *et al.*, 1990a). The environment sensitive mutants of spontaneous and induced origin have been extensively studied for their stability of sterility, fertility transformation, mode of inheritance and level of utility in hybrid breeding.

Photoperiod sensitive genic male sterility (PGMS)

Shi & Deng (1985) were the first to report photosensitive male sterile plant which was isolated as a spontaneous mutant in the field of a late maturing *japonica* variety Nongken 58 way back in 1973. The mutant designated as Nongken 58s was found to show total and stable sterility when it flowered under the day length of 14 h and above (before Sept 1^{st} or 2^{nd}). However, the male sterile plants flowering after this date, when the day length becomes less than 13.75 h showed spikelet fertility ranging from 10-40 per cent (Lu and Wang, 1986). The day length - influenced behaviour of the mutant was confirmed under controlled conditions as well. Genetic studies suggest a single recessive gene along with some modifiers to control the photoperiod sensitive male sterility (Shi and Deng 1985, Zhu and Yu 1987, Zhang and Zhu, 1990).

A study by Lu and Li (1990) of Nongken 58s and 105s, a derivative of the cross Nongken 58s / Gyi 105, while confirming that the trait was monogenic recessive, showed its fertility restoration to involve two pairs of independent major - genes. Nongken 58s for instance, has two pairs of independent genes in recessive state, the genotype being ms_1^{Ph} ms_2^{Ph} ms_2^{Ph} ms_2^{Ph}. Nongken 58 the fertile parent variety has a pair of dominant restorer genes with the genotype of MS_1^{Ph} MS_2^{Ph} ms_2^{Ph} ms_2^{Ph}. The other variety tested has two pairs of dominant restorer genes, their genotypes

being MS_1^{Ph} MS_1^{Ph} MS_2^{Ph} MS_2^{Ph}. Of these two pairs of dominant restorer genes MS_1^{Ph} shows complete fertility restoration while MS_2^{Ph} has partial restoration ability. MS_2^{Ph} is an incomplete dominant gene unlike MS_1^{Ph} but shows dosage effect in combination with MS_1^{Ph}.

Yet another indepth study of genetics of sterility behaviour of Nongken 58s by Mei *et al.* (1990) reveals it to be a bit more complex. Study of crosses of Nongken 58s with two japonica varieties showed in the F_2 a sort of continuous variation for fertility - sterility with 2 or 4 peaks suggesting the possibility of two pairs of genes to control the trait, instead of one as reported earlier. They opined that one pair could be photoperiod sensitive gene (ms), while the other pair fertility differentiating gene (Fd). Study of crosses between genotypes differing in their degree of phylogenetic affiliation reveals the presence of Fd genes in varying numbers. Postulation of Fd genes seems to be helpful in explaining more meaningfully the mode of inheritance of photoperiod sensitive male sterile lines in crosses within and between varietal groups. Continuous variation observed in the F_2 of several other combinations involving Nongken 58s by Xue and Deng (1991) can also be explained by *Fd* genes. The role of modifier genes along with the major gene(s) in the expression of sterility fertility grade cannot however, be underestimated. Using marker gene method the photosensitive male sterile gene ms^{Ph} of Nongken 58s was located on chromosome number 2 of Nishimura (Zhang *et al.*, 1990).

Subsequent to the identification of Nongken 58s several photosensitive steriles have been identified and some of them intensively studied for their mode of inheritance and usefulness in two-line breeding. MSR 54 A(B) isolated by Lu and Wang (1986) possesses a strong fertility transformation character, which decisively converts a male sterile line into a male fertile.

Genetic analysis reveals it also to be controlled by a pair of recessive genes as in Nongken 58s. Huang and Zhang (1991) isolated another valuable male sterile mutant of this kind - viz., CIS 28-10s. It possesses good ratooning ability with better agronomic traits and grain quality. Floral characters facilitate high percentage of outcrossing. It is considered as a true photosensitive male sterile, as it is not at all influenced by air temperature. Hence the sterility - fertility transformation is quite strong at critical period. Inheritance studies of this mutant by Huang (1991) revealed it to be controlled by a single dominant gene. Subsequent studies have shown the role of minor genes in modifying the effect of the dominant photo-period sensitive sterility gene.

Genetic analysis of EGMS, beyond suggesting that it is mono or digenically controlled with other major (Fd) or minor, modifier genes does not indicate whether it is different from either the nuclear fertility / sterility gene Fr/fr which in interaction with sterile (S) cytoplasm gene `*C*' induces male sterility or the genetic male sterility gene(s) which does not involve

cytoplasm in its expression of male sterility. Interaction effect of the gene(s) conferring PGMS and TGMS as well as cytoplasmic - genetic male sterility (CMS) in certain situation makes the topic much more complex warranting a systematic indepth study.

Dong *et al.* (1993) have reported a new PGMS source viz., Zhenong IS in *japonica* rice under long photoperiod of 14.75 h. Regardless of temperature, its self-sterility level was as high as 99.7 per cent and under short day length of 13.25 h it revealed to seed selfing. While the sterility induction exclusively by long day length, reversion to fertility at short day length is influenced by temperature. With lower temperature (25.7°C) the fertility percentage tends to increase and its genetics is yet to be studied.

Data from inheritance studies have established that male sterility of PGMS rice is controlled by a relatively simple genetic system (Jin and Li 1991). When Nongken 58s was crossed to its wild type progenitor (Nongken 58), fertility in the progenies segregated in a typical single locus mendelian ratio. A two - loci segregation ratio was typically obtained in progenies of Nongken 58s crossed with many other japonica varieties.

Zhang and his coworkers conducted a series of studies to determine the locations of the PGMS loci in the rice molecular marker linkage map. They extended the bulked segregant analysis to a two-step

approach for mapping the PGMS genes : 1) using bulked DNA from extreme plants to identify the chromosomal segments likely to carry sterility vs fertility alleles, which avoided classifying individuals into a fertile or a sterile class in a more or less continuously distributed population, and (2) determining the map locations of the genes using only the extreme sterile individuals. As pointed out by Zhang *et al.* (1994a) this approach in gene mapping had several advantages over routine segregation population analysis, including higher efficiency and reduced probability of this classification. In the first experiment, Zhang *et al.* (1994a) made a cross between two indica lines, 320013 (a PGMS line developed by transferring the PGMS genes from Nongken 58s) and Minhui 63 (a normal rice culture), that demonstrated a relatively high level of RFLP in a preliminary study. Large F_2 populations were planted annually in the field during the 1991-93 growing seasons under natural long-day conditions for fertility examination. A digenic ratio was consistently observed in all the years studied.

Two DNA bulks, F (fertile) and S (sterile) were made by selecting extreme individuals from the F_2 population of about 1,500 plants. These two bulks and the two parents were digested with 6-21 restriction enzymes, probed for RFLP with a total of 368 probes, and assayed using the 10 SSR (single sequence repeats) markers. These markers covered more than 90% of the rice molecular marker linkage map. The survey of

bulked extremes identified positive markers from three regions, located respectively on chromosomes 1, 3 and 7 that were probably linked to PGMS loci.

All available polymorphic probes surrounding the positive markers in the RFLP linkage map were added to the survey. Highly sterile plants from the F_2 population were assayed individually with all the positive markers to assess linkage. The results from the bulked extremes and recessive class analysis were confirmed using an additional large sample of 224 individuals from the same F_2 population and from data collected in different years. RFLP genotypes of these 224 individuals were determined using positive markers from chromsomes 1, 3 and 7. When a three-way analysis of variance was performed using the marker showing the largest effect from each of these three regions, all the effects involving the locus on chromosome 1 became insignificant. These analyses established the existence of two loci, designated as Pms 2 on chromosome 3 and Pms 1 on chromosome 7.

The recombination frequency between a positive marker and a target PGMS locus was calculated assuming that the highly sterile plants were homozygous for the recessive (sterility) allele at the PGMS locus. The recombination values were then converted to map distances. Genetic effects were estimated for these two loci based on the marker genotypes using a two - locus model. The effect of Pms 1 was two to

three times larger than that of Pms 2 and dominance was almost complete at both loci. The data also suggested that alleles of these two loci interacted more or less like alleles of duplicated loci, and highly sterile individuals were apparently homozygous for recessive alleles at both loci. Various genotypes containing at least one allele from the normal parent (Minghui 63) appeared to produce highly fertile individuals.

Physiological basis of sterility - fertility transformation in PGMS lines

Physiological basis of male sterility and fertility transformation in PGMS were intensively investigated in Nongken 58s under controlled short and long day conditions along with its parent, Nongken 58 during the sensitive stage (Primary and secondary rachis primordia to pistil and stamen primordia in the panicle development).

Light period, its quality and intensity, thermo - labile substances, the phenomenon of vacuolation and volume enlargement of anther in relation to nutrient use or microspore development are some of the aspects studied to relate with the sterility - fertility transformation.

The importance of day length in fertility transformation was very well brought out by Zhu *et al.* (1987). Under short day condition which is critical for fertility transformation, during the sensitive phase of panicle development (between secondary branch primorida stage and pistil - stamen primordia stage) if the short day treatment is interrupted by a flash

of light for even 5 minutes after every dark period, the PGMS continues to remain sterile.

Thermosensitive Genic Male Sterility

Limitations of PGMS system that it would be of no use in tropical conditions, where day length differences are small, prompted scientists to look for temperature sensitive male steriles so that wide temperature differences there could be made of use.

Annong-S was the first temperature sensitive plant identified. The spontaneous mutant identified in Hunan province of China, remains male sterile under high temperature conditions and fertile under low temperature. Its critical tempeature for fertility alteration is 27°C (Tan *et al.*, 1990).

Subseqeuntly another temperature sensitive male sterile viz., `Norin PL 12' was isolated in Japan from the variety Reimei irradiated with 20 kr gamma rays. The induced mutant and its derivative H 89-1 remains completely sterile at 31°-24°C, partially fertile at 28°-21°C and completely fertile at 25°-18°C (Maruyama, 1990a, b).

In 1988, when several breeding lines developed were subjected to different photoperiods and temperatures Fang & Lu found that the fertility expression of indica PGMS lines was controlled not only by photoperiod but also by temperature (Fang and Lu, 1990).

While their studies on several breeding lines Fang and Lu found that in some cases, the traits of fertility alteration in indica lines were controlled completely by temperature. The lines were called thermo sensitive genic male sterile (TGMS) lines.

Most rice-growing areas around the world are distributed in the tropics and warm temperature regions, where differences in photoperiod are marginal but differences in temperature over a year are striking. TGMS lines are more widely used geographically than PGMS lines. PGMS lines particularly *japonica* PGMS lines,are very useful at higher latitudes (above 30°N) because of their more stable sterility and easier multiplication than TGMS lines. The critical temperature (CTP) of fertility alteration is the most important in both TGMS and PGMS lines (Yuan, 1992).

Annong- 1S based TGMS lines like 5460s and R59TS developed in Fujian, China show fertility at 27°C / 22°C but turn sterile at 33 / 28°C (Yang and Wang, 1990, Sun *et al.*, 1989).

It is critical for the utilisation of EGMS rice to determine which are the main environmental factors influencing fertility changes. Cheng - Shi Hua *et al.* (1996) classified fertility responses to photoperiod and temperature in 76 dual purpose genic male sterile lines by studying under

nine controlled combinations of photoperiod / temperature. They grouped the male sterile lines by variance analysis into the following groups:

1. Photoperiod sensitive genic male sterility (PGMS) characterised by significant photoperiod (P) effect and PxT interaction and non-significant temperature (T) effect.
2. Thermosensitive genic male sterility (TGMS) by significant T effect, non-significant P effect, significant or non-significant PxT interaction and
3. Photothermosensitive genic male sterility (P-TGMS) by only significant PxT interaction.

Among the *Japonica* lines studied PGMS, TGMS, and P-TGMS account for 34.8, 13.0 and 47.8% respectively. Among the indica lines studied TGMS and P-TGMS account for 73.6 and 22.6% respectively and no PGMS was found.

According to Gao-Yong *et al.* (1996) fertility alteration in TGMS rice was mainly controlled by daily average temperature at the sensitive phase and not by photoperiod. The TGMS rice were subdivided into positive and negative types (p-TGMS and n-TGMS). Sterility in p-TGMS rice was induced by high temperature and fertility by low temperature, while the converse was true for n-TGMS rice. The critical temperature for fertility

alteration in Hengnong 3s was about 27°C i.e., plants were fertile at temperatures above 27° C and sterile at lower temperatures than 27° C.

The genetic relationship between developmental photoperiodic sensitivity (DPS) and male sterile photothermal sensitivity of photo and thermosensitive genic male sterile rice (PGMS and TGMS) was investigated by studying the photoperiodic reactions and photothermal responses of P(T) GMS plants of the F_2 generation of the cross W6 154Sx Nongken 5s. Results showed that it is possible that the fertility alteration characteristic is stable in lower generations, but modifiable genes which control the strength or weaknesses of photothermal sensitivity may be present (Zeng-Hanlai *et al.*, 1997).

Genetics of Thermosensitive Genic male sterility

Unlike the complicated genetics in PGMS rice, a single recessive gene controlled the thermosensitive male sterility in the mutants 5460S, H 89-1 and IR32364 which were produced through irradiation mutagenesis in China (Sun *et al.*, 1989), Japan (Maruyama *et al.*, 1991) and at IRRI (Virmani and Voc, 1991) (Maruyama *et al.*, 1991, Yang *et al.*, 1992, Borkakati and Virmani, 1996).

Genetic studies at IRRI (Borkakati and Virmani, 1993, 1996) indicated that the TGMS trait in Norin PL 12 and IR32364 TGMS was controlled by a single recessive gene. Allelic relationship studies

indicated that the TGMS genes in the two mutants were different (Borkakati and Virmani, 1996). Because the TGMS gene in the line 5460S from China was designated as tms 1, and one in Norin PL 12 from Japan as tms 2 (Kinoshita, 1992), the TGMS gene in the IR 32364 TGMS mutant was tentatively designated as tms 3(t). Its allelic relationship with tms 1 gene present in TGMS mutant 5460 S could not be studied due to non-availability of the mutant, 5460 S.

Recently the tms 3(t) TGMS gene has been located on the short arm of chromosome 6 using molecular markers (Subudhi *et al.*, 1997). Because the tms 1 gene of 5460 S TGMS is now known to be located on chromosome 8 (Wang *et al.*, 1995), it was concluded that tms 3(t) of IR 32364 TGMS is not allelic to tms1. Thus tms 3(t) was designated as tms 3.

Physiological behaviour of TGMS lines

Although temperature sensitive plants of spontaneous and induced origin, have been generally found to be sterile at high temperature and turn fertile at low temperature and there is no precise information on how the critical temperature and critical stage of development are sensitive to the temperature stress and what minimum period of effective temperature treatment is necessary for fertility reversion. As for determination of critical temperature, mean of minimum and maximum is taken by some, while for others it is the maximum. It was Dr. Maruyama (Maruyama *et al.*, 1990b) who studied this aspect intensively for his mutant H 89-1

through well designed experiment. By keeping under different but constantly maintained temperatures (31°C, 28°C 26°C, 24°C, 21°C) he found H 89 to respond to only maximum temperature for sterility (31°C) and fertility reversions (28°C) and not the mean of maximum, minimum temperature. To determine the exact thermosensitive stage of the mutant line he subjected to 3-day low temperature (25°-18°C) treatment of plants grown under high temperature (31°C-24°C) in batches right from panicle initiation stage and found the stage around 22-26 days before heading to be sensitive for fertility transformation. As for the duration of high temperature that would affect the fertility during fertility transformation phase, he reports that even high temperature for an hour would adversely affect the seed fertility. In respect of Annong S or its derivatives very little has been done to precisely understand their physiological behaviour as has been done in the case of H 89-1.

Photo - Thermo Sensitive Male Sterility:

For long since the discovery of Nongken 58S and development of PGMS lines using it was believed that the expression of fertility - sterility was determined by change of day length alone regardless of temperature variations. He *et al.* (1987) were the first to study and show that sterility fertility behaviour of PGMS lines is prone to the influence of temperature as well. Their observations were simultaneously confirmed by Zhang *et al.* (1987). The influence of temperature was found to persist to varied

level, in all the derivatives of Nongken 58S. For instance, study of expression of the PGMS gene in the genetic back ground of indica rices by Lu *et al.* (1992) has revealed that fertility alteration in indica MS lines such as W6154S, W6184S, W7415S and W6068S had different ranges of critical temperature. This indicated that temperature had as much influence as photoperiod on fertility transformation process in PGMS. Similar study in the background of japonica showed however, the PGMS gene to be less vulnerable to temperature changes as compared to MS lines of indica background (Bi *et al.*, 1990; Cheng *et al.*, 1990).

Subsequent studies by a number of workers suggest the expression of male sterility in all important PGMS sources like Nongken 58S, Annong IS, Eyi 105 S, W6154S etc., is controlled by temperature rather than photoperiod (Wan and Deng, 1990; Zhou *et al.*, 1990; Xue and Zhao, 1990; Xue and Shen, 1991; Zhang *et al.*, 1991a; Wu *et al.*, 1991) and the critical temperature range is determined by the genetic back ground in which the PGMS gene remains (He *et al.*, 1987; Bi *et al.*, 1990; Cheng *et al.*, 1990). More critical study by Sun *et al.* (1991) of Nongken 58 S and PGMSRS derived from it with Japonica background under controlled temperature and photoperiod finally proved that sterility -fertility transformation in PGMSRS occurred under certain temperature - day length combinations. The PGMSRS remained sterile under long photoperiod (15.0 h) and high temperature (29.6°C) and fertility

transformation under short photoperiod (12.0 h) and low temperature (23.6°C).

