PINSA **B65** No 6 pp 331-350 (1999)

Innovative Male Sterility Systems for Exploitation of Hybrid Vigour in Crop Plants
1 - Environment Sensitive Genic Male Sterility System

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(Received on 4 June 1999; Accepted after revision 6 August 1999)

The phenomenon of heterosis and its importance in crop improvement have been known since decades. Recognizing that commercial exploitation of hybrid vigour would, however, depend on economic viability of hybrid seed production. Varied genetic and non-genetic approaches for selective emasculation of one of the parents have been evolved and used. Cytoplasmic-genetic male sterility is the worldwide used system among them. Yet, its cumbersome nature, labour expensive seed production method and restricted parental choice warranting an alternative has led to the discovery and study of temperature or photoperiod influenced environment sensitive genic male sterility system in some of the cereal crops. Physiological characterization of various sources of photoperiod and temperature sensitive genic male sterility (TGMS/PGMS) in a model crop like rice has helped group them into 3-4 categories on the basis of their critical sterility and fertility points, while genetic analysis has revealed it to follow simple mendelian mode of inheritance and 2-3 putative genes (*pms 1, pms 2, tms 1, tms 2, tms 3*) to govern them. Development of stable and agronomically superior PGMS and TGMS lines using promising gene sources and identification of ideal locations/seasons for hybrid seed production and multiplication of parental TGMS or PGMS line has facilitated evolution of two-line hybrids of rice for commercial planting. It is hoped that more precise understanding of the physiology, biochemistry, genetics and molecular bases of these environment sensitive male sterility sources would greatly help extend this innovative system of hybrid breeding to a wide range of crop plants in the coming years.

Key Words: Male sterility, Photoperiod temperature sensitive male sterility, Physiological characterization, Genetics, Gene tagging, RAPD markers, Two-line hybrid, Hybrid seed production, Rice, Sorghum, Maize

Introduction

Ever since the domestication of economically important plant species in different parts of the world 12000-15000 years ago human beings have been devising, adopting and imporving various approaches to suit their agro-ecological and socioeconomic needs placing excessive emphasis on increased productivity. Pureline and mass selection in land races, pedigree/ bulk selection in cross-bred populations have been the common approaches until the importance of hybrid vigour was realized way back in the twenties. Even before the days of Mendel's Laws of Inheritance, it was known that the immediate progeny of crosses involving diverse parents were invariably superior to either of or both the parents in the expression of qualitative as well as quantitative traits. Exploitation of the excessive vigour in yield in the first generation of a cross is the principle behind heterosis breeding in crop plants. Development of cultivars and hybrids on a commercial scale in maize around 1930, is considered a landmark in the history of crop

breeding research. Genetic basis of heterosis has been the subject of controversy for long. Initially attributed to partial to complete dominance, over-dominance, epistasis and their combinations (Comstock & Robinson 1952), the phenomenon intensively investigated subsequently by several workers, is now believed to be as a result of all types of intra- and inter-allelic interactions along with the influence of cytoplasm. It would, therefore, amount to over simplification, if it is attributed to either dominance or overdominance (Sprague & Eberhart 1977, Jinks 1983, Hallauer & Miranda 1981, Pooni & Trehan 1994, Yu et al. 1997).

Commercial success of hybrid technology across crops depends on level of exploitable (standard) heterosis, which in turn depends on choice of parents, effective and economical means of hybrid seed production. Although exploitable heterosis is available to an appreciable extent in both auto and allogamous species, the bottleneck has always been in respect of seed production due to either absence of usable male sterility system or stability of the system wherever available. Male sterilization is achieved by (a) mechanical/ manual emasculation, (b) chemical emasculation, (c) genetic induction, and (d) cytoplasmic-nuclear interaction.

Mechanical emasculation is by physical removal of male flowers or stamens, which is practicable in those crops, where sex organs remain separated — as in corn, where flowers are of large size — as in cotton and where one pollination results in a large number of seeds — as in tomato. Chemical emasculation is through selective killing of pollen by use of specific gametocides widely known as chemical hybridizing agents (CHA). Inspite of having a wide range of CHAs of high efficiency, phytotoxicity, female fertility, difficulties in achieving total male sterility and crop/variety stage speacificity however, limit their use. (Naylor 1950, Brauer 1959, Hecker et al. 1972, Perez et al. 1973, Natrova 1973, Parmar et al. 1979, Shao & Hu 1986, Kaul 1988, Ali et al. 1990, Oard et al. 1991, Ali 1993, Horner & Palmer 1995).