Zhang *et al.* (1992) has developed a model to explain photo-thermo reaction of fertility alterations in photo-sensitive genic male sterile rices. It removes all the lacuna found in earlier research findings. According to them there is no absolute photosensitive or absolute thermosensitive sterility. Sterility induction - fertility alteration are as a result of interaction between photoperiod & temperature instead. When temperature is above a certain point pollen become sterile and when it is below another certain point, they become fertile regardless of photoperiod length. These two temperature points are referred to as critical sterility point (CSP) and critical fertility point (CFP).

There exists a temperature range of photoperiod sensitivity (TRPS) within which pollen fertility alteration of PGMS is regulated by photoperiod. This temperature range is between the critical sterility point (CSP) and critical fertility point (CFP). Even though in the TRPS, the fertility alteration is regulated by photoperiod, still the temperature can affect the critical light length (CLL) and the degree of fertility alteration such that as the CLL becomes shorter under short day, the fertility decreases as the temperature rises, and as the CLL becomes longer under short day the fertility increases as the temperature decreases. Thus photoperiod and

temperature are interdependent and inseparable in respect of fertility alteration (Zhang *et al.*, 1992c).

Critical light length and intensity of interaction effect between photoperiod and temperature are the main factors in determining the adaptability of a line. A line with longer CLL could be used at high latitudes and the one with shorter CLL at low latitudes. A line with large interaction effect between photoperiod and temperature would be adapted to a wider range of conditions, for high temperature, can complement insufficiency of photoperiod in low latitudes and longer photoperiod can complement the insufficiency of temperature in high latitudes.

For commercial hybrid rice production sterile lines, which have low critical fertility point (CFP); high critical sterility point (CSP) and wide TRPS are ideal (Yuan, 1992a; Zhang *et al.*, 1992c).

MATERIALS AND METHODS

The present investigation on the **Development and Evaluation of two and three line hybrids in rice** was conducted with the following objectives:

1. Evaluation of stability of TGMS lines and their morphological characterisation.
2. Evaluation of stability of CMS lines and their morphological characterisation.
3. Production and comparative evaluation of two and three line hybrids.

The experiments were carried out during kharif 1998 on farms of Indian Agricultural Research Institute, New Delhi, which is situated at 28°05' N latitude and 77°10'E longitude and 218m above mean sea level and the evaluation of hybrids was carried out at IARI Regional Station RBGRC, Aduthurai, Tamil Nadu during rabi '98-99. The details of materials used and methods employed for different experiments are described below:

Experiment-1

Evaluation of stability of TGMS lines and their morphological characterisation:

Materials : The materials for this study were obtained from IRRI, Philippines. They are the following eleven lines:

1. IR 68945-4-33-4-14-48
2. IR 68949-11-5-31-10-3
3. IR 68294-7-1-18-298
4. IR 32364-20-1-3-2

5. IR 68935-16-6-27
6. IR 71018-13-73-3-B
7. IR 68931-1-4-15-18-7
8. IR 68939-9-6-5-8
9. IR 68942-1-6-13-B-4
10. IR 68298-11-1-6-3B
11. *Norin PL 12*

Methods : Sowings were done in the second week of June during kharif 1998 and 25 days old seedlings were transplanted in a single row of 3m length per variety with a plant to plant spacing of 20 cm and row to row spacing of 30 cm. The crop was raised under uniform and standard agronomic practices. Observations on the following morphological characters were recorded on 5 randomly selected competitive plants:

1. Days to 50% flowering
2. Plant height (cm)
3. Panicle length (cm)
4. Number of effective tillers
5. Total number of spikelets / panicle

Pollen sterility studies:

The above mentioned eleven TGMS lines were screened for stability for sterility in two seasons namely kharif 1998, under IARI, New Delhi conditions, and rabi 1998-99, under RBGRC, Aduthurai, Tamil Nadu conditions.

Pollen studies were carried out at the time of flowering. 10 plants were randomly selected from each line and anthers from the spikelets representing top, middle and bottom portion of the main panicle were used for preparing the slides to study under the microscope. This was done on two dates on each line during the flowering time. The spikelets thus collected were fixed in 1:3 acetic acid alcohol solution. All the anthers of the spikelets collected were smeared in 1% Iodine - Potassium Iodide (I-KI) solution and pollen grains were observed under binocular microscope for the presence of any fertile pollen. Deep dark stained and round pollen grains were considered as fertile, while lightly stained round pollen as well as those with one half stained and the other half unstained were considered as partial sterile, whereas deshaped and unstained pollen grains were grouped as aborted sterile and round pollen which were not stained were considered as round sterile. The percentage of pollen sterility is calculated by using the formula:

$$\text{Pollen sterility (\%)} = \frac{\text{Number of sterile pollen grains}}{\text{Total number of pollen grains}} \times 100$$

Experiment-II

Evaluation of stability of CMS lines and their morphological characterisation

Materials : The materials for this study include the following eight CMS lines out of which the first five were imported from IRRI, Philippines and the rest were taken from the breeding material present at IARI.

CMS lines

1. IR 68897 A
2. IR 68886 A
3. IR 68888 A
4. IR 68899 A
5. IR 70372 A
6. IR 58025 A
7. Pusa 5A
8. Pusa 3A

Methods : The method followed for evaluation of CMS lines is similar to that mentioned for TGMS lines:

The morphological characters recorded on CMS lines are the following:

1. Plant height (cm)
2. Panicle length (cm)
3. Number of effective tillers
4. Total number of spikelets / panicle

Experiment-III

a) Production of two line (TGMS based) hybrids:

Materials : The lines used for the production of ten two line hybrids are given below:

Female parents:

1. IR 32364-20-1-3-2
2. IR 68939-9-6-5-8
3. IR 68298-11-1-6-3B

Pollen parents:

CMS restorers

1. IR 54
2. PRR 78

Elite breeding lines

3. PS-18
4. Pusa 1080

Check variety

5. Jaya

Method : Hybridisation was carried out through hand pollination method. In this method top 1/3rd portion of spikelets of a female line panicle which is likely to open next day were clipped off before one day to enhance the anthers and stigma exsertion. As soon as clipping was over, panicle was covered with a butter paper bag. After anthesis in male lines, well opened panicles were

collected between 9 a.m. to 11 a.m. and pollen grains were dusted on the clipped panicle. Dusting was repeated for two or three times and the panicle was kept closed in butter paper bag till maturation of seed.

b) Production of three line (CMS based) hybrids:

Materials : The lines used for the production of nine three line hybrids are given below:

Female parents:

1. IR 580 25A
2. Pusa 5A
3. PMS 2A

Pollen parents / restorers:

1. IR 54
2. PRR 72
3. PRR 78
4. PRR 22

Method : The procedure followed in the production of CMS based hybrids is similar to that mentioned for TGMS based hybrids.

c) Comparative evaluation of two and three line hybrids:

The hybrids produced were evaluated in a trial at IARI Regional Station, RBGRC, Aduthurai, Tamil Nadu, along with the pollen parents, B-lines and check varieties during rabi '98-99.

Materials:

CMS based hybrids	-	9
TGMS based hybrids	-	10
Pollen parents	-	6
B-lines	-	3
Check varieties	-	2

The details of the parentage of the hybrids and other lines are given in Table 6.

Method : The above 30 genotypes were planted in a randomised block design (RBD) with 3 replications during rabi 98-99. Seedlings were planted at the rate of one seedling / hill with a spacing of 20x20 cm. A 4m row was maintained for each genotype. Fertilizers were given in the ratio of NPK: 90:50:40. Basal -- 30:50:40 and the remaining nitrogen was given in 2 splits of 30 and 30, one at maximum tillering stage and the other was given at boot leaf stage. Observations were recorded on 5 randomly selected competitive plants in each genotype in each replication for the eleven characters as follows:

1. **Days to 50% flowering (DTF)** : Number of days from sowing to panicle emergence in 50 per cent of the population was recorded.

2. **Days to maturity (DTM)** : Days required for 85% of the grains in the panicle to mature were recorded.

3. **Plant height (PH)** : The culm height at maturity was measured in centimetres from the ground level to the tip of the panicle excluding awns if any.

4. **Number of tillers (Tiller No.)** : Total number of tillers per plant were counted.

5. **Number of effective tillers (NET)** : Total number of effective tillers in a plant were counted at the time of harvest.

6. **Panicle length (PL)** : The length of the main panicle of each plant was measured in centimeters from panicle base to the tip excluding awns if any.

7. **Number of spikelets / panicle (NSP)** : Total number of spikelets in the main panicle of each plant were counted and recorded.

8. **Number of filled spikelets / panicle (NFSP)** : Number of well filled grains in the main panicle of each plant were counted and recorded.

9. **Per cent spikelet fertility (%SPF)** : This was calculated by the formula:

$$\% \text{ spikelet fertility} = \frac{\text{Number of filled spikelets/panicle}}{\text{Total number of spikelets/panicle}} \times 100$$

10. **1000 grain weight (TGWT)** : The weight of one thousand well filled grains in each plant was measured in grams and was recorded.

11. **Grain yield per plant (GYP)** : Weight of well filled grains of each plant which was dried to a constant moisture level of 12 per cent was measured in grams and was recorded.

Estimation of Heterosis

Heterosis was calculated by using the over all mean of each hybrid for each character. The magnitude of heterosis was expressed as superiority over check variety (Standard Heterosis) Sc. Heterosis was calculated as percentage increase or decrease of F_1's mean performance over the mean performance of standard check variety:

$$Sc\% = \frac{F_1 - CV}{CV} \times 100$$

where, F_1 is the average performance of hybrid
CV is the average performance of check variety

Test of significance:

The test of significance of different yield and its component characters was calculated by adopting 't' test as suggested by Wynne *et al.* (1970).

$$t \text{ (standard heterosis)} = \frac{F_{ij} - CV}{(3/8\ EMS)^{1/2}}$$

where, Fij = mean of the i^{th}, j^{th} cross
CV = mean of the check variety
EMS = Estimates of the error variance

The calculated `t' value was compared with the table value of `t' at (n_1+n_2-2) 4 d.f at 5% level and 1% level of significance.

Statistical analysis:

The data with respect to the yield and its related characters was subjected to the following analysis with the help of standard statistical procedures:

- Analysis of variance
- Parameters of variability - Mean
- DMRT analysis
- Orthogonal contrast
- Correlation matrix

Parameters of variability:

Mean : The mean value of each character was calculated by dividing the total value of observations by total number of observations:

$$X = \frac{1}{n} (\sum^{n}_{i} = {}_1X_j)$$

where, Xi = value of i^{th} observation

n = total number of observations

DMRT analysis:

Mean comparisons were done by Duncan's Multiple Range Test (DMRT) method.

Orthogonal contrast:

The analysis of variance is only the first step in studying the results. The next step is to examine the class means and the sizes of differences among them.

Often, particularly in controlled experiments, the investigator plans the experiment in order to estimate a limited number of specific qualities. Orthogonal contrast is used when group comparisons are made which is not possible through simple pair wise comparisons like DMRT, LSD, SNK etc. For the group comparison it is usually best to study each contrast of interest i.e., each question in logical order. The method of contrast though old is still recommended.

In each contrast the sum of the coefficients should be zero. For example, in the present study of the 3 groups [CMS based hybrids (9), TGMS based hybrids (10) and inbreds (11)], the aim was to compare CMS with TGMS, CMS with inbreds and TGMS with inbreds. The coefficients for the above contrast are given as follows:

Contrast	CMS	TGMS	Inbreds	Total
CMS VS TGMS	10(9)	-9(10)	0	= 0
CMS Vs Inbreds	11(9)	0	-9(11)	= 0
TGMS Vs Inbreds	0	11(10)	-10(11)	= 0

If the sum of the multiplication of the coefficient of different contrasts is zero, then these contrasts are called as orthogonal. The variance of each contrast is calculated by the summation of the product of the mean of each group with the

respective co-efficient. The sum of squares contributed by each has one degree of freedom and is equal to :

$$\frac{rQ^2}{\sum aj^2}$$

r = No. of replications

Q = Value (on effect of contrast)

aj = The co-efficient of each treatment (environment) in the contrast.

$Q = \sum aj\, x_j$

Xj = The mean of j^{th} treatment.

Once the sum of squares has been found, F-test can easily be carried out to find out the significance of the contrast (Pearce, 1992).

Correlation analysis:

The correlation was calculated by using the following formula

$$r(x, y) = \frac{C_oV(xy)}{V_{(x)}\, V_{(y)}}$$

where, $r(X, Y)$ = correlation coefficient between X & Y

$C_0V(X,Y)$ = Covariance between X and Y.

$V_{(x)}, V_{(y)}$ = variance of X and Y respectively

The significance of correlation coefficients were tested against table values of 'r' given by Fisher and Yates (1938).

RESULTS

Experiment-I

1. Screening of TGMS lines for stability of sterility both at New Delhi and Aduthurai conditions:

Eleven TGMS lines which were found to be male sterile under IRRI, Philippines, conditions were imported. These eleven TGMS lines were sown on 16th of June 1998 and 25 days old seedlings w ere transplanted. Two of the lines viz., IR 68949-11-5-31-10-3 and Norin PL 12 did not germinate. Panicle initiation stage was observed around 15th August and most of the lines came to flowering between September 1st to 11th. During this entire period the mean temperatures were found to be in the range of 24.2°C to 32.5°C (Table 2). At the time of flowering pollen sterility of these lines were evaluated as per the standard procedure. It was found that except one line viz., IR 68294-7-1-18-29B, all others exhibited complete pollen sterility (Table 1; Plate 1, 2 & 3).

By the third week of October the temperatures dropped to 25°C and below. At these temperatures three lines viz., IR 32364-20-1-3-2, IR 68935-16-6-27 and IR 68298-11-1-6-3B showed 50% transformation to fertility,whereas IR 68939-9-6-5-8, IR 71018-13-73-3-B, IR 68942-1-6-13-B-4 and IR 68931-1-4-15-18-7 showed complete transformation to fertility (Plate 4).

Table 1. Percent Pollen Sterility of thermo-sensitive genic male sterile (TGMS) lines

S.No.	TGMS Line	Delhi (Kharif 1998)	Aduthurai (Rabi 1998-99)
1.	IR 68945-4-33-4-14-48	100	100
2.	IR 68949-11-5-31-10-3	did not germinate	
3.	IR 68294-7-1-18-29 B	fertile	fertile
4.	IR 32364-20-1-3-2	100	100
5.	IR 68935-16-6-27	100	100
6.	IR 71018-13-73-3-B	100	90
7.	IR 68931-1-4-15-18-7	100	100
8.	IR 68939-9-6-5-8	100	100
9.	IR 68942-1-6-13-B-4	100	100
10.	IR 68298-11-1-6-3B	100	100
11.	Norin PL12	Did not germinate	100

Table 2. Weather data of August, September and October during Kharif 1998 at Delhi

Date	August			September			October		
	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean
1	36.2	28.2	32.2	35.2	28.0	**31.6**	34.4	24.4	29.4
2	36.0	28.0	32.0	36.4	28.0	**32.2**	34.8	24.8	27.8
3	36.2	28.2	32.2	35.4	26.6	**31.0**	34.0	20.8	27.4
4	36.6	24.8	30.7	34.2	26.0	**30.1**	30.2	20.8	25.5
5	35.8	28.0	31.9	34.2	26.0	**30.1**	31.6	22.6	27.1
6	35.8	27.8	31.8	35.0	27.0	**31.0**	31.8	22.0	26.9
7	36.0	27.8	31.9	37.0	28.0	**32.5**	31.8	22.0	26.9
8	36.4	27.6	32.0	36.4	28.0	**32.2**	32.0	22.0	27.0
9	36.0	26.8	31.40	36.0	26.0	**31.0**	33.0	22.0	27.0
10	36.2	27.2	31.70	36.8	36.0	**31.4**	33.0	22.2	27.1
11	36.0	27.8	31.90	37.4	25.2	**31.3**	33.8	21.0	27.0
12	36.0	26.8	31.40	34.2	25.0	29.1	33.4	21.0	27.2
13	35.8	24.0	29.39	29.6	24.8	27.2	33.2	20.0	26.5
14	34.6	25.0	29.30	32.0	24.0	28.1	32.2	20.0	26.5
15	34.0	25.0	**29.00**	31.5	24.0	27.2	31.8	23.0	27.4
16	31.0	26.0	**31.50**	29.4	26.0	27.7	26.6	21.0	23.8
17	28.4	25.0	**26.70**	330.	26.4	29.2	23.0	20.0	21.5
18	31.4	27.0	**29.20**	34.4	27.4	30.4	23.8	20.0	21.9
19	34.0	27.0	**30.00**	34.2	27.0	30.6	24.0	20.4	22.2
20	31.4	28.0	**29.70**	34.0	27.0	30.5	28.2	19.0	**23.6**
21	30.0	28.0	**29.00**	30.0	25.0	27.5	30.0	19.6	**24.8**
22	28.0	26.0	**27.00**	32.0	26.0	29.0	30.4	19.8	**25.1**
23	32.4	26.0	**29.20**	31.4	25.0	28.2	31.0	18.0	**25.0**
24	33.4	26.6	**30.00**	27.6	25.0	26.3	31.2	18.0	**24.6**
25	30.4	27.0	**24.20**	28.0	26.0	27.0	31.3	19.0	**24.9**
26	32.4	26.0	**29.20**	31.0	26.0	27.0	31.6	17.4	**24.5**
27	30.0	27.0	**26.50**	33.6	25.0	29.3	31.6	17.0	**24.3**
28	32.6	26.0	**29.20**	33.2	24.8	29.0	31.6	14.0	**22.8**
29	30.4	26.0	**28.20**	34.4	24.4	29.4	30.4	14.0	**22.2**
30	32.6	25.8	**29.20**	35.2	26.0	30.6	30.0	14.0	**22.0**
31	35.2	26.8	**31.80**				29.0	14.2	**21.6**
Mean	**40.2**	**23.6**	**27.3**	**33.4**	**25.9**	**28.7**	**30.7**	**19.6**	**25.2**