Genetic male sterility is of three kinds viz., nuclear gene induced sterility, cytoplasmic-nuclear interaction-based sterility and maternally controlled cytoplasmic sterility, which is maintainable only by vegetative propagation. Male sterility conditioned by nuclear genes is referred to as genetic male sterility (GMS). Governed by one or two mendelian genes, genetic male steriles have been reported in a wide range of crop species and made use of commercially in a few crops like cotton, pigeonpea, sesame, chillies etc., Their success, however, depends on linking the GMS with an easily identifiable seedling marker so that fertile plants (50%) in a test-crossed progeny could be removed at early stages.

Cytoplasmic-genetic male sterility is the widely utilized system for exploitation of hybrid vigour. The sterility factor *S* found in certain cytoplasms, when interacts with the nuclear fertility gene in its recessive state (*rf rf*) results in male sterility. The male sterile line (*S/rf rf*) is maintained by crossing with the maintainer lines (*N/rf rf*); and crosses between male sterile line and pollinator/ restorer line (*S/Rf Rf* or *N/Rf Rf*) result in hybrid (*S/Rf rf*).

The above systems have their own limitations, which include (a) very narrow genetic base for male-sterility-fertility restoration system, (b) lack of either of or both male sterility and fertility restorer gene sources in many a crop, and (c) cumbersome method of seed production. Of various approaches contemplated to overcome these limitations, environment sensitive genic male sterility (EGMS) and genetically engineered male sterility are considered promising. Whereas the EGMS induced by either temperature or day length variation has already assumed field reality in rice in China (Yuan 1992), the approach of engineering into parental lines of chosen crop species, the novel gene (s), which is capable of inducing male sterility/ restoring fertility, would go a long way in extending the technology to many crops, that are hitherto found non-suitable for hybrid breeding. This paper (part I) has been devoted

exclusively to the environment sensitive genic male sterility system. In subsequent paper (part-II), genetically engineered male sterility and prospects of its utilization in commercial hybrid seed production will be discussed.

Environment Sensitive Genic Male Sterility

A plant remaining male sterile in one set of conditions and turning male fertile in another, akin to conditional mutations in bacteria, which are lethal in restrictive environment but viable in permissive environment, may be attributed to the principle that phenotypic expression of a gene(s) is specific to a given environment. Environment-influenced male sterility-fertility transformation is known since long, although it has assumed applied importance only after the discovery and use of photoperiod and temperature sensitive genic male steriles in rice in the eighties in China. Shi (1982, 1985) Rick (1948) and Martin and Crawford (1951) were the first to report on the occurrence of temperature and light sensitive reversible male sterile mutants in tomato and pepper. Subsequently, Rundfeldt (1960) reported isolation of a cabbage mutant that remained male sterile in summer and turned fertile in winter. Among cereals, first report on recovery of such environment-influenced mutants affecting male fertility was in wheat (Jan 1974). Later Ahokas and Hockett (1977) reported the barley mutant 'MS9', which was completely sterile in Finland, while remaining partially sterile in the USA.

Inspite of all such reports on environment-influenced expression of male sterility/fertility in a variety of crop plants, the phenomenon has hardly been exploited for commercial hybrid seed production. It was following the discovery of a photoperiod sensitive male sterile mutant of spontaneous origin in rice by Prof. Min Shong Shi of Hubei Province, China in the early eighties that systematic research was mounted there to develop environment sensitive genic male sterility system as an alternative approach to cytoplasmic-genetic male sterility-fertility restoration system for hybrid seed production (Shi 1981, 1985, Shi & Deng 1986). Consequent upon this discovery and demonstration of its potential in commercial seed production, there has been worldwide interest resulting in the identification of several additional and alternate sources of photoperiod sensitive, temperature sensitive, photoperiod-influenced temperature sensitive and *vice-versa* male steriles in China, Japan, India and at IRRI, the Philippines (Lu & Wang 1988, Yuan et al. 1988, Wang et al. 1990, Maruyama et al. 1990b,Virmani 1992, Ali et al. 1995). They were of either spontaneous or induced origin as detailed in table 1.

Physiological Characterization, Biochemical Basis and Genetics of EGMS

In-depth understanding of the physiology, genetics and biochemical basis of environment sensitive male sterility has been possible so far in rice. Hence, using largely rice as the model crop, this sterility system has been discussed in this paper.