(Table 2 contd..) Weather data of March and April during Rabi 98-99 at RBGRC, Aduthurai, Tamil Nadu

Date	March '99			April '99		
	Max.	Min.	Mean	Max.	Min.	Mean
1	32.0	22.0	27.50	36.6	21.2	**28.90**
2	32.6	22.5	27.55	36.6	19.6	**28.10**
3	32.0	21.2	26.60	35.0	19.6	**27.30**
4	32.4	21.8	29.70	34.5	23.0	**28.70**
5	33.5	21.0	27.25	34.6	23.8	**29.20**
6	33.0	28.8	53.80	33.9	39.9	**28.90**
7	32.6	17.5	25.00	35.0	25.0	**30.00**
8	33.2	17.4	25.30	36.2	25.7	**31.00**
9	33.6	17.0	25.30	36.5	25.5	**31.00**
10	33.6	16.6	25.10	37.7	25.6	**31.60**
11	33.2	16.5	24.80	37.7	25.5	**31.60**
12	33.2	21.8	27.50	38.6	25.6	**32.10**
13	34.8	24.0	29.40	39.0	26.5	**32.70**
14	35.6	24.4	30.00	38.2	25.0	**31.60**
15	35.0	24.8	29.90	38.7	27.5	**33.10**
16	33.8	23.0	28.40	39.0	26.6	**32.80**
17	33.6	22.0	27.80	38.0	26.1	**32.05**
18	34.6	22.5	28.50	38.0	26.6	**32.30**
19	35.8	21.8	28.80	35.2	23.2	**29.20**
20	35.8	21.9	28.50	36.2	24.8	**30.50**
21	35.6	22.9	29.25	35.6	26.4	**30.50**
22	35.7	23.2	29.45	35.6	25.2	**30.40**
23	35.6	23.0	29.30	36.8	26.7	**31.75**
24	35.6	23.9	**29.75**	35.3	24.1	29.70
25	36.0	23.5	**29.75**	32.1	23.4	27.75
26	35.8	23.5	**29.6**	31.0	22.2	26.60
27	36.2	23.0	**29.60**	30.5	21.1	25.80
28	36.8	24.5	**30.60**	30.1	20.8	25.45
29	37.2	23.4	**30.30**	29.2	20.4	24.80
30	37.2	23.5	**30.3**	30.4	22.6	26.50
31	36.4	22.0	**29.20**			

Plate 1 : The pollen micrographs of the TGMS lines showing complete sterility:

a) IR 68945-4-33-4-14-48

b) IR 32364-20-1-3-2

c) IR 68935-16-6-27

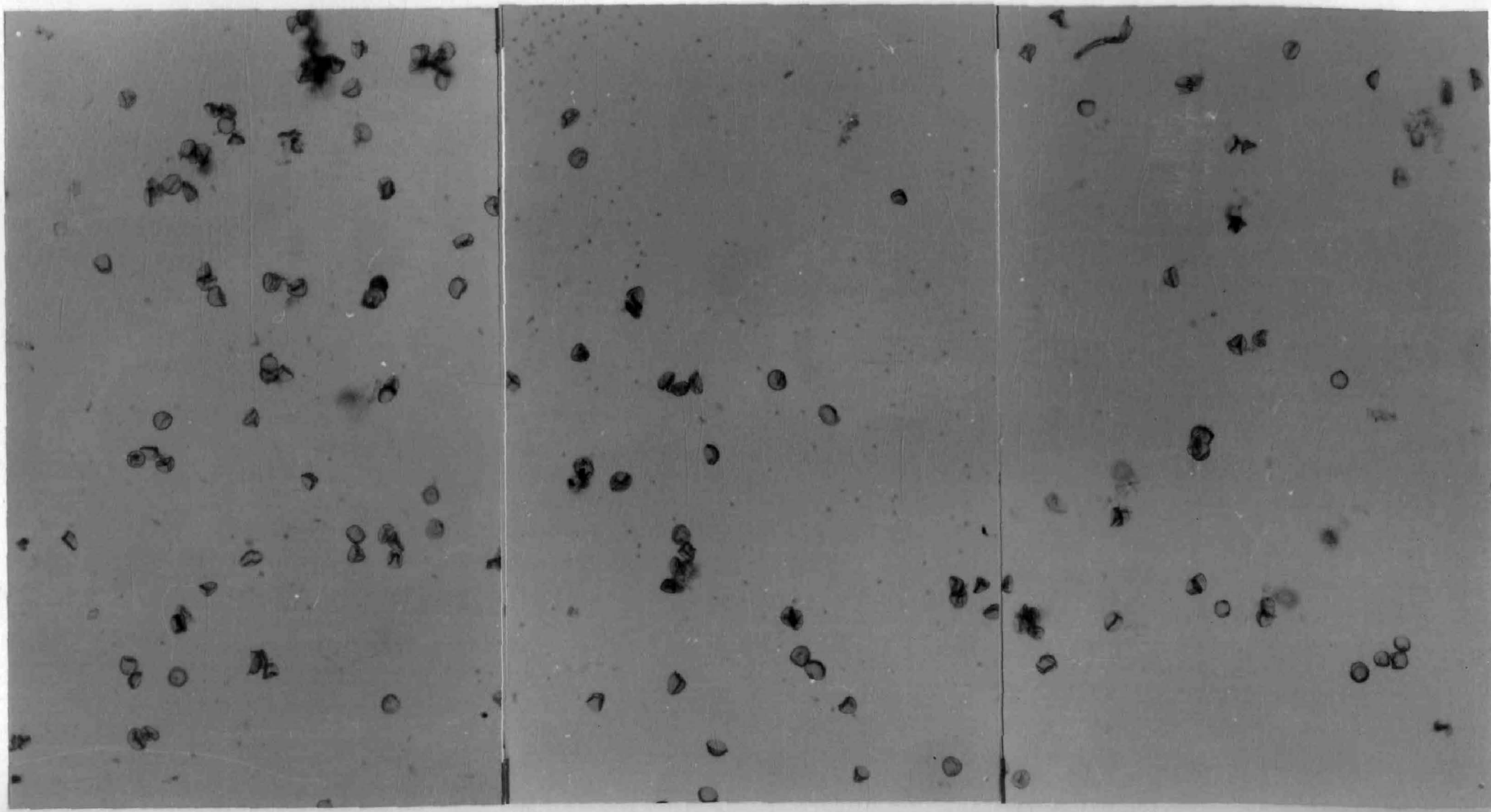


Plate 2 : The pollen micrographs of the TGMS lines showing complete sterility:

d) IR 71018-13-73-B

e) IR 68931-1-4-15-18-7

f) IR 68939-9-6-5-8

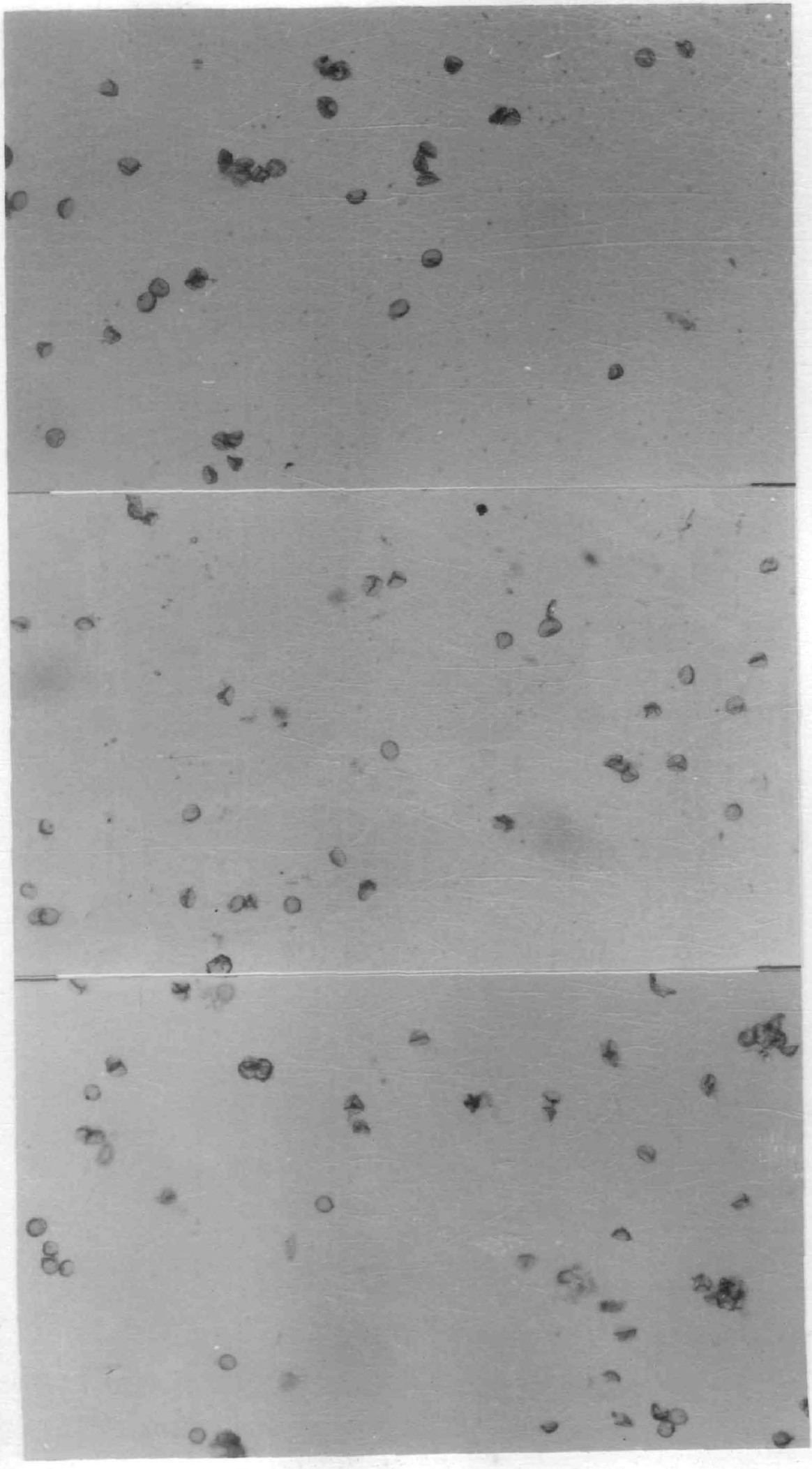
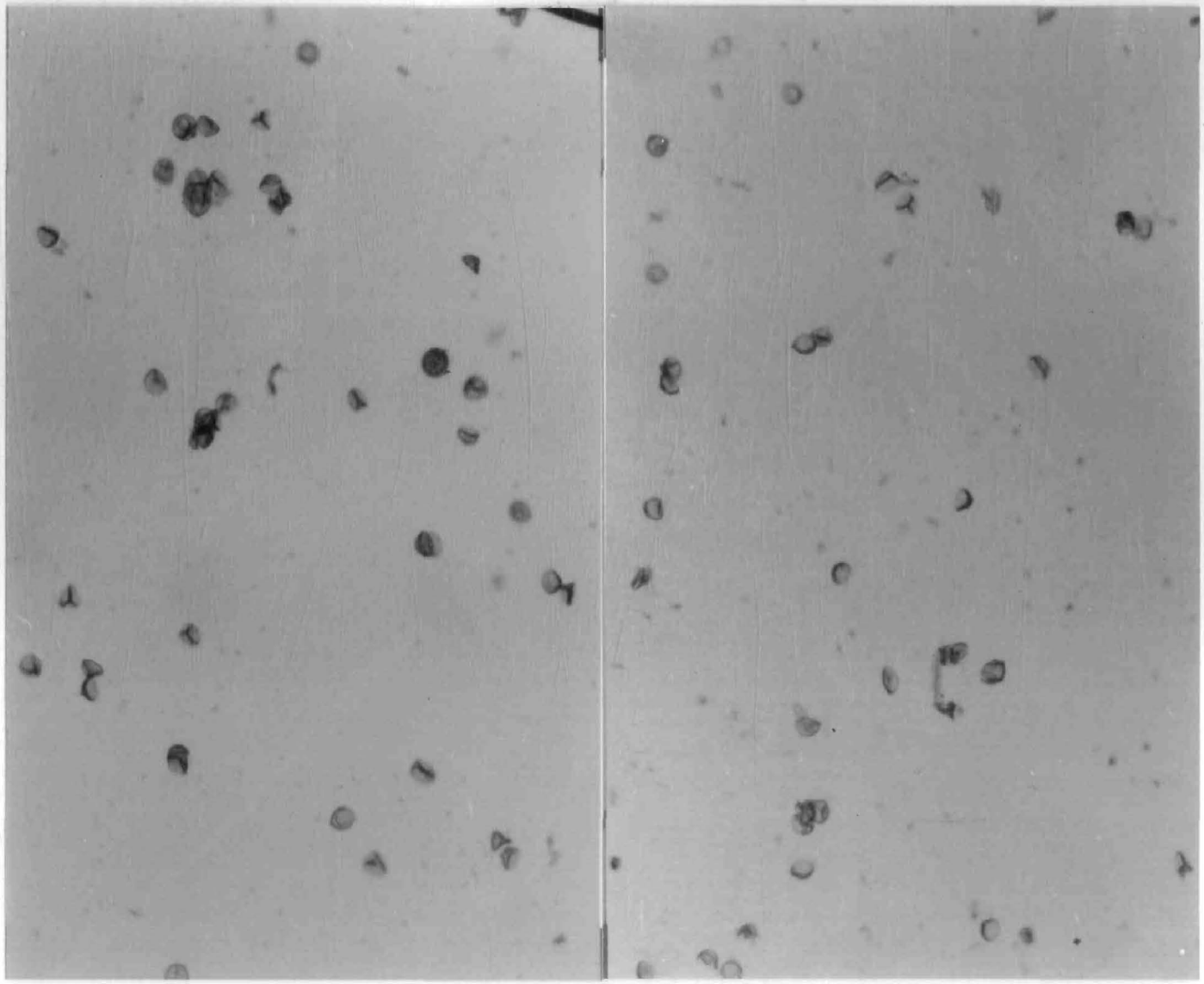


Plate 3 : The pollen micrographs of the TGMS lines showing complete sterility:

g) IR 68942-1-6-13-B-4

h) IR 68298-11-1-6-3B



The same eleven TGMS lines were sown in the third week of January in the subsequent rabi. These lines came to panicle initiation stage around 24th March and flowered between April 8th and 24th April. During this entire period i.e., from March 24th to 24th April, the daily mean temperatures ranged from 29.2°C to 31.6°C (Table 2). Pollen sterility studies showed that except IR 68294-7-1-18-29B, all the rest were completely male sterile.

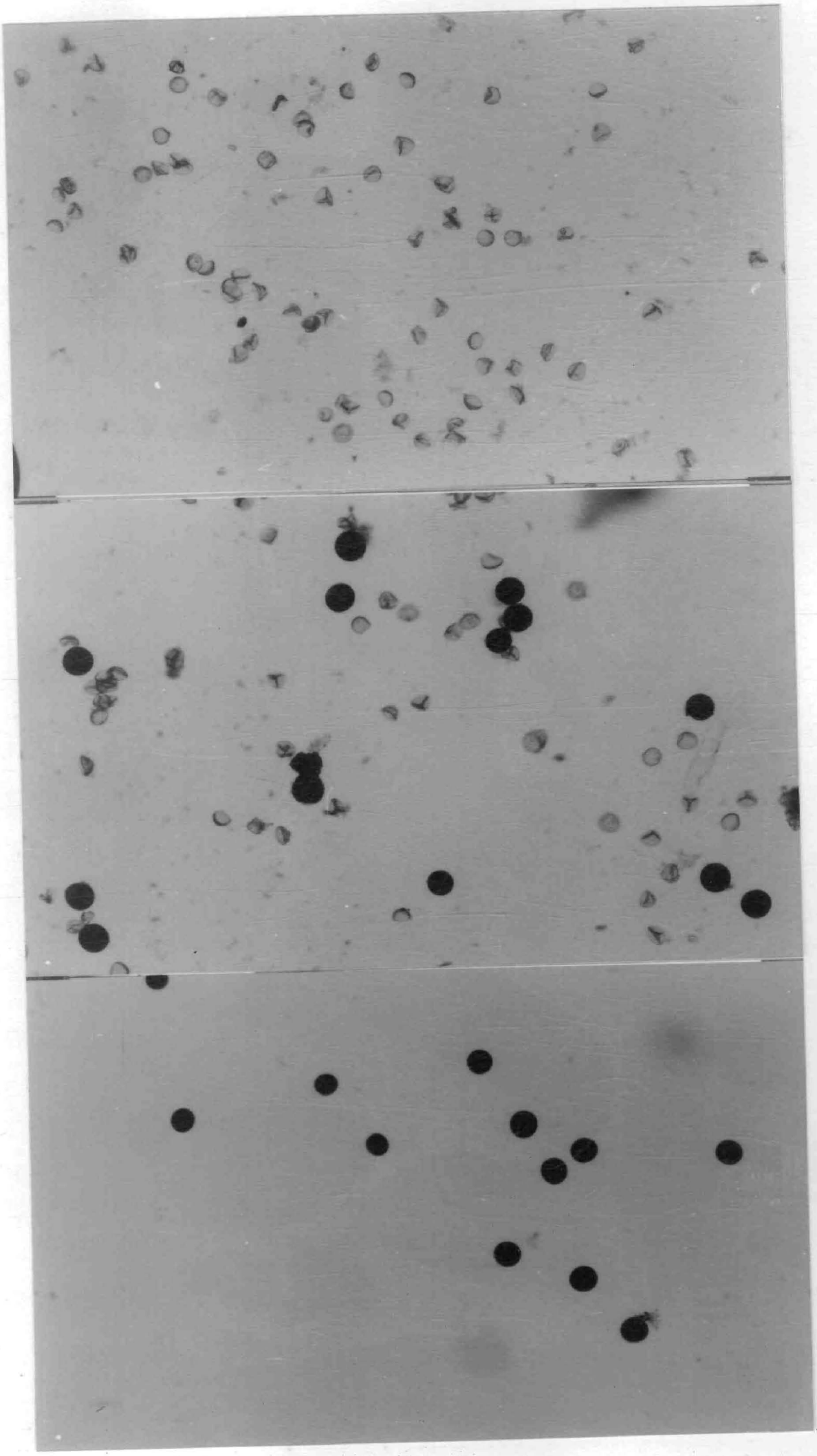
Morphological characterisation of TGMS lines

Out of the eleven TGMS lines, two did not germinate and the data on various morphological characters viz., days to 50% flowering,plant height (cm), number of effective tillers, panicle length (cm) and number of spikelets/panicle was recorded in rest of the nine lines (Table 3). It was observed that IR 68945-4-33-4-14-48 took minimum days (86.0) and IR 68939-9-6-5-8 took maximum days (110) to 50% flowering. Plant height ranged from 70.8 cm in IR 68942-1-6-13-B-4 to 103.8 cm in IR 68294-7-1-18-29B. Panicle length varied from 18.4 cm in IR 68942-1-6-13-B-4 to 28.8 cm in IR 68294-7-1-18-29B. Number of effective tillers varied from 16.8 in IR 32364-20-1-3-2 to 6.2 in IR 68935-16-6-27. Number of spikelets / panicle ranged from 81.0 in IR 68294-7-1-18-29B to 172.6 in IR 68939-9-6-5-8.

Table 3. Agronomic characters of the thermo-sensitive genic male sterile (TGMS) lines sown during Kharif 1998, Delhi

S.No	TGMS line	Days to flowering	Plant height (cm)	No. of effective tillers	Panicle length (cm)	No. of spikelets/ panicle
1.	IR 68945-4-33-4-14-48	86.0	86.0	13.0	21.5	113.0
2.	IR 68294-7-1-18-29 B	86.0	103.8	9.6	28.8	81.0
3.	IR 32364-20-1-3-2	108.0	89.8	16.8	26.4	164.2
4.	IR 68935-16-6-27	86.0	73.4	6.2	23.4	162.2
5.	IR 71018-13-73-3-B	89.0	80.0	15.5	26.5	157.0
6.	IR 68931-1-4-15-18-7	100.0	75.6	15.0	24.1	135.0
7.	IR 68939-9-6-5-8	110.0	79.6	11.2	21.25	172.6
8.	IR 68942-1-6-13-B-4	96.0	70.8	11.2	18.4	134.0
9.	IR 68298-11-1-6-3B	90.0	82.8	7.2	22.9	170.6

Plate 4 : The pollen micrographs of the TGMS lines showing i) Complete sterility (at 30°C) j) 50% transformation to fertility (at 24°C)and k) complete transformation to fertility (at 24°C):
i) IR 68939-9-6-5-8
j) IR 32364-20-1-3-2
k) IR 68939-9-6-5-8



Experiment-II

2. Screening of CMS lines for stability of sterility and their morphological characterisation:

From the pollen sterility studies conducted on the eight CMS lines, it was found that all of them showed complete sterility both under Delhi and Aduthurai conditions and were found to be stable (Table 4).

Data on various morphological characters recorded on the eight CMS lines in kharif 98, under New Delhi conditions are given in the Table 5. It was observed that the character plant height ranged from 64.8 cm in IR 68886A to 88.8 cm in IR 68897A. Panicle length varied from 20.2 cm in Pusa 3A to 29.0 cm in IR 68897A. Number of effective tillers varied from 16.4 in IR 68897A to 36.0 in IR 68886A. Number of spikelets/panicle varied from 129.0 in IR 68886A to 264.3 in IR 58025A.

Experiment-III

3(a). Production of CMS based hybrids:

Out of the eight CMS lines evaluated for pollen sterility the following three were found to be stable for pollen sterility and vigorous and so were selected as female parents for producing the hybrids.

Female parents:

1. IR 58025A
2. Pusa 5A
3. PMS 2A

Table 4. Percent Pollen Sterility of cytoplasmic male sterile (CMS) lines

S.No.	CMS Line	Delhi (Kharif 1998)	Aduthurai (Rabi 1998-99)
1.	IR 68897 A	100	100
2.	IR 68886 A	100	100
3.	IR 68888 A	100	100
4.	IR 68899 A	100	100
5.	IR 70372 A	100	100
6.	IR 58025 A	100	100
7.	Pusa 5A	100	100
8.	Pusa 3A	100	100

Table 5. Agronomic characters of the cytoplasmic male sterile (CMS) lines sown during Kharif 1998, Delhi

S.No.	CMS line	Plant height (cm)	Panicle length (cm)	No. of effective tillers	No. of spikelets /panicle
1.	IR 68897 A	88.8	29.0	16.4	172.8
2.	IR 68886 A	64.8	22.8	36.0	129.0
3.	IR 68888 A	65.6	23.8	17.8	149.8
4.	IR 68899 A	67.2	22.6	20.8	148.4
5.	IR 70372 A	69.4	24.6	20.4	152.3
6.	IR 58025 A	84.6	26.0	27.7	264.3
7.	Pusa 5A	75.3	22.0	27.0	137.7
8.	Pusa 3A	72.1	20.2	25.2	131.2

and the following four CMS restorers were used as male parents:

Male parents:

1. IR 54
2. PRR 72
3. PRR 78
4. PRR 22

By using the above female and male parents nine CMS based hybrids were produced, the details of which are presented in Table 6.

3(b). Production of TGMS based hybrids:

Out of the eleven TGMS lines screened for pollen sterility under New Delhi, kharif '98 conditions the following three were identified as stable for pollen sterility and were found to be vigorous. These were subsequently used as female parents for producing the hybrids:

Female parents:

1. IR 32364-20-1-3-2
2. IR 68939-9-6-5-8
3. IR 68298-11-1-6-3B

and the following lines were used as male parents.

Male parents:

CMS restorers

1. IR 54
2. PRR 78

Table 6. **Details of the 30 genotypes sown in RBD during the Rabi 98-99 at RBGRC fields, Aduthurai, Tamil Nadu for evaluating the performance of hybrids**

S. No.	Genotypes
	Three line hybrid
C-1	IR 58025A x IR 54
C-2	IR 58025A x PRR22
C-3	IR 58025A x PRR78
C-4	Pusa 5A x IR54
C-5	Pusa 5A x PRR 22
C-6	Pusa 5A x PRR72
C-7	PMS 2A x IR54
C-8	PMS 2A x IR54
C-9	PMS 2A x PRR22
	Two line Hybrids
T-10	IR 32364-20-1-3-2 x IR54
T-11	IR 32364-20-1-3-2 x PRR78
T-12	IR 32364-20-1-3-2 x Pusa1080
T-13	IR 32364-20-1-3-2 x PS-18
T-14	IR 32364-20-1-3-2 x Jaya
T-15	IR 68939-9-6-5-8 x IR54
T-16	IR 68939-9-6-5-8 x PS-18
T-17	IR 68939-9-6-5-8 x Pusa1080
T-18	IR 68298-11-1-6-3B x PS-18
T-19	IR 68298-11-1-6-3B x PRR78
	Pollen Parents
20	IR 54
21	PRR 78
22	PRR 22
23	PRR 72
24	Pusa 1080
25	PS-18
26	**Check - Jaya**
	B-Lines
27	IR 58025B
28	Pusa 5B
29	PMS 2B
30	Check - Pusa 834

N.B. : For simplicity in further coding of the hybrid all the CMS based hybrids will be represented with a prefix 'C' and TGMS based with a prefix 'T'

Plate 5 : **The TGMS lines used as female parents for the production of hybrids:**

a) **TG 2063 - IR 32364-20-1-3-2**
b) **TG 2000 - IR 68939-9-6-5-8**
c) **TG 2056 - IR 68298-11-1-6-3B**


TG 2056

Elite breeding lines

3. PS - 18
4. Pusa 1080

Check variety

5. Jaya

By using above male and female parents ten TGMS hybrids were produced, the details of which were presented in Table 6.

3(c). Comparative evaluation of CMS and TGMS based hybrids with pollen parents and checks in a trial at IARI Regional Station, RBGRC, Aduthurai, Tamil Nadu

During rabi 98-99 nineteen hybrids (ten TGMS based and nine CMS based) along with six pollen parents, three B-lines and two checks which all together form a total of 30 genotypes were sown in RBD with 3 replications. The details of the genotypes are given in Table 6. At maturity data was recorded on the following grain yield and its eleven component characters and the data is presented in the Table 8.

ANOVA:

Analysis of variance was carried out on the eleven characters recorded for the thirty genotypes. There were significant differences among the genotypes for all the characters studied at 5% level except for the character panicle length, the values of which were at par in all the thirty genotypes (Table 7).

Table 7. Analysis of variance

Source	d.f.	Days to 50% flowering	Days to maturity	Plant height	Tiller number	Number of effective tillers	Panicle length	Numebr of spikelets/ panicle	Number of filled spikelets/ panicle	%spikelet fertility	1000 grain weight	Grain yield per plant
Replications	2	20.88 (0.0105)	20.23 (0.0019)	95.91 (0.0091)	13.96 (0.1503)	28.22 (0.0270)	39.95 (0.0000)	2837.54 (0.0363)	1729.80 (0.0193)	143.21 (0.0997)	12.08 (0.0145)	144.77 (0.0077)
Genotypes	29	90.94 (0.0000)*	85.360 (0.0000)*	98.58 (0.0000)*	33.484 (0.0000)*	30.99 (0.0000)*	2.872 (0.1206)	2538.40 (0.0001)*	1196.75 (0.0002)*	132.69 (0.0049)*	21.14 (0.0000)*	77.56 (0.0004)*
CMS vs TGMS	1	37.57 (0.004)*	29.95 (0.002)*	850.34 (0.0000)*	4.984 (0.1206)	12.49 (0.197)	6.47 (0.077)	7731.43 (0.003)*	1568.70 (0.055)	55.75 (0.274)	211.85 (0.0000)*	194.03 (0.010)*
CMS vs Inbred	1	175.49 (0.0000)*	158.73 (0.0000)*	94.24 (0.029)*	28.94 (0.049)*	35.09 (0.033)*	2.169 (0.362)	5065.96 (0.015)*	1336.79 (0.076)	3.852 (0.065)	56.86 (0.0000)*	176.03 (0.014)*
TGMS vs Inbred	1	402.92 (0.0000)*	350.27 (0.0000)*	427.59 (0.0000)*	62.12 (0.005)*	96.27 (0.001)*	17.556 (0.004)*	370.42 (0.459)	16.31 (0.040)*	66.75 (0.295)	56.99 (0.0000)*	800.70 (0.0000)*
Error	58	4.234	2.900	18.78	7.128	7.34	2.002	807.82	408.89	59.69	2.651	27.360

Note : * indicates significance at 5% level

In the case of CMS based hybrids compared with TGMS hybrids significant differences were found in all the characters except for the characters tiller number, number of effective tillers and panicle length, the values of which were at par.

When CMS based hybrids were compared with the inbreds (parents & checks) significant differences were found in all the characters except for the characters panicle length, number of filled spikelets / panicle and percent spikelet fertility whose values were at par.

When TGMS based hybrids were compared with inbreds (parents & checks) significant differences were found in all the characters except for the characters number of spikelets / panicle and percent spikelet fertility whose values were at par.

Duncan's Multiple range test (DMRT):

From the results of the DMRT (Table 8) the following inferences are made.

The TGMS based hybrid T-18 took minimum number of 85.33 days to flower whereas the B-line PMS 2B took a maximum number of 107.3 days to flower. Among the thirty genotypes the most early performing group (i.e. the group with genotypes possessing the ranks with `A' or `B' in common) is constituted by T-18, T-17- T-16, C-1, T-13 and Pusa 834. For the character days to maturity the TGMS hybrid T-18 took minimum number of days (116.3) where as the B-line PMS 2B took maximum

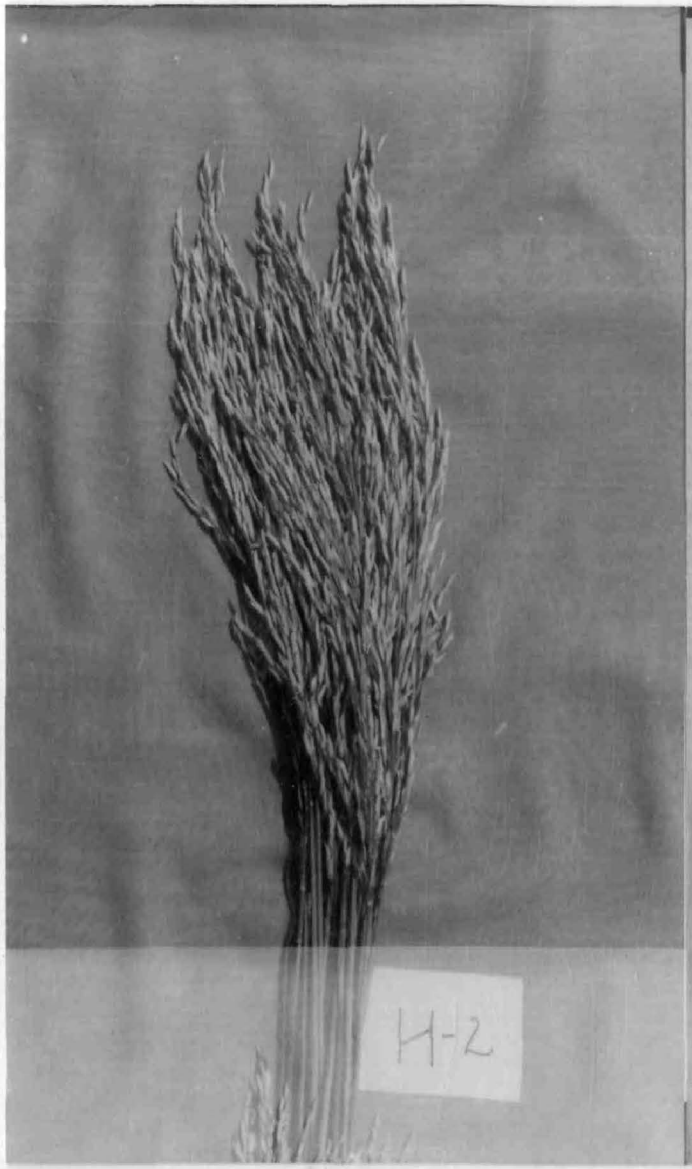
Table 8. **Mean comparisions (DMRT) for yield and its related characters of 30 genotypes (CMS and TGMS based hybrids, Pollen Parents and Check varieties).**

Strain		DTF	Rank	DTM	Rank	PH	Rank	Tiller no.	Rank	NET	Rank
1	C	91.00	**B**	121.3	**A**	103.50	GHIJK	14.20	CDEF	12.27	CDEFG
2	C	100.70	HIJKL	130.7	EFGH	105.50	DEFGHIJ	14.47	CDEF	10.87	CDEFG
3	C	99.33	EFGHI	129.7	DEFG	102.30	HIJK	13.93	CDEF	11.00	CDEFG
4	C	95.67	**CDE**	126.0	**BC**	99.20	JKL	13.53	DEF	11.93	CDEFG
5	C	101.00	HIJKLM	130.7	EFGH	107.90	BCDEFGHI	12.67	DEF	10.00	DEFG
6	C	99.67	FGHIJ	129.3	DEFG	109.30	BCDEFGH	16.27	**BCDE**	14.47	**BCDE**
7	C	96.33	**CDEFG**	126.7	**BCD**	93.93	L	19.80	**B**	17.93	**B**
8	C	97.67	DEGFH	128.7	CDEF	102.30	HIJK	13.47	DEF	10.73	CDEFG
9	C	100.00	GHIJK	130.7	EFGH	102.90	HIJK	14.07	CDEF	11.40	CDEFG
10	T	104.70	MNOP	134.7	IJKL	114.50	**ABC**	19.07	BC	15.07	**BCD**
11	**T**	100.70	HIJKL	131.0	EFGHI	118.30	**A**	27.37	**A**	24.20	**A**
12	T	105.30	OP	136.0	KL	111.60	**ABCDEFG**	14.13	CDEF	12.60	CDEF
13	**T**	91.00	**B**	121.3	**A**	107.80	BCDEFGHI	15.47	**BCDE**	12.93	**BCDEF**
14	T	105.00	NOP	135.0	JKL	115.30	**AB**	13.33	DEF	11.40	CDEFG
15	**T**	96.00	**CDEF**	126.7	**BCD**	108.00	BCDEFGHI	14.60	CDEF	13.40	**BCOEF**
16	T	90.33	**B**	120.3	**A**	112.00	**ABCDEF**	14.67	CDEF	13.80	**BCDEF**
17	T	90.00	**B**	121.0	**A**	103.80	FGHIJK	12.93	DEF	11.67	CDEFG
18	T	85.33	**A**	116.3	**A**	103.40	GHIJK	09.467	F	07.067	G
19	T	94.67	**CD**	125.0	**B**	112.40	**ABCDE**	12.00	EF	10.13	DEFG
20		104.30	LMNOP	134.0	HIJK	104.80	EFGHIJK	13.73	DEF	10.73	CDEFG
21		101.30	HIJKLMN	131.7	FGHIJ	109.30	BCDEFGH	11.20	EF	08.867	FG
22		103.30	JKLMNO	133.7	HIJKL	113.70	**ABCD**	11.00	EF	10.00	DEFG
23		102.30	IJKLMNO	132.3	GHIJKL	106.50	CDEFGHIJ	11.60	EF	09.933	DEFG
24		97.67	DEFGH	128.3	CDEB	96.47	KL	15.73	**BCDE**	11.60	CDEFG
25		95.67	**CD**	124.7	**B**	101.60	HIJKL	13.80	DEF	08.667	FG
26		100.30	HIJK	129.7	DEFG	112.70	**ABCDE**	11.53	EF	09.400	EFG
27		105.70	OP	135.7	KL	103.30	GHIJK	13.20	DEF	11.60	CDEFG
28		103.7	KMNOP	134.0	HIJK	104.50	EFGHIJK	14.80	**BCDEF**	11.33	CDEFG
29		107.3	P	137.3	L	100.20	IJKL	11.87	EF	10.20	DEFG
30		93.67	**BC**	124.7	**B**	107.30	BCDEFGHIJ	18.00	**BCD**	15.93	**BC**

(Table 8 contd....)