Photoperiod Sensitive Genic Male Sterility

The first EGMS discovered in rice was photoperiod sensitive male sterility (PGMS). The mutant of spontaneous origin isolated from the *Japonica* variety Nongken 58 in 1973 and designated as Nongken 58 S was characterized by complete and stable male sterility under day length of 14hr and above and reverting to fertile phase (10-40% spikelet fertility) during day length of less than 13.75hr (Shi & Deng 1986, Lu & Wang 1988). Recently Dong et al. (1993) have reported yet another PGMS source viz., *'Zhenong 1S'* in the *japonica* varietal group itself. It also remains completely male sterile under long day conditions and turns fertile under short day conditions (<13.25 hr). However, it differs from Nongken 58 S in that it is influenced to an extent by temperature. Under relatively low temperature (25.7°C) conditions percentage fertility increases significantly. This finding necessitated review of the long-held notion that expression of fertility-sterility alteration was affected by change in day length alone and without any

Table 1 *Origin and fertility-sterility transformation behaviour of photoperiod and temperature sensitive male sterile sources in rice*

Source	Varietal Group	Origin	CDL (hr)/ CSP/CFP (°C)	Reference
Photoperiod sensitive male sterile				
Nongken 58 S	*japonica*	Spontaneous, China	14-00-13.45	Shi & Deng 1986
MSR 54A (B)	—	China	14-00-13.00	Lu & Wang 1988
CIS 28-10 S	*indica*	Spontaneous, China	14-00-12.00	Huang & Zhang 1991
26 ZhaiZao	*indica*	Induced (R), China	14-00-12.00	Shen et al. 1994
EGMS	*japonica*	USA	14-00-13.00	Rutger & Chalter 1989
M 201	*japonica*	Induced (?), USA	14-00-12.00	Qard & Hu 1995
Temperature sensitive male sterile				
5460 S	*indica*	Induced (R), China	28.0-26.0	Yang & Wang 1988
IR 32364	*indica*	Induced (R), IRRI	32.0-24.0	Virmani & Voc 1991
H89-1	*japonica*	Induced (R), Japan	31.0-28.0	Maruyama et al. 1990b
Annong IS	*indica*	Spontaneous, China	30.0-27.0	Tan et al. 1990
R 59TS	*indica*	China	—	Yang et al. 1990b
Xinguang	*indica*	Breeding population, China	30.0-24.0	Cheng et al. 1995
26 Zhi Zao S	*indica*	Induced (R), China	23.0-25.0	Shen et al. 1993
5088 S*	*indica*	Introgression from Nongken 58 S, China	30.0-22.0	Zhang et al. 1994
SM 5	*indica*	Spontaneous mutation, India	32.3-22.0	Ali et al. 1995
SM3	*indica*	Spontaneous mutation, India	32.0-22.0	Ali et al. 1995
JP 2	*indica*	Spontaneous mutation, India	33.9-23.0	Ali et al. 1995
SA 2	*indica*	Induced mutation, India	31.7-20.0	Ali et al. 1995
F 61	*indica*	Induced mutation, India	30.9-22.0	Ali et al. 1995
JP 8-1A -12	*indica*	Breeding population,, India	30.9-20.0	Ali et al. 1995
JP 24 A	*indica*	CMS, India	33.8-23.0	Ali 1993
JP 38	*indica*	Spontaneous mutation, India	24.0-30.5	Ali 1993
Dianxin/A	*japonica*	CMS, China	23.0-20.0	Lu et al. 1994
Hennong S	*indica*	Cross breeding, China	30.0-29.0	Lu et al. 1994
IV A	*indica*	Cross breeding, China	24.0-28.0	Zhang et al. 1991

*Several introgressed forms from Nongken 58 S and Annong 1S developed by Yang et.al. 1996 and Mou et.al. 1996 not included here.

CDL, critical day length; *CSP,* critical sterility point; *CFP,* critical fertility point.