Strain		PL	Rank	NSP	Rank	NFSP	Rank	%SFP	Rank	TGWT	Rank	GYP	Rank
1	C	28.73	BC	191.4	BCDEFG	99.20	DEFGH	51.85	F	22.43	EFG	24.00	BCDE
2	C	30.87	AB	224.5	ABC	152.90	A	68.12	ABCDE	18.88	HI	22.43	BCDE
3	C	30.00	ABC	203.4	ABCDEF	117.70	ABCDEFG	57.68	BCDEF	21.48	GH	20.73	DE
4	C	29.47	ABC	156.7	EFG	88.60	GH	56.90	CDEF	24.28	BCDEFG	24.23	BCDE
5	C	28.20	BC	244.4	AB	150.60	AB	62.88	ABCDEF	21.63	FGH	23.43	BCDE
6	C	28.73	BC	202.4	ABCDEF	109.70	CDEFG	54.80	CDEF	24.27	BCDEFG	27.57	ABCD
7	C	28.00	C	158.4	EFG	102.40	DEFGH	64.24	ABCDEF	19.05	HI	20.12	DE
8	C	30.07	ABC	217.7	ABCD	121.80	ABCDEFG	56.04	CDEF	21.40	GH	22.93	BCDE
9	C	28.00	C	246.5	AB	138.70	ABCD	56.57	CDEF	21.59	GH	26.87	ABCD
10	T	31.73	A	175.4	CDEFG	98.07	EFGH	54.43	CDEF	24.94	ABCDE	27.49	ABCD
11	T	30.47	ABC	174.9	CDEFG	106.70	CDEFG	60.93	ABCDEF	26.29	ABC	31.28	ABC
12	T	30.27	ABC	184.8	CDEFG	93.33	EFGH	50.39	F	26.09	ABC	26.90	ABCD
13	T	29.93	ABC	193.1	BCDEFG	101.30	DEFGH	58.46	BCDEF	26.59	ABC	35.07	A
14	T	30.07	ABC	202.2	ABCDEF	110.90	BCDEFG	54.62	CDEF	24.28	BCDEFG	23.53	BCDE
15	T	28.87	BC	180.9	CDEFG	126.10	ABCDEFG	69.10	ABCD	25.54	ABCDE	32.60	AB
16	T	28.07	BC	165.2	DEFG	90.00	FGH	54.01	DEF	26.07	ABC	29.17	ABCD
17	T	29.13	ABC	195.5	BCDEF	122.70	ABCDEFG	67.67	ABCDE	26.69	AB	27.97	ABCD
18	T	29.87	ABC	159.6	EFG	118.20	ABCDEFG	74.27	A	25.98	ABCD	14.80	EF
19	T	29.53	ABC	185.6	CDEFG	129.30	ABCDEF	69.64	ABC	22.83	DEFG	24.07	BCDE
20		27.73	C	200.1	ABCDEF	114.50	ABCDEFG	57.31	BCDEF	21.71	FGH	19.73	DE
21		28.47	BC	137.9	G	66.33	H	49.11	F	25.85	ABCD	09.367	F
22		29.53	ABC	253.7	A	133.50	ABCDE	53.30	EF	24.16	BCDEFG	21.53	CDE
23		29.20	ABC	176.4	CDEFG	118.8	ABCDEFG	67.79	ABCDE	26.75	AB	22.63	BCDE
24		28.30	BC	150.3	FG	88.40	GH	58.73	BCDEF	27.80	A	21.13	CDE
25		28.27	BC	148.1	FG	93.40	EFGH	72.44	AB	23.43	CDEFG	19.50	DE
26		28.73	BC	192.4	BCDEFG	119.30	ABCDEFG	62.73	ABCDEF	24.79	ABCDEF	22.07	CDE
27		29.00	ABC	204.7	ABCDEF	120.80	ABCDEFG	57.93	BCDEF	18.42	I	20.67	DE
28		29.07	ABC	171.1	CDEFG	100.60	DEFGH	58.98	BCDEF	23.99	BCDEFG	21.83	CDE
29		27.93	C	209.0	ABCDE	146.80	ABC	58.75	BCDEF	18.51	I	19.63	DE
30		29.87	ABC	208.7	ABCDE	115.1	ABCDEFG	55.16	CDEF	24.50	BCDEFG	23.53	BCDE

Plate 6 : Comparison of the best performing CMS based hybrid C-2 (Photograph a) with its pollen parent PRR 22 (Photograph b). The Female Parent IR 58025A (Photograph c) is also presented


H-2


PRR 22

number of 137.3 days to mature and the early maturing group is constituted by T-18, T-16,T-17, C-1 and T-13.

The TGMS based hybrid T-11 showed a maximum plant height of 118.3 cm and CMS based hybrid C-7 showed a minimum plant height of 93.93 cm. The group of genotypes that showed lower plant heights constitute C-7, Pusa 1080, C-4, PMS 2B and PS-18.

The hybrid T-11 showed the highest number of tillers where as the hybrid T-18 showed the least number of tillers. The genotypes T-11, C-7, T-10, Pusa 834, C-6, Pusa 1080, T-13 and Pusa 5B constituted the group with high tiller number.

The same hybrid T-11 showed the highest number of effective tillers while T-18 showed least number of effective tillers. The highly performing group consists of T-11, C-7, Pusa 834, T-10, C-6, T-16, T-15 and T-13. Highest panicle length was shown by hybrid T-10 while least length was observed in IR 54. Since there were no significant differences among the genotypes in panicle length 17 genotypes came under the highly performing group.

The CMS restorer PRR 22 showed the highest number of spikelets / panicle where as PRR 78 had the least number. The group that is having the genotypes with the highest number of spikelets / panicle constitute PRR 22, C-9, C-5, C-2, C-8, PMS 2B, Pusa 834, IR 58025B, C-6, T-14 and IR 54. The CMS based hybrid C-2 had the higest

number of filled spikelets/panicle where as PRR 78 had the least number of filled spikelets/panicle. The highly performing group constituted the genotypes C-2, C-5, PMS 2B, C-9, PRR 22, T-19, T-15, T-17, C-8, IR 58025B, Jaya, PRR 72, T-18, C-3, Pusa 834 and IR 54. The hybrid T-18 showed the highest percent spikelet fertility whereas PRR 78 showed the least percent spikelet fertility. And the group with highest performance for this character constituted the genotypes T-18, PS-18, T-19, T-15, C-2, PRR 72, T-17, C-7, C-5, Jaya and T-11.

The highest thousand grain weight was observed in Pusa 1080 and the lowest in IR 58025B. The highly performing group was constituted by the genotypes Pusa 1080, PRR 72, T-17, T-13, T-11, T-12, T-16, T-18, PRR 78, T-15, T-10 and Jaya.

The TGMS based hybrid T-13 showed the highest grain yield / plant where as PRR 78 showed the least grain yield / plant. The highly performing group for the grain yield / plant was constituted by the genotypes T-13, T-15, T-11, T-16, T-17, C-6, T-10, T-12 and C-9.

Orthogonal Contrast:

The present study was carried out for evaluation of the performance of TGMS and CMS based hybrids and finding out their vigour over the parents and checks. For this the thirty genotypes under study were grouped into CMS group of hybrids (9), TGMS group hybrids (10) and Inbred group (parents and checks) (11). To find out which group

Plate 7 : Five randomly selected competitive plants bulk of the TGMS based hybrid T-11 (IR 32364-20-1-3-2 x PRR 78)


H-12

is performing well when compared to other two groups individually orthogonal contrast was done in 3 steps:

1. CMS based hybrids group vs TGMS based hybrids group
2. CMS based hybrids group vs Inbreds group
3. TGMS based hybrids group vs Inbreds group

The results are presented in the Table 9.

CMS based hybrids group vs TGMS based hybrids group:

1. **Days to 50% flowering** : The positive effect for this trait indicates that CMS is having a higher value of days to 50% flowering i.e., CMS group is late flowering compared to TGMS group of hybrids.
2. **Days to maturity** : The positive effect for this character also indicates that CMS group is the late maturing one and TGMS group is early maturing in nature.
3. **Plant height** : A negative effect of this character indicates that CMS group is having lower plant height compared to TGMS group.
4. **Tiller number** : Negative effect of this character indicates that CMS group is not favourable because of lower tiller number compared to TGMS group.
5. **Number of effective tillers** : Negative effect of this trait indicates that TGMS group is better than CMS group because of higher number of effective tillers in TGMS group.

Table 9. Orthogonal contrast

		DTF	DTM	PH	TL	NET	PL	NSP	NFSP	%SPF	TGWT	GYP
CMS vs	TGMS											
	MSS	37.57	29.95	850.34	4.984	12.49	6.47	7731.43	1568.70	55.75	211.85	194.03
		(0.004)	(0.002)	(0.0000)	(0.0001)	(0.197)	(0.077)	(0.003)	(0.055)	(0.274)	(0.0000)	(0.010)
	Effect	0.086	0.076	-0.407	-0.031	-0.049	-0.036	1.228	-0.002	-0.098	-0.203	-0.194
CMS vs	Inbreds											
	MSS	175.48	158.73	94.24	28.94	35.09	2.169	5065.96	1336.79	3.852	56.86	176.03
		(0.0000)	(0.0000)	(0.029)	(0.049)	(0.033)	(0.302)	(0.015)	(0.076)	(0.065)	(0.0000)	(0.014)
	Effect	-0.172	-0.163	-0.126	0.070	0.077	0.019	0.924	0.474	-0.025	-0.098	0.172
TGMS vs	Inbreds											
	MSS	402.92	350.27	427.59	62.12	96.27	17.556	370.42	16.31	66.75	56.99	800.70
		(0.0000)	(0.0000)	(0.0000)	(0.005)	(0.001)	(0.004)	(0.459)	(0.040)	(0.295)	(0.0000)	(0.0000)
	Effect	-0.241	-0.225	0.248	0.095	0.118	0.050	-0.231	-0.049	0.098	0.091	0.340

6. **Panicle length** : Negative effect of this character indicates that TGMS group is more favourable than CMS group because of higher value of panicle length.

7. **Number of spikelets / panicle** : Positive effect of this character indicates that CMS group is better than the TGMS group of hybrids because of high value of number of spikelets / panicle.

8. **Number of filled spikelets / panicle** : Positive effect of this trait indicates that CMS group is better than the TGMS group for this particular trait.

9. **% spikelet fertility** : Negative effect of this trait indicates that TGMS group of hybrids is better than the CMS group of hybrids.

10. **1000 grain weight** : Negative effect of this character indicates that TGMS group is better than the CMS group of hybrids for this particular trait.

11. **Grain yield / plant** : Negative effect of this trait indicates that TGMS group is better than the CMS group of hybrids for this particular trait.

CMS based hybrids group vs Inbreds group:

1. **Days to 50% flowering** : A negative effect of this character indicates that CMS group is having lower value of days to 50% flowering and which is favourable.

2. **Days to maturity** : Negative effect of this character also indicates that CMS group of hybrids are of early maturing type and hence are favourable compared to inbreds (parents & checks) group.
3. **Plant height** : Negative effect of this character indicates CMS group of hybrids show lower value of plant height.
4. **Tiller number** : Positive effect of this character indicates that CMS group of hybrids have higher tiller number compared to parents-checks group and hence are favourable.
5. **Number of effective tillers** : Positive effect of this character indicates that CMS group of hybrids are better than the parents - checks group for this particular character.
6. **Panicle length** : Positive effect of this trait shows that CMS group is favourable.
7. **Number of spikelets / panicle** : Positive effect of this trait shows that CMS group is better than the parents - checks group.
8. **Number of filled spikelets / panicle** : Positive effect of this trait shows that CMS group is better than the parents - checks group.
9. **% spikelet fertility** : Negative effect of this trait indicates that parents - checks group is better than the CMS group of hybrids.
10. ***1000 grain weight*** : Negative effect of this trait indicates that parents - checks group is better than the CMS group for this particular trait.

11. **Grain yield / plant** : Positive effect of this trait shows that CMS group is better than the parents - checks group for this particular trait.

TGMS based hybrids group vs Inbreds group

1. **Days to 50% flowering** : Negative effect of this trait shows that TGMS group of hybrids are early flowering compared to inbreds group.
2. **Days to maturity** : Negative effect of this trait shows that TGMS group of hybrids are early maturing compared to inbreds group.
3. **Plant height** : Positive effect of this trait shows that TGMS group of hybrids are having greater plant height compared to inbreds (parents - checks) group.
4. **Tiller number** : Positive effect of this trait shows that TGMS group is having better tiller number compared to parents - checks group.
5. **Number of effective tillers** : Positive effect of this character shows that TGMS group of hybrids is having more number of effective tillers than parents - checks group.
6. **Panicle length** : Positive effect of this character indicates that TGMS group is having higher value of panicle length than the parents - checks group.
7. **Number of spikelets / panicle** : Negative effect of this character shows that parents - checks group is better than the TGMS group for this character.

8. **No. of filled spikelets / panicle** : Negative effect of this trait indicates that parents - checks group is better than the TGMS group.
9. **% spikelet fertility** : Positive effect of this trait indicates that TGMS group is better performing than the parents - checks group for this particular trait.
10. **1000 grain weight** : Positive effect of this trait shows that TGMS group is better than the parents & checks group.
11. **Grain yield / plant** : Positive effect of this trait shows that TGMS group is better than the parents - checks group.

Correlation

Data pertaining to correlation analysis is presented in Table 10

1. **Days to 50% flowering** : It showed highly significant positive correlation with days to maturity and a significant positive correlation with number of spikelets / panicle. Whereas it showed a significant negative correlation with thousand grain weight and % spikelet fertility. It showed a significant negative correlation with grain yield / plant.
2. **Days to maturity** : It showed a significant positive correlation with number of spikelets / panicle and a significant negative correlation with 1000 grain weight and % spikelet fertility.
3. **Plant height** : It showed a significant positive correlation with tiller number, number of effective tillers and panicle length and showed a

Table 10 Correlation matrix for yield and its components

	DTF	DTM	PH	TL	NET	PL	NSP	NFSP	%SPF	TGWT	GYP
DTF	1.0000 (0.0000)	0.9864 (0.0001)*	0.1576 (0.1378)	0.0218 (0.8381)	-0.0161 (0.8801)	0.033 (0.7553)	0.2792 (0.0077)*	0.1675 (0.1145)	-0.2688 (0.0104)*	-0.3253 (0.0018)*	-0.1207 (0.2568)
DTM		1.0000 (0.0000)	0.1532 (0.01494)	0.0414 (0.6982)	0.0066 (0.9506)	0.0701 (0.5112)	0.2788 (0.0078)*	0.1815 (0.0868)	-0.2543 (0.0155)*	-0.3134 (0.0026)*	-0.1175 (0.2700)
PH			1.0000 (0.0000)	0.1771 (0.0949)	0.2532 (0.0160)*	0.2146 (0.0422)*	0.1497 (0.1590)	0.0936 (0.3798)	-0.0149 (0.8885)	0.3199 (0.0021)*	0.3934 (0.0001)*
TL				1.0000 (0.0000)	0.9191 (0.0001)*	0.0940 (0.3778)	-0.0877 (0.4107)	-0.1435 (0.1770)	-0.0831 (0.4361)	0.0190 (0.8587)	0.4509 (0.0001)*
NET					1.0000 (0.0000)	-0.0065 (0.9509)	-0.0956 (0.3699)	-0.1660 (0.1178)	-0.1239 (0.2446)	0.0401 (0.7070)	0.4622 (0.0001)*
PL						1.0000 (0.0000)	0.1721 (0.1047)	0.1596 (0.1329)	0.0923 (0.3867)	0.0515 (0.6295)	0.0687 (0.5197)
NSP							1.0000 (0.0000)	0.4763 (0.0001)*	-0.0927 (0.3847)	-0.3864 (0.0002)*	0.1988 (0.0602)*
NFSP								1.0000 (0.0000)	0.4763 (0.0001)*	-0.4795 (0.0001)*	0.1689 (0.1114)*
%SPF									1.0000 (0.0000)	-0.0926 (0.3850)	0.0730 (0.0938)
TGWT										1.0000 (0.0000)	0.02038 (0.0540)*
GYP											1.0000 (0.0000)

* indicates significance at 5% level
- values in parentheses indicate probability

highly significant positive correlation with 1000 grain weight and grain yield / plant. Whereas it showed a negative correlation with % spikelet fertility.

4. **Tiller number** : It showed a highly significant positive correlation with number of ear bearing tillers and grain yield / plant.

5. **Number of effective tillers** : It showed a highly significant positive correlation with grain yield / plant.

6. **Number of spikelets / panicle** : It showed a highly significant positive correlation with number of filled spikelets / panicle and a significant positive correlation with grain yield / plant. Whereas it showed a highly significant negative correlation with 1000 grain weight.