influence of temperature. He et al. (1987) were the first to make an in-depth study and report that sterility/fertility transformation of this system could be prone to the influence of temperature as well. Significantly, their findings have been simultaneously confirmed by Zhang et al. (1987) as well. Characterization of PGMS lines of *indica* background such as W 6154 S, W 6184 S, W 7415 S, W 6068 S etc., has indicated that these lines differ in their critical temperature range and their fertility-sterility alterations vary accordingly with day length, as the major factor (Lu et al. 1992, 1994). Although Bi et al. (1990) and Cheng et al. (1990) reported that the PGMS gene of Nongken 58 S was less vulnerable to temperature changes, studies by other workers suggest that this line as well as others sources like Annong 1 S, EIYA 105 S and W 6154 were influenced more by temperature than photoperiod (Wan & Deng 1990, Zhou et al. 1990, Xue & Zhou 1990, Wu et al. 1991, Xue & Shen 1991, Zhang et al. 1993, 1994). More critical study of Nongken 58 S and its PGMS derivative lines under controlled conditions of temperature and photoperiod by Sun et al. (1991) revealed sterility-fertility transformation to occur under certain combinations only of temperature and day length. Under long day (15hr) and high temperature (29^0C) combinations, for instance, they remain sterile and turn fertile under short day (13 hr) and low temperature (23^0C) combination.

Based on 10 year long study of PGMS lines Yuan et al. (1993) reported two photoperiod reactions to govern their growth and development. The first photo-periodic reaction (FPR), which is common in all varieties either accelerates or delays panicle differentiation and heading while the second photoperiod reaction (SPR) determines fertility-sterility of pollen. According to them, SPR requires more critically timed photoperiod, higher light intensity and higher temperature than does FPR. The PGMS lines derived from Nongken 58 S differ in critical light length (CLL), critical temperature point (CTP), temperature range for photoperiod sensitivity (TRPS) and intensity of interaction between temperature range and photoperiod sensitivity, which determine the suitability of a line for commercial seed production. If CFP of a line is not low enough sterility induction would not be complete and thereby it would prove risky in hybrid seed production. On the other hand, if CSP is not high enough multiplication of male sterile line would be difficult under short day-cum-high temperature condition. A line characterized by longer CLL could be used at high altitude regions and the one with shorter CLL in low altitude. Lines characterized by photoperiod and temperature interaction would adapt well to a wide range of conditions, as high temperature would compensate insufficient photoperiod in lower altitudes and longer photoperiod at higher altitudes. Lu et al. (1994) classifies such temperature influenced PGMS lines based on their fertility-sterility response over a range of temperature as under:

(i) Nongken 58 S type: Characterized by relatively low critical sterile temperature (CST) high critical fertile temperature (CFT) and wider temperature range for photoperiod sensitivity (TRPS). This type can be easily multiplied but it is slightly risky to use in hybrid seed production. This include 3111 S, 5088 S, 1541 S, 7001 S etc.,

(ii) Peiai 64 S type: Characterized by low CST and low CFT with narrow TRPS. This type poses no problem in hybrid seed production but its multiplication is difficult. This includes 735 S, HN 52 S, 8906 S, M 901 S, K 14 S etc.,

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CFP, Critical fertility point (Critical temperature required for realizing highest fertility in TGMS lines; *CSP*, Critical sterility point (Critical temperature required for induction of complete male sterility in temperature sensitive genic male sterile (TGMS) lines; *CFT*, Critical fertility temperature (Critical temperature required for interaction with day length for reversion to fertility phase in temperature dependent PGMS lines; *CST*, Critical sterility temperature (Critical temperature for induction of complete pollen sterility in the photoperiod sensitive genic male sterile lines (PGMS)); *CLL*, Critical light length (Critical day length which is sensitive to the temperature effects in PGMS lines.

(iii) 8902 S type: Characterized by high CFT and relatively higher CST. 31302 S, 32001 S, 5047 S, Shuangung S and W 7415 S belonging to this type can be multiplied with ease but hybrid seed production using them would be risky.

(iv) W 6154 S type: Characterized by high CST, low CFT and narrow TRPS. Lines like W6154 S, 8801 S etc., manifest complex thermo-sensitive sterility. Transformation to fertile phase is achievable by photoperiod within TRPS. It can be used more as thermo-sensitive genic male sterile germplasm.

The foregoing types bring out the fact that ideal PGMS lines characterized by low CST, low CFT and wide TRPS are yet to be identified. Nevertheless for commercial seed production a PGMS line should fulfill the following:

- Pollen sterility of sterile plants should be no less than 99.5%
- Period of sterility induction phase should be atleast 30 days in a year.
- Fertility transformation phase be clearly defined.
- Seed setting should be more than 30% during fertile phase.

Even the widely used PGMS lines in China like 5088 S and 7001 S do not meet all these requirements.