7. **Number of filled spikelets / panicle** : It showed a highly significant positive correlation with % spikelet fertility and also showed a highly significant negative correlation with 1000 grain weight.

8. **1000 grain weight** : It showed a significant positive correlation with grain yield / plant.

9. **Grain yield / plant** : It showed a highly significant positive correlation with plant height, tiller number and number of effective tillers and a significant positive correlation with number of spikelets / panicle and 1000 grain weight.

Studies on the level of heterosis in three line hybrids:

The data on yield and its component characters is presented in Table 11a. Heterosis as per cent deviation in the performance of hybrid over the check variety Jaya has been calculated and the values are presented in Table 11b.

Days to 50% flowering (DTF) : The mean value of days to 50% flowering of three line hybrids ranged from 91.00 days in C-1 to 101 days in C-5. The check (Jaya) had a mean value of 100.3 days.

The heterosis for days to 50% flowering over Jaya ranged from -0.29% to -9.27%. C-1 showed highly significant negative heterosis whereas C-4 and C-7 showed significant negative heterosis.

Days to maturity (DTM) : Mean duration of hybrids ranged from 130.7 days in C-9 to 121.3 days in C-1. The mean duration of Jaya is found to be 129.7 days. The heterosis for this trait ranged from 0 to -6.47%. C-1 showed highly significant negative heterosis whereas C-4 and C-7 showed significant negative heterosis.

Plant height (PH) : The mean values for plant height ranged from 93.93 cm in C-7 to 107.9 cm in C-5. Jaya showed a plant height of 112.7 cm. The heterosis for this trait ranged from -3.01% in C-6 to -16.65% in C-7. Hybrids C-7 & C-4 showed highly significant negative heterosis whereas hybrids C-1, C-3, C-8 and C-9 showed significant negative heterosis.

Tiller Number (Till No) : Mean values of tiller number ranged from 12.67 in C-5 to 19.80 in C-7. The mean value of Jaya was found to be 11.53. Heterosis for this trait ranged from 9.88% in C-5 to 71.72% in C-7. C-7 showed highly significant positive heterosis whereas C-6 showed significant positive heterosis.

Number of effective tillers (NET) : The mean values for this trait ranged from 10.00 in C-5 to 17.93 in C-7. The mean value of Jaya was found to be 9.40. Heterosis for this trait ranged from 6.38% in C-5 to 90.74% in C-7. Highly significant positive heterosis was shown by C-7 only. All other hybrids have shown positive heterosis which is not significant.

Panicle length (PL) : The mean values for this trait ranged from 28.00 cm in C-9 to 30.87 cm in C-2. Jaya showed a mean value of 28.73 cm for this trait. Heterosis for this trait ranged from 0.1% in C-1 and C-6 to 7.44% in C-2. Except C-5 and C-7, rest of the hybrids showed insignificant positive heterosis.

Number of spikelets / panicle (NSP) : The mean values for this trait ranged from 156.7 in C-4 to 246.5 in C-9. Jaya showed a mean value of 192.4. Heterosis for this trait ranged from -0.51% in C-1 to 27.02% in C-5. Significant positive heterosis was shown by C-5 and C-9.

Number of filled spikelets / panicle (NFSP):

The mean values for this trait ranged from 88.60 in C-4 to 152.9 in C-2. Jaya showed a mean value of 119.3. Heterosis for this trait ranged

from 1.34% in C-3 to 26.23% in C-5. C-2, C-5 and C-9 showed insignificant positive heterosis. No hybrid is significantly heterotic with respect to this trait.

Percent spikelet fertility (%SPF) : The mean values of this trait ranged from 51.85 in C-1 to 68.12 in C-2. Jaya showed a mean value of 62.73 for this trait. Heterosis for this trait ranged from 0.23% in C-5 to -17.34% in C-1. Except C-2, C-5 and C-7 all others showed negative insignificant heterosis.

Thousand grain weight (TGWT) : The mean values for this trait ranged from 18.88g in C-2 to 24.28g in C-4. Jaya showed a mean value of 24.79g. Heterosis for this trait ranged from -2.05% in C-4 to -23.15% in C-7. C-7 showed highly significant negative heterosis. All the hybrids without any exception showed negative heterosis.

Grain yield / plant (GYP) : The mean value for this trait ranged from 20.12g in C-7 to 27.57g in C-6. Jaya showed a mean value of 22.07g. Heterosis for this trait ranged from 1.63% in C-2 to 24.92% in C-6. Except C-3 all the other hybrids showed insignificant positive heterosis.

Studies on the level of heterosis in two line hybrids:

The data on yield and its component characters of the ten two line hybrids is represented in Table 12a . Heterosis as percent deviation in the performance of hybrid over the check Jaya has been calculated and the values are presented in the Table 12b.

Days to 50% flowering (DTF) : The mean values for this trait among the two line hybrids ranged from 85.33 in T-18 to 105.3 in T-12. Jaya, the check variety showed the mean value of 100.3. Heterosis for this trait ranged from 0.39% in T-11 to -14.9% in T-18. Highly significant negative heterosis was shown by the hybrids T-18, T-17, T-16 and T-13. Whereas significant negative heterosis was shown by T-15 and T-19. T-10, T-12 and T-14 showed significant positive heterosis.

Days to maturity (DTM) : The mean value of this trait ranged from 120.3 days in T-16 to 136.0 in T-12. Jaya showed a mean value of 129.7 days. Heterosis for this trait ranged from 1.00% in T-11 to -10.33% in T-18. T-13, T-16, T-17 and T-18 showed highly significant negative heterosis. Except T-10, T-11, T-12 and T-14 all others showed negative heterosis.

Plant height (PH) : The mean values of this trait ranged from 103.4 cm in T-18 to 118.3 cm in T-11. Jaya showed a mean value of 112.7 cm. Heterosis for this trait ranged from -0.26% in T-19 to -8.25% in T-18. T-17 and T-18 showed significant negative heterosis. Whereas hybrids T-12, T-13, T-15, T-16 and T-17 showed insignificant negative heterosis.

Tiller number (Till No.) : The mean values for this trait ranged from 9.467 in T-18 to 27.37 in T-11. Jaya showed a mean value of 11.53. Heterosis for this trait ranged from 4.07% in T-19 to 137.38% in T-11. All the hybrids showed positive heterosis except T-18. T-10 and T-11 showed highly significant positive heterosis.

Number of effective tillers (NET) : The mean values for this trait ranged from 7.076 in T-18 to 24.20 in T-11. Jaya showed a mean value of 9.40. Heterosis for this trait ranged from 7.76% in T-19 to 157.4% in T-11. T-11 showed highly significant positive heterosis. All the hybrids except T-18 showed positive heterosis. T-10 showed significant positive heterosis.

Panicle length (PL) : The mean value of this trait ranged from 28.07 cm in T-16 to 31.73 cm in T-10. Jaya showed a mean value of 28.73 cm. Heterosis for this trait ranged from 0.48% in T-15 to 10.44% in T-10. All the hybrids except T-16 showed positive heterosis. T-10 showed significant positive heterosis.

Number of spikelets / panicle (NSP) : The mean values for this trait ranged from 159.6 in T-18 to 202.2 in T-14. Jaya showed a mean value of 192.4. Heterosis for this trait ranged from 0.36% in T-13 to -17.04% in T-18. Except T-13 and T-14 all others showed negative heterosis. None of the hybrids is significantly heterotic with respect to this trait.

Number of filled spikelets / panicle : The mean value of this trait ranged from 90.0 in T-16 to129.3 in T-19. Jaya showed a mean value of 119.3. Heterosis for this trait ranged from -0.92% in T-18 to -24.55% in T-16. Except T-15, T-17 and T-19, rest of the hybrids showed negative heterosis. None of the hybrids showed significant positive heterosis for this trait.

Percent spikelet fertility (% SPF) : The mean values for this trait ranged from 50.39 in T-12 to 74.27 in T-18. The mean value of Jaya was found to be 62.73. Heterosis for this trait ranged from -2.86% in T-11 to 18.39% in T-18.

Table 11a. Mean performance of CMS based hybrids for yield and its components

S.No.	Hybrid	DTF	DTM	PH	Tiller No.	NET	PL	NSP	NFSP	%SPF	TGWT	GYP
C-1	IR 58025A x IR 54	91.00	121.3	103.50	14.20	12.27	28.73	191.4	99.20	51.85	22.43	24.00
C-2	IR 58025A x PRR 22	100.70	130.7	105.50	14.47	10.87	30.87	224.5	152.90	68.12	18.88	22.43
C-3	IR 58025A x PRR 78	99.33	129.7	102.30	13.93	11.00	30.00	203.4	117.70	57.68	21.48	20.73
C-4	Pusa 5A x IR 54	95.67	126.0	99.20	13.53	11.93	29.47	156.7	88.60	56.90	24.28	24.23
C-5	Pusa 5A x PRR 22	101.00	130.7	107.90	12.67	10.00	28.20	244.4	150.60	62.88	21.63	23.43
C-6	Pusa 5A x PRR 72	99.67	129.3	109.30	16.27	14.47	28.73	202.4	109.70	54.80	24.27	27.57
C-7	PMS 2A x IR 54	96.33	126.7	93.93	19.80	17.93	28.00	158.4	102.40	64.24	19.05	20.12
C-8	PMS 2A x PRR 78	97.67	128.7	102.30	13.47	10.73	30.07	217.7	121.80	56.04	21.40	22.93
C-9	PMS 2A x PRR 22	100.00	130.7	102.90	14.07	11.40	28.00	246.5	138.70	56.57	21.59	26.87
Check	Jaya	100.30	129.7	112.70	11.53	9.40	28.73	192.4	119.30	62.73	24.79	22.07

Table 12a. Mean performance of TGMS based hybrids for yield and its components

T-10	IR 32364-20-1-3-2xIR54	104.70	134.7	114.50	19.07	15.07	31.73	175.4	98.07	54.43	24.94	27.49
T-11	**IR 32364-20-1-3-2xPRR78**	**100.70**	**131.0**	**118.30**	**27.37**	**24.20**	**30.47**	**174.9**	**106.7**	**60.93**	**26.29**	**31.28**
T-12	IR 32364-20-1-3-2xPusa1080	105.30	136.0	111.60	14.13	12.60	30.27	184.8	93.33	50.39	26.09	26.90
T-13	**IR 32364-20-1-3-2xPS-18**	**91.00**	**121.3**	**107.80**	**15.47**	**12.93**	**29.93**	**193.1**	**101.30**	**58.46**	**26.59**	**35.07**
T-14	IR 32364-20-1-3-2xJaya	105.00	135.0	115.30	13.33	11.40	30.07	202.2	110.9	54.62	24.28	23.53
T-15	**IR68939-9-6-5-8xIR54**	**96.00**	**126.7**	**108.00**	**13.60**	**13.40**	**28.87**	**180.9**	**126.1**	**69.10**	**25.54**	**32.60**
T-16	IR68939-9-6-5-8xPS-18	90.33	120.3	112.00	14.67	13.80	28.07	165.2	90.00	54.01	26.07	29.17
T-17	IR68939-9-6-5-8xPusa1080	90.00	121.0	103.80	12.93	11.67	29.13	195.5	122.7	67.67	26.69	27.97
T-18	IR68298-11-1-5-3BxPS-18	85.33	116.3	103.40	9.467	7.06	29.87	159.6	118.2	74.27	25.98	14.80
T-19	IR68298-11-1-5-3BxPRR78	94.67	125.0	112.40	12.00	10.13	29.53	185.6	129.3	69.64	22.83	24.07
Check	Jaya	100.30	129.7	112.70	11.53	9.40	28.73	192.4	119.30	62.73	24.79	22.07

Table 11b. Percent heterosis of CMS based hybrids over national check Jaya

S.No.	Hybrid	DTF	DTM	PH	Tiller No.	NET	PL	NSP	NFSP	%SPF	TGWT	GYP
C-1	IR 58025A x IR 54	-9.27**	-6.47**	-8.16*	23.15	30.53	0.00	-0.51	-16.84	-17.34	-9.51	8.74
C-2	IR 58025A x PRR 22	0.39	0.77	-6.38	25.49	15.63	7.44	16.68	28.16	8.59	-23.84	1.63
C-3	IR 58025A x PRR 78	-0.96	0.00	-9.22*	20.81	17.02	4.42	5.71	-1.34	-8.05	-13.35	-6.07
C-4	Pusa 5A x IR 54	-4.61*	-2.85*	-11.9**	17.34	26.91	2.57	-18.55	-25.73	-9.29	-2.05	9.78
C-5	Pusa 5A x PRR 22	0.69	0.77	-4.25	9.88	6.38	-1.84	27.02*	26.23	0.23	-12.74*	6.18
C-6	Pusa 5A x PRR 72	-0.62	-0.30	-3.01	41.11*	53.93	0.00	5.19	-8.04	-12.64	-2.09	24.92
C-7	PMS 2A x IR 54	-3.95*	-2.31*	-16.65**	71.72**	90.74**	-2.54	-17.67	-14.16	2.40	-23.15**	-8.83
C-8	PMS 2A x PRR 78	-2.62	-0.77	-9.22*	16.82	14.14	4.66	13.14	2.01	-10.66	-13.67*	3.89
C-9	PMS 2A x PRR 22	-0.29	0.77	-8.69*	22.02	21.27	-2.54	28.11*	16.26	-9.82	-12.91*	21.74

Table 12b. Percent heterosis of TGMS based hybrids over Naitonal Check Jaya

T-10	IR 32364-20-1-3-2xIR54	4.38*	3.85**	1.59	65.39**	60.31*	10.44*	-8.84	-17.79	-13.23	0.60	24.55
T-11	**IR 32364-20-1-3-2xPRR78**	**0.39**	**1.00**	**4.96**	**137.38****	**157.4****	**6.05**	**-9.09**	**-10.56**	**-2.86**	**6.05**	**41.73***
T-12	IR 32364-20-1-3-2xPusa1080	4.98*	4.85**	-0.97	22.54	34.04	5.36	-3.95	-21.63	-18.81	5.24	21.88
T-13	**IR 32364-20-1-3-2xPS-18**	**-9.27****	**-6.47****	**-4.34**	**34.17**	**37.55**	**4.17**	**0.36**	**-14.94**	**-6.80**	**7.26**	**58.90***
T-14	IR 32364-20-1-3-2xJaya	4.68*	4.08**	2.30	15.61	21.27	4.66	5.09	-6.88	-12.93	-2.05	6.61
T-15	**IR68939-9-6-5-8xIR54**	**-4.28***	**-2.31***	**-4.17**	**26.62**	**42.55**	**0.48**	**-5.97**	**5.67**	**10.15**	**3.02**	**47.71***
T-16	IR68939-9-6-5-8xPS-18	-9.94**	-7.24**	-0.62	27.23	46.81	-2.29	-14.13	-24.55	-13.75	5.16	32.17
T-17	IR68939-9-6-5-8xPusa1080	-10.26**	-6.70**	-7.89*	12.14	24.14	1.39	16.11	2.84	7.87	7.66	26.73
T-18	IR68298-11-1-6-3BxPS-18	-14.9**	-10.33**	-8.25*	-17.89	-24.81	3.96	-17.04	-0.92	18.39	4.80	-32.94
T-19	IR68298-11-1-6-3BxPRR78	-5.61*	-3.62*	-0.26	4.07	7.76	2.78	-3.53	8.38	11.01	-7.91	9.06

Note: ** indicates significance at 1% level & * indicates significance at 5% level

Plate 8 : Comparison of T-17 (IR 68939-9-6-5-8 x Pusa 1080) (Photograph a) and T-19 (IR 68939-9-6-5-8 x PRR 78) (Photograph b) with check variety Jaya (Photograph c)


H-18


JAYA


H-20

Hybrids T-15, T-17, T-18 and T-19 showed positive insignificant heterosis, rest of the hybrids showed negative heterosis.

Thousand grain weight (TGWT) : The mean values for this trait ranged from 22.83g in T-19 to 26.69g in T-17. Jaya showed a mean value of 24.79g. Heterosis for this trait ranged from 0.60% in T-10 to -7.91% in T-19. Except T-14 and T-19 all the other hybrids showed insignificant positive heterosis amongst which T-17 showed the highest value. No hybrid showed significant heterosis for this particular trait.

Grain yield / plant (GYP) : The mean value for this trait ranged from 14.80g in T-18 to 35.0g in T-13. Jaya showed a mean value of 22.07g. Heterosis for this trait ranged from 6.61% in T-14 to 58.90% in T-13. The hybrids T-11, T-13 and T-15 showed significant positive heterosis. Except T-18 all the hybrids showed positive heterosis.