Thermosensitive Genic Male Sterility

Annong 1 S is the first ever-identified TGMS source. It is a spontaneous mutant isolated in the Hunan Province, China. The mutant and its derivatives like 5460 S, R 59TS etc., remain sterile under relatively higher temperature (33-28 °C) and turn fertile under low temperature (27-22 °C) (Yang & Wang 1990, Sun et al. 1989). Closely following its discovery, Japanese reported successful induction of the TGMS mutant 'Norin PL 12' in the parent variety Reimei irradiated with gamma rays. The mutant and lines derived therefrom manifest male sterility at 31-24°C and partial fertility at 28-21°C and complete fertility at 26-18°C (Maruyama et al. 1990 a, b, Virmani 1996). Independent efforts by Ali et al. (1995) in India and Virmani and Voc (1991) at the International Rice Research Institute (IRRI) have led to the identification of additional/alternate sources of TGMS of spontaneous and induced origin. Ali et al. (1995) and Reddy (1997) characterized TGMS lines under controlled temperature and light conditions on the basis of CSP and CFP and photoperiod range for temperature activity (PRTA) into three types viz., High CSP-High CFP, Low CSP-Low CFP and Low CSP-High CFP as detailed below and illustrated in figure 1.

Norin PL 12-based IRRI TGMS lines such as IR68945 and Annong 1 S based lines have been found to belong to Type-1 while the induced

	Type	Source	Features
High temperature sterility & low temperature fertility			
1.	High CSP — High CFP (> 32°C — >24°C)	JP 2	CSP of >32°C and CFP of <24°C with no influence of photoperiod
2.	High CSP — Low CSP (>32°C — <24°C)	SM 3, SM 5	CSP of > 32°C and CFP of <24°C
3.	Low CSP — Low CFP (<32°C — <24°C)	SA 2, F61, ID 24, JP 8-8-1 S	CSP of < 32°C & CFP of <24°C with varied PRTA (12.0 to 13.5h) suited to a wide range of areas of low temperature condition
4.	Low CSP — High CFP (<32°C — >24°C)	not found yet	CSP of < 32°C and CFP of >24°C; Rare to find
High temperature fertility & low temperature sterility			
5.	CSP — CFP (<24°C — <32.0)	JP 38	Reverse TGMS with CSP of 24°C and CSP of 30.5°C

TGMS source of IRRI, IR 32364 to Type-2. Many sources alternative to Norin PL 12 and Annong 1 S having desirable CSP/CFP are available. Unlike the normal TGMS lines that are sterile at high temperature and turn fertile at low temperature, a new source such as JP 38 remains sterile at low temperature and fertile at high temperature. Such reverse TGMS has also been reported earlier by Chinese though not as yet used in commercial hybrid seed production (Jiang 1988, Zhang et al. 1991 b).

There is no precise information on how the critical temperature at critical stage of development sensitive to temperature stress is determined and what minimum period of effective temperature treatment is needed for transformation to fertile phase. As for critical temperature, mean of minimum and maximum is taken by some, while that of maximum by others. Intensive study of H 89-1 (Norin PL12), by Japanese scientists reveals the response to be to maximum temperature and not to either mean of maximum temperature or to mean of maximum-minimum temperature (Maruyama et al. 1990b). As regards sensitive stage, they report stamen-pistil primordial initiation stage i.e., 22-26 days before heading to be the stage most sensitive to temperature effect. In the case of Nongken 58 S, the sensitive stage has been reported to be between stamen-pistil primordia differentiation and pollen formation stages and for W 6154 S it is between pollen mother cell formation and single nuclear pollen stages (Zhang et al. 1994, Zhang et al. 1993). Using tracking technique Ali et al. (1995) reported sensitive stage in the indigenous TGMS sources to vary from 24 days before heading as in SM 5 to 17 days in SA 2. In terms of developmental stage, F 61 has been found to be sensitive at stamen-pistil primordia stage, while SA 2 at meiotic stage and ID 24 between stamen-pistil primordia and uninucleate stage of pollen.

Biochemical Basis of Environment Sensitive Male Sterility-Fertility

Investigations to understand the biochemical basis of sterility-fertility transformation have been confined to the PGMS line Nongken 58 S in comparison to the stable fertile parent variety Nongken 58. Although a variety of biochemical parameters, which broadly include content, presence-absence and activity of proteins, enzymes, amino acids, hormones and minerals has been used, no single parameter appears to be the causal factor for the day length dependant sterility-fertility transformation. Whereas Chen and Xiao (1987) have attributed pollen abortion under long day condition to decreased proline content in anthers, Qian and Zhu (1987) relate sterility with reduced peroxidase activity and significant changes in the activity of Pox, Cox and esterases in the post microspore mother cell formation phase. Electrophoretic pattern of protein of panicle samples at pistil-formation stage by Cao et al. (1987) reveals appearance of 15 additional bands in lieu of disappearance of an equal number of bands at sterile phase in Nongken 58 S as against 15 additional bands and loss of 9 bands in normal Nongken 58. Observations on carbohydrate level (starch, sucrose and reducing sugar) with changing phases of sterility and fertility by Wang et al. (1990) suggest that impaired transport of carbohydrates from leaves to stamens under long day condition could be the reason for induced male sterility. Yang and Zhu (1987) trying to relate reversible sterility-fertility phenomenon to minerals have shown the increasing level of Ca, Mn, K and Ti in short day-treated resulting in fertile phase as against their decreasing level in long day-treated resulting in sterile phase. Also, their study revealed fluctuating amounts of Na, K and Zn on the surface of pollen grains under different photoperiod treatments to be related to pollen abortion. The role of hormonal balance as related to sterility-fertility was also studied and showed that accumulation and depletion of IAA were attributable to day length-dependant fertility and sterility (Xu et al. 1990, Yang et al. 1990, Luo et al. 1993). Changes in the equilibrium among hormones like GA, ABA and ZT, which appeared to be the cause of sterility-fertility, were the result of IAA