Comparison of standard heterosis of two and three line hybrids with common pollen parent:

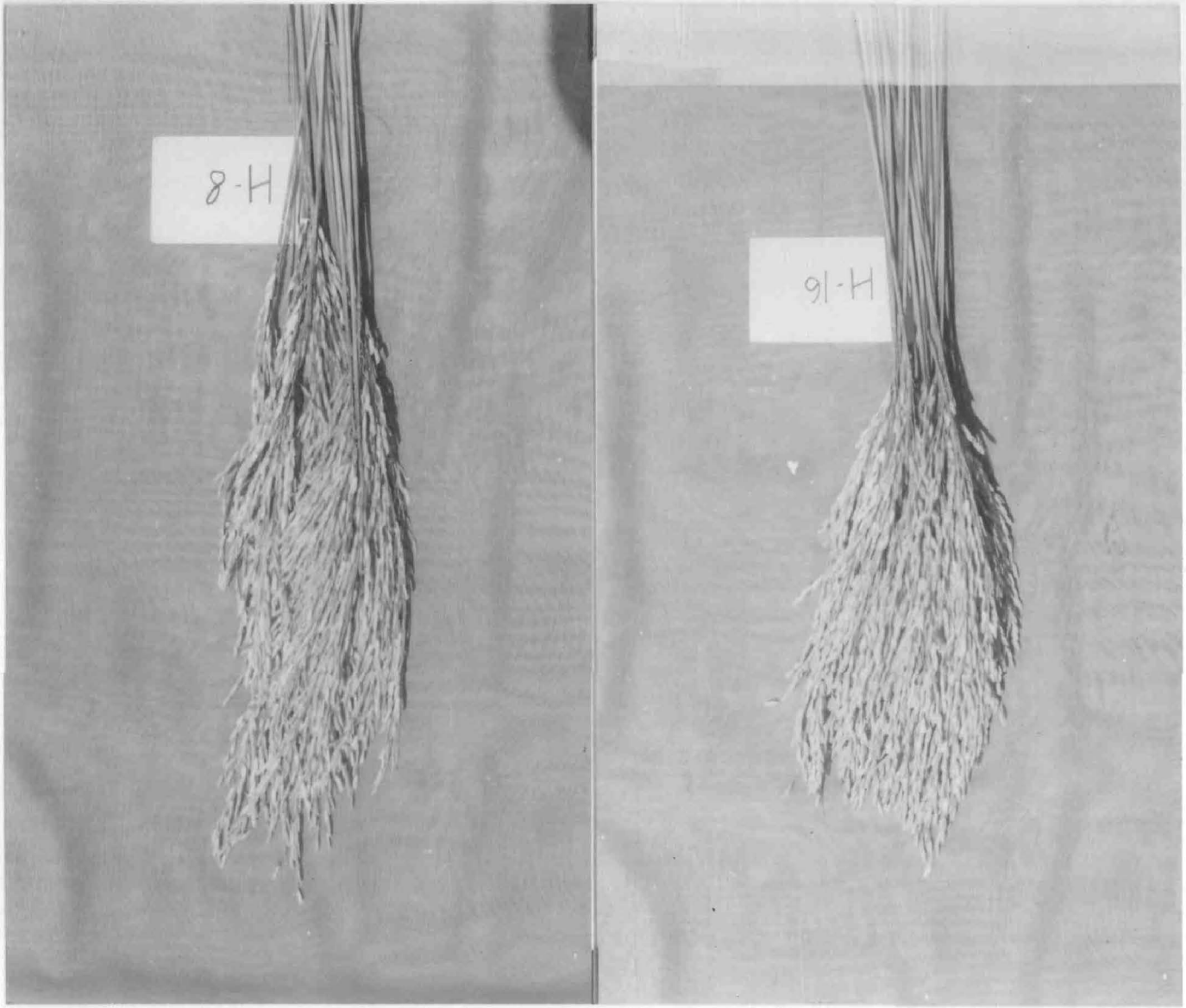
The comparison of two and three line hybrids with IR 54 as common pollen parent showed that among all the hybrids (C-1, C-4, C-7, T-10 and T-15) compared, only T-15 was found to be significantly heterotic. Similarly, when both two and three line hybrids (C-3, C-8, T-11 and T-19) with PRR78 as common pollen parent were compared, it was found that only T-11 was significantly heterotic (Table 13).

Table 13. Comparison of percent heterosis of cytoplasmic male sterile (CMS) and thermosensitive genic male sterile (TGMS) based hybrids with *common pollen parent* over national check Jaya for yield and its components

S.No	Hybrid	DTF	DTM	PH	Tiller No.	NET	PL	NSP	NFSP	%SPF	TGWT	GYP
	CMS hybrids											
C-1	IR 58025A x **IR 54**	-9.27**	-6.47**	-8.16*	23.15	30.53	0.00	-0.51	-16.84	-17.34	-9.51	8.74
C-4	Pusa 5A x **IR 54**	-4.61*	-2.85*	-11.9**	17.34	26.91	2.57	-18.55	-25.73	-9.29	-2.05	9.78
C-7	PMS 2A X **IR 54**	-3.95*	-2.31*	-16.65**	71.72**	90.74**	-2.54	-17.67	-14.16	2.40	-23.15**	-8.83
	TGMS hybrids											
T-10	IR 32364-20-1-3-2 x **IR54**	4.38*	3.85**	1.59	65.39**	60.31*	10.44*	-8.84	-17.79	-13.23	0.60	24.55
T-15	**IR 68939-9-6-5-8 x IR54**	**-4.28***	**-2.31***	**-4.17**	**26.62**	**42.55**	**0.48**	**-5.97**	**5.67**	**10.15**	**3.02**	**47.71***
	CMS hybrids											
C-3	IR 58025A x **PRR78**	-0.96	0.00	-9.22*	20.81	17.02	4.42	5.71	-1.34	-8.05	-13.35	-6.07
C-8	PMS 2A x **PRR 78**	-2.62	-0.77	-9.22*	16.82	14.14	4.66	13.14	2.01	-10.66	-13.67*	3.89
	TGMS hybrids											
T-11	**IR32364-20-1-3-2xPRR78**	**0.39**	**1.00**	**4.96**	**137.38****	**157.4****	**6.05**	**-9.09**	**-10.56**	**-2.86**	**6.05**	**41.73***
T-19	IR68298-11-1-6-3Bx**PRR78**	-5.61*	-3.62*	-0.26	4.07	7.76	2.78	-3.53	8.38	11.01	-7.91	9.06

Note : **, * indicates significance at 1% & 5% level respectively

Plate 9 : Comparative performance of T-15 (IR 68939-9-6-5-8 x IR 54) (Photograph a) and C-7 (PMS2A x IR 54); (Photograph b) hybrids with a *common pollen parent* IR 54


H-8
H-16

DISCUSSION

Ever since the first report of the phenomenon of heterosis by Jones (1926) and role of cytoplasm in inducing male sterility (Sampath and Mohanty, 1954) there have been efforts to explore the possibilities of exploiting hybrid vigour in rice (Shinjyo and Omura, 1966; Athwal and Virmani, 1972; Carnahan *et al.*, 1972; Swaminathan *et al.*, 1972). Chinese were the first to discover a stable male sterility source and to develop commercially viable hybrids using it (Lin and Yuan, 1980). More than half of the rice area in China is under hybrid rice today. From 1976 to 1995, hybrid rice helped China to increase rice production from 129 million tonnes to 200 million tonnes. In recent years, rice hybrids have yielded about 6.6 t ha^{-1} compared to 5 t ha^{-1} yield of conventional high yielding rice varieties. Many new cytoplasmic male sterile (CMS) lines with a high outcrossing rate and good grain quality have been developed recently.

Research on the commercial use of heterosis in rice has made tremendous achievements during the past 20 years. From a strategic point of view, however, it is still in the juvenile stage because the high yield potential of hybrid rice has not yet been fully tapped. In all the rice hybrids developed so far, the level of yield harvested has stagnated for years. This means that we have already reached the yield plateau for rice

hybrids. It would be difficult to further increase the yield potential in new rice hybrids if no new methods and materials are invented and adopted.

Hybrid rice breeding still has a bright future. Based on our studies, to derive full benefit from hybrid rice breeding, future developments may involve intensifying research on breeding methods and increasing the degree of heterosis. Two approaches are involved:

1. The three-line method using the CMS system.
2. The two-line method using the photoperiod-sensitive genic male sterility (PGMS) or thermosensitive genic male sterility (TGMS) system.

The rice hybrids used in commercial production belong to the category of intervarietal hybrids based on the CMS system. Many years of practice and experience have proved that the CMS system or three-line method is an effective way to develop rice hybrids and will continue to play an important role in this country. But this system has some constraints and problems. The sources of male sterility-inducing cytoplasm that can be used to develop better CMS lines are poor. Currently, about 85% of the A - lines used in commercial production still belong to the wild abortive (WA) type. The dominant cytosterility situation of the WA type could produce crisis in the long run, which could make hybrid rice susceptible to destructive pests.

Taking the long-range strategy of rice heterosis breeding into account, many Chinese rice scientists have been exploring new technological approaches to replace the CMS system. So far, the most successful outcome is the development of two-line hybrids.

This method is based on two new kinds of rice genetic tools; photoperiod-sensitive genic male sterile (PGMS) lines and thermosensitive genic male sterile (TGMS) lines that have been successfully developed in China recently. Their male sterility is mainly controlled by one or two pairs of recessive nuclear genes, and it has no relation to cytoplasm. Exploitation of these P(T) GMS lines to develop rice hybrids has the following advantages over the classical three-line or CMS system:

- The maintainer line is avoided. The PGMS lines under longer day length or the TGMS lines under higher temperature show complete pollen sterility, therefore, they can be used for hybrid seed production in these conditions. Under shorter day length or moderate temperature conditions, they show almost normal fertility and can thus multiply themselves by selfing.
- The choice of parents in developing heterotic hybrids is greatly broadened. Studies showed that more than 95% of varieties tested (with in the same sub-species) can restore such TGMS

lines. In addition, PGMS and TGMS genes can be easily transferred into almost any rice line with desirable characteristics.

- No negative effects are caused by sterile cytoplasm and the dominant cytoplasm situation of WA will be avoided.

Several achievements in this research area have been made.

To develop P(T)GMS lines that can be used commercially, one important criteria is that the male-sterility-inducing temperature (critical temperature) must be relatively low (mean temperature 23°C in the temperate zone and 24°C in the subtropics). If the critical temperature is relatively high (such as 26°C) for these male sterile lines, regardless of PGMS or TGMS, it is not safe in hybrid seed production because a temperature below this point, which may some times occur in the hot season can transform sterile pollen into fertile pollen. After nine years of research, considerable progress has been made. Now more than 20 P(T) GMS lines have been registered in China. Among these, two japonica lines belong to PGMS and the others are indica TGMS lines. Seven combinations have been certified and released for commercial production. The area under the two-line system for hybrid rice has been increasing steadily. Experimental tests and commercial practices have proved that the best two-line hybrids out yield three-line hybrids by 5-10%.

Another advantage of the two-line system over the three-line system is that the field area ratio of P(T)GMS line multiplication, seed production, and commercial use of F_1 is 1:100:12000-15000 as against 1:50:5000 in case of CGMS system.

Keeping in view of the success achieved by China in hybrid rice, the present study of Development and Evaluation of two and three line hybrids in Rice (*Oryza sativa* L.) was aimed at the following objectives:

1. Screening of TGMS lines for stability of sterility and their morphological characterisation.
2. Screening of CMS lines for stability of sterility and their morphological characterisation.
3. Production and comparative evaluation of two and three line hybrids.

I. **Screening of TGMS lines and their morphological characterisation**

The eleven TGMS lines which were imported from IRRI, and sown under kharif 98, New Delhi conditions were screened for pollen sterility at the time of flowering. Two lines viz., IR 68949-11-5-31-10-3 and Norin PL12 did not germinate. The rest nine lines which showed an average of 96 days to 50% flowering came to panicle initiation stage around 15th August and flowered between September 1st to 11th. The daily mean temperatures during this entire period were found to be in the range of 24.2°C to 32.5°C. Due to this higher temperatures except one line IR

68294-7-1-1829B, all the rest showed complete sterility when the pollen were observed under microscope.

Pollen sterility studies were also done on the same lines during the third week of October. During this time the daily mean temperatures were continuously found to be 25°C and below. Because of the lower temperature conditions the lines IR 32364-20-1-3-2, IR 68935-16-6-27 and IR 68298-11-6-3B showed 50% transformation to fertility whereas IR 68939-9-6-5-8, IR 71018-13-73-3-B, IR 68942-1-6-13-B-4 and IR 68931-1-4-15-18-7 showed complete transformation to fertility.

Morphological characters of all the nine TGMS lines were measured and their relative performance was observed under field conditions. All the lines performed well and some were found to be comparatively vigorous than the other lines. The lines IR 68294-7-1-18-29B, IR 68945-4-33-4-14-48 and IR 68935-16-6-27 flowered earlier than the others. The lines IR 71018-13-73-3-B, and IR 68298-11-1-6B showed the lowest plant height compared to others. The lines IR 32364-20-1-32 and IR 68931-1-4-15-8-7 showed maximum number of effective tillers. The lines IR 68294-7-1-18-29B, IR 32364-20-1-3-2 and IR 71018-13-73-3B showed longer panicle length. Lines IR 68298-11-1-6-3B, IR 32364-20-1-3-2 and IR 68935-16-6-27 showed the largest number of spikelets / panicle.

The same eleven TGMS lines were also sown during Rabi 98-99 under Aduthurai, T.N. conditions. The TGMS line IR 68949-11-5-31-10-3 did not germinate. The lines sown in the 3rd week of January came to panicle initiation stage around March 24th and flowered between 8th to 24th April under daily mean temperatures range of 29.2°C to 31.6°C. These higher temperatures caused the complete sterility of all the lines except one line IR 68294-7-1-18-29 B. This line IR 68294-7-1-18-29B was found to be fertile both under New Delhi and Aduthurai conditions and so it may not be suitable for Indian conditions. All the lines showed poor performance in the morphological characters under Aduthurai conditions.

II. Screening of CMS lines and their morphological characterisation

Exploitation of cytoplasmic-genetic male sterility led to the commercialization of hybrids in many crops, especially in self pollinated crop like rice. The CMS lines to be used in hybrid rice breeding should possess complete pollen sterility and should be stable in pollen behaviour over varied environments 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 reduced performance of the hybrid. Instability of male sterile line defeats the very objective of fixing the lines for hybrid seed production and lessens the economic importance and chances of success of hybrids.

CMS lines developed at IRRI and China were evaluated in several other countries to study their adaptability and stability over environments. Many of these lines were found to be unsuitable due to lack of stability in pollen sterility.

CMS lines in which pollen grains abort at uninucleate stage were found to be more stable than those in which pollen grains abort at bi- or tri-nucleate stage. Among the various sources of cytosterility in rice, CMS lines derived from the WA system have been found to be the most stable ones for their complete pollen sterility (Lin and Yuan, 1980; Chaudhary *et al.*, 1981). Lack of stability of male sterility in a genotype may be due to the presence of minor genes for fertility restoration in the maintainer genotype or due to incomplete sterilization or due to breakdown of male sterility in different environments as reported by Virmani and Wan (1988). Out of the CMS lines developed so far IR 58025A has been found to be the most stable.

For the present study five CMS lines imported from IRRI and three CMS lines from IARI breeding material were used. These lines were sown in the second week of June, kharif '98 and pollen studies were carried out at the time of flowering. All the lines studied have shown complete sterility without any exception and were found to be stable under Delhi conditions.

Same lines were also sown during rabi '98-99 under Aduthurai, T.N. conditions and pollen studies were conducted at the time of flowering. All the lines were found to be completely sterile without any exception. Chaudhary *et al.* (1981) and Pradhan *et al.* (1990b) reported that pollen abortion at uninucleate stage leads to withered or shrivelled pollen grains where as abortion at tri-nucleate stage will result in stained round sterile pollen grains. As the proportion of shrivelled and unstained pollen grains is high in both the CMS and TGMS lines we can infer that the pollen grains aborted at uninucleate stage.

Morphological characters of all the eight CMS lines were measured and their relative performance was observed under field conditions. Lines IR 68886 A and IR 68888 A had shown the lowest plant height compared to others. Lines IR 68897 A and IR 58025 A had shown longer panicles. Lines IR 68886 A, IR 58025 A and Pusa 5A showed higher number of effective tillers than others. IR 58025A and IR 68897A showed maximum number of spikelets / panicle. The agronomic performance of the lines IR 68897A and IR 58025A was found to be superior on the whole.

III. Production and comparative evaluation of two and three line hybrids

Out of the eleven TGMS lines screened three lines which were found to be stable and vigorous were selected and were used as female parents in producing the hybrids. Elite breeding lines and two CMS

restorers were used as male parents and crosses were effected by hand pollination during kharif 1998 at New Delhi. Thus a total of ten TGMS based hybrids were produced. Out of the eight CMS lines screened for pollen sterility two lines IR 58025A and Pusa 5A were found to be stable and vigorous and were selected as female parents. One more CMS line PMS 2A was also used in crossing. By using identified restorers nine CMS based hybrids were produced. Two of the pollen parents were common to both TGMS and CMS lines which helped in the comparative evaluation of the two and three line hybrids. These 19 hybrids along with 6 pollen parents, 3 B-lines and 2 checks were evaluated in a trial at IARI regional station, RBGRC, Aduthurai during rabi 98-99.

Inferences drawn from the DMRT analysis:

From the comparison of means of all the characters of the 30 genotypes under study in the DMRT analysis it was found that the highly performing group (i.e. the group with genotypes possessing the ranks with A in common) for each character studied is mostly composed of TGMS based hybrids.

If we analyse character by character for days to 50% flowering and days to maturity, 75% of the genotypes of the highly performing groups are of the TGMS based hybrids. The hybrids T-18 and T-17were found

to be the earliest flowering and maturing hybrids amongst the 30 genotypes compared.

Similarly for the character plant height the least performing group composed of 50% CMS based hybrids. The highest performing group (8 genotypes) for the character number of tillers is composed of three TGMS based hybrids, two CMS based hybrids and three inbreds. For the character number of effective tillers the highest performing group (9 genotypes) is composed of 55.55%of TGMS based hybrids and the other 44.55% is composed by CMS based hybrids and Inbreds. The panicle length did not vary much in all the genotypes and the highly performing group is composed of 17 genotypes. The top most performing hybrids for this trait are T-11 and C-7.

The highly performing group (12) for the character, number of spikelets/panicle was found to be composed of 50%of CMS based hybrids and 50% of the inbreds. The top most better performing hybrids for this trait are C-9 and C-5. The CMS based hybrids C-2 and C-5 had the highest number of filled spikelets/panicle, of which C-2 had the highest. The high performing group is constituted by the C-2, C-5 and C-9 hybrids followed by T-19, T-15, T-17 and T-18. T-18 showed the highest value of percent spikelet fertility and the group with highest performance consisted of six TGMS based hybrids and 3 CMS based hybrids followed by Jaya.