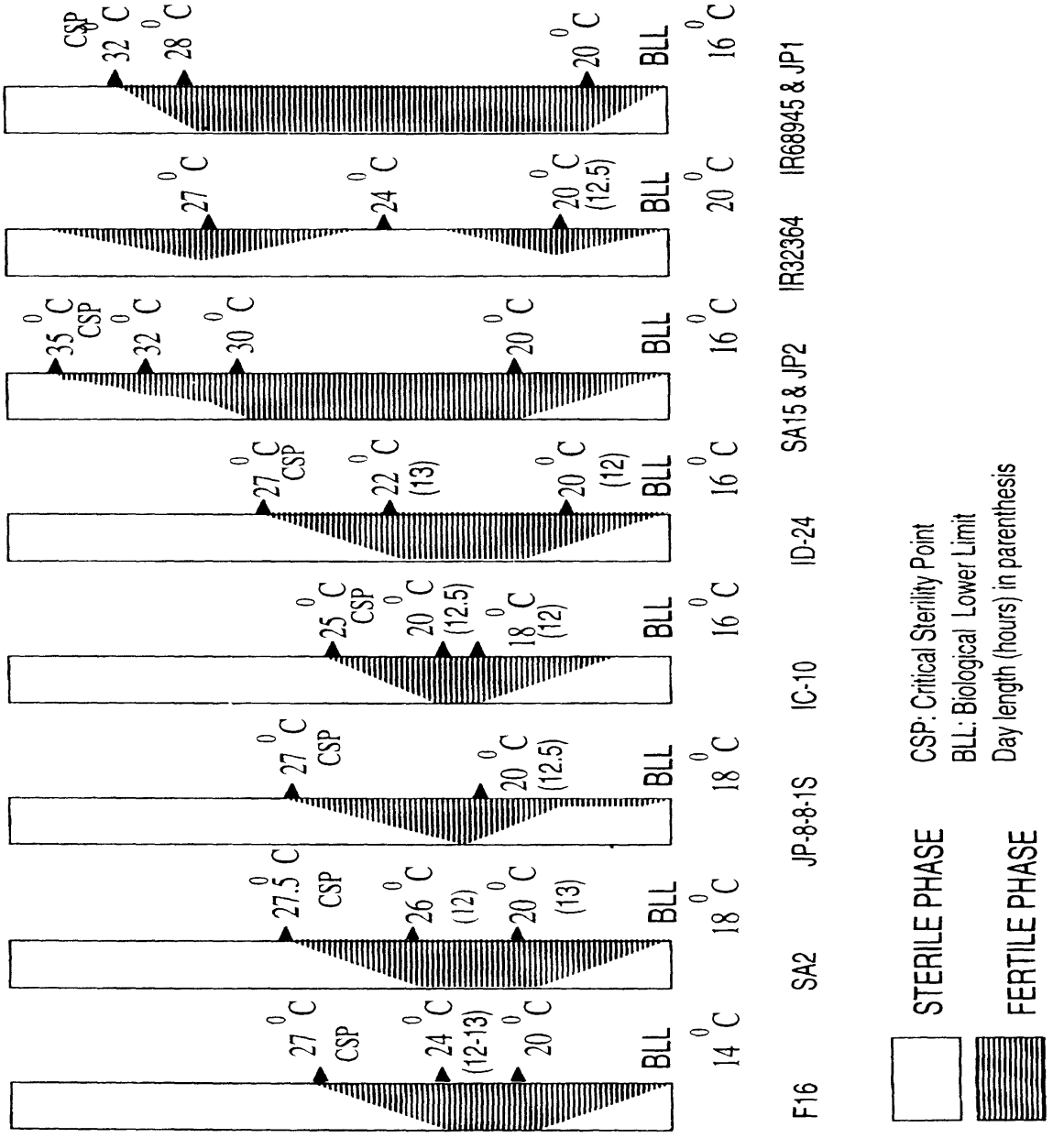

Figure 1 Mode of fertility alteration in diverse TGMS lines

deficiency. Luo et al. (1993) related higher ethylene release rate of young panicles during long day conditions to pollen sterility. This was confirmed from restoration of fertility following treatment with amino ethoxy glycine (AEG), an inhibitor of ethylene increase. Influence of temperature on ethylene release rate was in line with their model of light-temperature action in fertility change. Findings of Yang and Zhu (1990b) suggested that a low molecular weight RNA fraction, observed in plants under long day condition, might be associated with fertility transformation. By and large biochemical basis of day length-induced sterility-fertility transformation appears quite complex and it is not yet clearly understood whether it would be the same with temperature-influenced transformation as well. In the case of cytoplasmic-genetic male sterility system pollen shedding often experienced across crops could be the manifestation of breakdown of sterility attributable to environmental variation and more specifically fluctuation in temperature. The phenomenon influenced by either temperature or photoperiod or combination of both might have the same bio-chemical basis. However, it needs to be confirmed. The knowledge so gained would help induce and preacise manner complete sterility in the most.

Reverse Temperature Sensitive Male Sterility

It is just opposite of normal TGMS sources like Annong 1 S and Norin PL 12, wherein high temperature induces male sterility while low temperature restores fertility. Dian-xin 1A reported for the first time of its kind behaves just opposite of the normal TGMS by remaining sterile under low temperature (24°C) and fertile under high temperature (27°C) (Jiang 1988). Recently Chinese scientists have developed yet another reverse TGMS viz, 'IVA', which is similar in its response to Dian-xin-1A (Zhang et al. 1991b). They have been reported to remain male sterile at 22-24°C and fertile at 27°C. JP 38 is one more addition from India to the reverse TGMS source. (Ali 1993). Isolated as a spontaneous mutant in a farmer's field at Katrain in Himachal Pradesh, it is characterized by complete male sterility at temperature below 24°C and very high pollen and spikelet fertility above 30.5°C. Mutant genes of this kind can be profitably utilized by undertaking hybrid seed production at high altitude low temperature regions while seed multiplication of reverse TGMS in the plains of high temperature.

Environment-influenced Cytoplasmic-Genetic Male Sterility

One of the problems often encountered in hybrid seed production in 3 lines approach is pollen shedding in some of the male sterile lines under certain environments. This kind of instability in male sterile lines is largely on account of temperature extremes. It amounts to saying that genes involved in nuclear-cytoplasmic interaction inducing male sterility could also be vulnerable to reversion to fertile phase due to the influence of environment change. Such reversible phenomenon could be as a result of weakening of the interaction with environment change influencing either nuclear (*rf*) or cytoplasmic (*ms*) genes. Differences in the level of stability among male sterile lines, which are based on a common sterile cytoplasm, tend to suggest that nuclear background is largely contributing to such differential stability.

Cytoplasmic-genetic male sterile lines in crops like *Capsicum, Pearl millet, rye, Secale* etc., manifest enhanced percentage male sterility following low temperature treatment during premeiotic stage and reduced level of sterility following treatment with high temperature (Martin & Crowford 1951). In grain *Sorghum*, high temperature (40°C) treatment at premeiotic stage has been reported to show high degree of male fertility. (Kongtian & Hongyi 1981, Zhang & Fu 1982). Such instances of reverse response on CMS lines at low and high temperature regimes have been reported in many more crops, which include besides, petunia, sorghum etc., (Duvick 1966, Hayase