The highest value of thousand grain weight was shown by Pusa 1080. The highly performing group of 12 genotypes consisted of 8 TGMS based hybrids i.e. 75% of the highly performing genotypes are of TGMS based.

The TGMS based hybrid T-13 showed the highest grain yield / plant. The best performing group (9) of genotypes consisted of T-13, T-15, T-11, T-16, T-17, C-6, T-10, T-12 and C-9 in the descending order of performance.

From the above discussion we can infer that the TGMS based hybrids dominated the better performing group for the characters days to flowering, days to maturity, number of tillers, number of effective tillers, percent spikelet fertility, thousand grain weight and grain yield / plant which are the most influencing characters on the yield. CMS hybrids were found to be inferior to the TGMS hybrids for these particular characters. However, CMS based hybrids dominated the better performing group for the characters, number of spikelets / panicle and number of filled spikelets / panicle along with the pollen parents. Hence we can conclude that the per se performance of the TGMS based hybrids is better than the CMS based hybrids, pollen parents and check varieties.

Orthogonal contrast study showed the overall performance of two line hybrids group, three line hybrids group and inbreds (Parents &

Checks) group. On the basis of the results it was found that the TGMS based hybrids are the earliest flowering group followed by CMS based hybrid group and inbreds group and similar trend was found for the trait days to maturity. CMS group of hybrids showed lower plant height followed by inbreds group which in turn was followed by TGMS group. For multiple cropping situation earliness and dwarfness are desirable where as in ill drained and deep water situations late maturing and tall varieties perform better.

For the traits tiller number, number of effective tillers and panicle length TGMS group of hybrids showed the best performance followed by CMS based hybrids group which in turn is followed by inbreds group.

Three line hybrids group performed better for the characters number of spikelets per panicle and number of filled spikelets per panicle followed by inbreds group which inturn is followed by two line hybrids group. These two are the direct yield influencing characters, lesser performance in these characters may lower the yield levels of the TGMS based hybrids.

Highest levels of the trait thousand grain weight was shown by two line hybrids followed by inbreds group which is followed by three line group of hybrids. A higher amount of grain yield per plant was shown by

two hybrids followed by three line hybrids which inturn are followed by inbreds.

The suspected lower yield levels of two line hybrids because of lower ranking of the characters, number of spikelets per panicle and number of filled spikelets per panicle was compensated by the higher levels of the characters number of total tillers, effective tillers, percent spikelet fertility and 1000 grain weight which enhanced the yield levels of two line hybrids.

The correlation analysis showed significant negative correlation between the characters days to 50% flowering, days to maturity and thousand grain weight which implies that early strains had higher thousand grain weight. Tiller number showed significant positive correlation with number of effective tillers and grain yield per plant. Number of spikelets and filled spikelets per panicle showed significant negative correlation with 1000 grain weight which implies that as the number of spikelets and filled spikelets per panicle increased thousand grain weight decreased because of the distribution of photosynthates to more number of grains. Grain yield per plant showed a highly significant positive correlation with tiller number, number of effective tillers, and significant positive correlation with number of spikelets / panicle and thousand grain weight.

Studies on the level of heterosis in the two and three line hybrids

Shull, who coined the term heterosis, gave an explicit and precise definition of heterosis as "the increased vigour, size, fruitfulness, speed of development, resistance to diseases and pests manifested in crossbred organism as compared to corresponding inbreds,as the specific result of unlikeliness in the constitution of uniting gametes". In plant breeding heterosis refers to any increase/decrease in the performance of F_1 hybrid over its parents. Heterosis is measured as deviation from mid parent (relative heterosis), better parent (heterobeltiosis) and superiority as percent improvement in performance of hybrid over the standard check. The superiority over check or standard heterosis is more relevant from the plant breeding point of view.

The phenomenon of heterosis in rice was first reported by Jones (1926) for number of culms and yield. Studies conducted afterwards have provided evidence of significant heterosis for various agronomic traits. The most convincing reports on the presence and possibilities of exploiting heterosis on commercial scale have come from Chinese workers (Lin and Yuan, 1980). On an average, the commercial rice hybrids were reported to show 20-30 % standard heterosis for grain yield (Lin and Yuan, 1980; Yuan *et al.*, 1989). Yuan *et al.* (1994) also reported a 29 to 45 % yield advantage of hybrids over conventional rice varieties in

China. Yield component analysis indicated that yield heterosis is imparted by heterosis in one or more of its components.

Nature and magnitude of heterosis over check variety Jaya differ from character to character depending on the cross combination. Heterosis has been observed both in positive and negative direction.

DMRT analysis was done for all the eleven characters measured.

For the trait days to 50% flowering, within the three line hybrids C-1 showed highly significant negative heterosis where as, within the two line hybrid group T-13, T-16, T-17 and T-18 showed highly significant negative heterosis compared to check variety Jaya. Between two line and three line hybrids all the above two line hybrids showed greater negative heterotic value than C-1. The highly significant negative heterosis showed by two line hybrids indicates the earliness of these hybrids compared to Jaya and also three line hybrids. Similar trend was observed for days to maturity also. The hybrids T-16, T-17 and T-18 matured 9 days, 8 days and 13 days earlier than Jaya respectively, where as C-1 matured 8 days earlier than Jaya.

For optimally managed and multiple crop growing situations, earliness is preferred in general where as in ill drained and deep water situations generally late varieties fit better.

The results show that the magnitude of negative heterosis is higher for the character plant height in case of three line hybrids when compared to two line hybrids. All the three line hybrids showed negative heterosis for the plant height without any exception and the hybrids C-7 and C-4 showed highly significant negative heterosis whereas C-1, C-3, C-8, and C-9 showed significant negative heterosis.

For optimally managed and multiple cropping situation short height or dwarfness of the plant is preferred where as in ill drained and deep water situations tall varieties perform better.

Both positive and negative heterosis for plant height has been reported by several researchers (Singh, 1980b; Srivastava and Seshu, 1982; Amrithadevarathinam, 1984; Nijaguna and Mahadevappa, 1983; Sharma and Mani, 1990). Taller plants with weak culm are more prone to lodging and hence affect the yield considerably. Most of the traditional basmati varieties are tall and heavy lodging leads to reduction in grain yield. Hence breeding for semi-dwarf varieties with stronger culms leading to non lodging habit is of importance. Non lodging habit will also allow mechanised harvesting. However, Ponnuthurai *et al.* (1984) reported that taller plants may have better plant canopy for photosynthesis. As plant height is a component of straw yield, reduction in culm length will lead to low straw yield (Nijaguna and Mahadevappa, 1983).

In case of number of tillers within the two line hybrids T-10 and T-11 showed highly significant positive heterosis of 65.39% and 137.38% respectively. Where as within the three line hybrids C-7 showed highly significant positive heterosis of 71.72%. Same trend was observed for the trait, number of effective tillers, whereas within the two line hybrids T-10 and T-11 showed a highly significant positive heterosis of 60.31% and 157.4% where as within the three line hybrids C-7 showed a highly significant positive heterosis of 90.74%.

Reports on significant positive and negative heterosis for this trait are available. The lowest value of -43.90% was reported by Ravikumar (1983) and highest standard heterosis value of 242.85 % was reported by Rangaswamy and Natarajamurty (1988). The number of effective tillers per plant, an important yield component is known to have strong association with grain yield. Significant positive heterosis for productive tillers results in heterosis for grain yield also (Pandey *et al.*, 1995; Reddy and Nerkar, 1995; Padmavathi *et al.*, 1996; Rao *et al.*, 1996a). However, mere presence of high heterosis for productive tillers per plant may not give higher yield, particularly in those hybrids where spikelet fertility is greatly affected due to the varying levels of fertility restoration. Thus, number of filled grains per panicle would also influence the grain yield with varying degrees in different hybrids.

For the trait panicle length among the two-line hybrids only T-10 showed significant positive heterosis. All the others have shown insignificant positive heterosis where as among the three line hybrids no hybrid showed significant positive heterosis. Among two and three line hybrids even the best performed hybrids have not shown significant heterosis for panicle length.

Hence information on panicle length alone may not be sufficient in predicting the grain yield. Considerable amount of heterosis has been reported for this trait by Anandakumar and Sree Rangasamy (1984), Sarial (1994) and Pandey *et al.* (1995). However, Nijaguna and Mahadevappa (1983), Paramasivam and Sree Rangasamy (1988) and Manuel and Palanisamy (1989) observed the higher magnitude of negative heterosis than positive heterosis.

Considerable amount of heterosis for number of spikelets / panicle has been reported in earlier studies (Sharma and Mani, 1990; Patnaik *et al.*, 1990; Sarial, 1994). Among the three line hybrids C-5 and C-9 showed significant positive heterosis for this trait where as all the two line hybrids except T-13, T-14 & T-17 showed insignificant negative heterosis. This implies that the total number of spikelets / panicle is comparatively less in TGMS based hybrids than CMS based hybrids. This trait has the direct influence on final yield of the plant. Both two and three line hybrids have not shown any significant positive or negative heterosis for the trait

number of filled spikelets / panicle. More than five three line hybrids and seven two line hybrids showed negative heterosis for this trait which has got direct influence on the final yield of the plant. Number of filled spikelets / panicle seems to be the most important factor contributing to grain yield especially in hybrids. Virmani *et al.* (1982) concluded that heterosis in yield was primarily due to increased filled spikelets / panicle. High heterosis for filled spikelets per panicle resulting in higher grain yield has been reported by several workers (Sharma and Mani, 1990; Patel *et al.*, 1994; Pandey *et al.*, 1995; Rao *et al.*, 1996a).

Test weight or 1000 grain weight is yet another important yield component having direct association with higher grain yield. Virmani *et al.* (1982), Ponnuthurai *et al.* (1984), Patnaik *et al.* (1990), Pandey *et al.* (1995), Rao *et al.* (1996a) and Padmavathi *et al.* (1996) reported that increased yield in heterotic hybrids in rice is due to heterosis for 1000 grain weight, to some extent. All the three line hybrids with, out any exception showed negative heterosis for the trait thousand grain weight where as all the two line hybrids except T-14 and T-19 showed significant positive heterosis. This is one of the major cause for the decreased yield of CMS based hybrids compared to TGMS based hybrids.

The extent of heterosis for grain yield in rice has been reported in various studies. Ravikumar (1983) reported the highest negative value of 98% for standard heterosis. The highest positive standard heterosis for

grain yield per plant was observed by Sarial (1994) and the value was 284.55%. In the present study the superiority over Jaya ranges from 1.68 to 24.92 among the CMS based hybrids whereas it ranged from 6.61 to 58.90 among the TGMS hybrids. No CMS based hybrid showed significant positive heterosis for grain yield per plant. The hybrid C-7 showed highly significant tiller number and effective tillers. But was not able to show significant grain yield per plant because of the negative heterosis shown by it for the characters number of spikelets / panicle, number of filled spikelets per panicle and thousand grain weight. The C-6 showed significant tiller number but it could not show significant grain yield because of the negative heterosis shown by the other yield component characters, number of filled spikelets / panicle, percent spikelet fertility and thousand grain weight. But still it is the highly performing hybrid amongst the three line hybrids for the character grain yield per plant.

In the absence of increased photosynthetic efficiency, increase in one component would occur at the cost of that in another component. The phenomenon is known as yield compensation. However, this compensation will not be always complete and increase in the end product may still be substantial. The three two line hybrids T-11, T-13 and T-15 showed significant positive heterosis for grain yield per plant and were found to be superior to other two line hybrids and also CMS based

hybrids. The highest value of heterosis in the three significantly heterotic two line hybrids is shown by T-13 followed by T-15 and T-11. The superiority of the T-13 is because of high heterosis in the characters number of tillers and number of effective tillers and negative heterosis in days to flowering, maturity and plant height and positive heterosis for the trait number of spikelets per panicle which was shown by only two of the TGMS based hybrids and also due to comparatively higher heterosis for the trait thousand grain weight. All the above levels of heterosis together made the hybrid T-13 the top performing one of all the 30 genotypes studied. Hence the cross combinations:

IR 32356-20-1-3-2 x PS-18 (T-13),

IR 68939-9-6-5-8 x IR 54 (T-15) and

IR 32364-20-1-3-2 x PRR 78 (T-11) can be considered as the three two line hybrids which outyielded the rest of the two line and three line hybrids.

In test crosses made at IRRI, the frequency of heterotic rice hybrids derived from the TGMS system was higher than from the CMS system. Some two line hybrids yielding 1t / ha higher than inbred check varieties were identified in preliminary yield trials at IRRI [Lopez and Virmani (unpublished data)], Lu Xinggui *et al.*, 1996.

Lopez and Virmani (Lu Xinggui *et al.*, 1996) got 17 heterotic CMS hybrids out of 103 crosses made, i.e. 16% heterotic hybrids were obtained. Where as they got 47 heterotic TGMS based hybrids out of 131 crosses made i.e. 36% heterotic hybrids were obtained. In the present study out of 9 CMS crosses made, none were heterotic which account 0% and out of 10 TGMS crosses made 3 were heterotic which accounts for about 30% heterotic hybrids.

A number of two-line hybrid rice combinations with a yield increase of 5-10% over the three line combinations have been developed and commercialised by Chinese scientists who produced the TGMS based hybrids using CMS restorers and several cultivars as TGMS restorers (Wang and Li, 1992; Luo *et al.*, 1994; Wang *et al.*, 1995; Cheng *et al.*, 1996).

In the present study two-line hybrid rice combinations with a yield increase of 4.97-36.4% (Table 14a & 14b) over three-line combinations have been developed. Hence the results obtained from the present study are in concordance with the results obtained by several Chinese scientists as quoted previously.

Table 14a. Increase of yield of two line hybrids when compared with the *best performing* three line hybrid (Pusa 5A x IR 54) all having *common pollen parent*

	Hybrid	GYP (g)	Increase
	TGMS		
T-10	IR 32364-20-1-3-2 x IR54	27.49	13.5 %
T-15	IR 68939-9-6-5-8 x IR 54	32.60	34.7%
	CMS		
C-4	Pusa 5A x IR 54	24.23	0

Table 14b. Increase of yield of two line hybrids when compared with the *best performing* three line hybrid (PMS 2A x PRR 78) all having *common pollen parent*

	Hybrid	GYP (g)	Increase
	TGMS		
T-11	IR 32364-20-1-3-2 x PRR 78	31.28	36.4%
T-19	IR 68298-11-1-6-3B x PRR78	24.07	4.97%
	CMS		
C-8	PMS 2A x PRR 78	22.93	0

SUMMARY AND CONCLUSIONS

In the light of the success achieved by Chinese in the field of two line breeding, and being acquainted with the advantages of two line system of hybrid rice breeding over that of three line system, the present investigation on the **Development and evaluation of two and three line hybrids in Rice** was aimed at the objective of producing two and three line hybrids and their comparative evaluation.

For the production of two line hybrids, eleven TGMS lines which were found to be sterile at IRRI conditions were imported and screened for pollen sterility and were characterised morphologically under Delhi conditions during kharif '98. Out of these, three best performing lines were selected as female parents and by using elite lines and CMS restorers as male parents, ten two line hybrids were produced.

For obtaining three line hybrids, five CMS lines were imported from IRRI and three CMS lines were selected from IARI breeding material and these were screened for pollen sterility at the time of flowering and also characterised morphologically under field conditions. Three CMS lines which are stable for pollen sterility and with best agronomic performance were selected as female parents. By using identified CMS restorers as male parents, nine CMS hybrids were produced.

The ninteen hybrids thus produced were evaluated along with parents and check variety Jaya in a trai at RBGRC, Aduthurai, T.N. The experiment was laid out in RBD with three replications during rabi '98-99 and the observations were recorded on yield and its components. The data was analysed statistically and the relative merits and demerits of two and three line hybrids for different characters studied were found out and the best performing hybrids were reported.

From the statistical analysis, it was evident that there were significant differences among all the genotypes for all the characters studied except for the character panicle length. From the DMRT analysis, it was evident that the frequency of the two line hybrids was higher in the top ranked group (the group of genotypes possessing `A' or `B' in their rank) for the characters early maturity, early flowering, plant height, total number of tillers, number of effective tillers, per cent spikelet fertility, thousand grain weight and grain yield per plant. The lower values of the two line hybrids for the characters, number of spikelets per panicle and number of filled spikelets per panicle were compensated by the higher values of their total number of tillers and effective tillers which enabled them to outyield the three line hybrids.

Group wise comparison of two and three line hybrids and inbreds (pollen parents and checks) also revealed the out ranking performance of the two line hybrids with regard to yield and its component characters.

From the correlation analysis, it was found that grain yield / plant had a highly significant positive correlation with tiller number and effective tillers and significant positive correlation with number of spikelets per panicle and thousand grain weight.

When the levels of standard heterosis for different characters of different genotypes were estimated, the superiority of the two line hybrids over three line hybrids and Jaya for grain yield and its components was proved. Out of the nine CMS lines evaluated, none was found to be significantly heterotic over Jaya but still many of them showed positive heterosis over Jaya. But out of the ten two line hybrids evaluated three were found to be significantly heterotic over Jaya. When both two and three line hybrids with common pollen parents were compared once again the hybrids with TGMS line as female parent were found to be superior in grain yield. When the frequency of heterotic hybrids obtained from all the crosses made is calculated, it is found to be nil in case of three line hybrids where as a frequency of 30% was shown by two line hybrids. This is in accordance with the results obtained earlier by Chinese scientists. The per cent yield increase of two line hybrids over the best performing three line hybrids is in the range of 4.97 to 36.4%. These results are in accordance with the work done by the Chinese scientists early in this decade. From this we can conclude that the TGMS lines imported from IRRI performed better than the existing CMS lines.

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