et al. 1969, Izhar 1975, Sawada & Kinoshita 1977, Murty 1997). In rice, sensitivity to low temperature has been reported to be maximum at microspore release stage, when *ms/rf* genes interact (Satake & Hayase 1974), while exposure to high temperature (35-41^{0}C) at anthesis induces pollen sterility (Satake &Yoshida 1977) in majority of the CMS lines. Certain temperature regimes either suppress or delay the action of *rF* genes in the CMS lines of crops such as onion, alfalfa, pearlmillet and rye resulting in the breakdown of male sterility. Recently Murai et al. (1992) of Japan have reported all alloplasmic lines of Norin 26 (*Triticum aertivum*), especially *Aegilops crassa* cytoplasm to express male sterility under long day (15 hr) and fertility under short day (14.5 hr) conditions, interestingly with no influence whatsoever of temperature. Such a system has been designated as photoperiod sensitive cytoplasmic male sterility (PCMS). This finding is indicative of the fact that *ms/fr* interaction could be influenced as much by photoperiod as temperature extremes. Unlike nuclear gene-based temperature sensitive male sterility (TGMS) sources, which are already in commercial exploitation in rice in China, environment sensitive CMS lines could dispense with cumbersome seed production of male sterile lines by taking advantage of temperature or/and photoperiod-triggered partial reversion to fertile phase by raising male sterile lines in appropriate season/location, which facilitate with desired temperature/day length range for reversion. It appears that a distinct possibility exists in sorghum (Murty 1995, 1997) and maize (Zwngyum & Zong-long 1997). In sorghum, unlike the widely used 'milo' cytoplasm-based male sterile lines, which are stable over varied environments, behaviour of the A-2 cytoplasm-based CMS lines has been found to vary with the environment. Like the reverse TGMS in rice, these lines remain completely male sterile during winter months and turn fertile to different degrees during summer months. The safe period for using a line as female is as long as 60 days. The National Research Center for Sorghum at Hyderabad maintains as many as 11 lines that show total sterility in winter and total fertility in summer (table 2).

In case of maize, Chinese scientists are exploiting commercially the environment, especially temperature-influenced fertility-sterility system in hybrid seed production (Zwngyum & Zong-long 1997). The line Qun-6-ms for instance, remains sterile under high temperature and becomes fertile under low temperature. The critical point of sterility-fertility conversion has been reported to be around 24.8^{0}C daily mean temperature. Though it is mainly a temperature sensitive male sterility system with percentage sterility ranging between 67 and 100, the fact that it is influenced to an extent by day length, could be taken advantage of for ensuring 100% male sterility by subjecting the line to longer sun shine hours between planting and tasseling period. Unlike in rice, there is no intensive study in sorghum, maize and wheat to determine the critical sterility-fertility points, sensitive stage, locations and precise periods for commercial production of CMS and hybrid seed. Such information would greatly help make the hybrid breeding 2-line approach a viable technology.

Genetics of Environment Sensitive Male Sterility

Genetic analyses of all known sources of PGMS and TGMS reveal them to follow simple mode

Table 2 Promising temperature sensitive A-2 cytoplasm-based CMS lines in Sorghum

CMS line	origin	Pedigree
St 26	Paired back-crossing	CKPA 2 x MR 750
St 28	"	CKPA 2 x MR 840
St 29	"	CKPA 2 x SB 1085
St 30	"	CKPA 2 x CS 3541
St 32	"	CKPA 2 x SPV 918
St 34	"	CKPA 2 x ICSR 161
St 37	"	CKPA 2 x ICSR 162
St 38	"	CKPA 2 x ICSR 165
St 40	"	CKPA 2 x ICSR 172
St 49	"	CKPA 2 x RS 79

Source: Murty et al. (1999)

of inheritance governed by one or two major genes in recessive state. Also, skewed distribution of segregating populations does not, however, rule out the role of minor/ modifier gene complexes. Study of the PGMS Nongken 58 S by Mei et al. (1990) suggests two pairs of independent genes in recessive state to govern the sterility behaviour. Designating these genes as ms_1^{Ph}, ms_1^{Ph} ms_2^{Ph}, ms_2^{Ph}, they opined that one of the pairs could be photoperiod sensitive gene '*ms*', while the other fertility differentiating gene '*Fd*'. It appears from the study of crosses involving diverse genotypes that '*Fd*' genes could remain in varied numbers. Postulation of '*Fd*' genes helps in explaining meaningfully the varied distribution patterns of segregating populations for photoperiod sensitive male sterility. Studies subsequently of other PGMS sources like MSR 54A also reveal sterility behaviour to be governed by similar pair of recessive genes as reported in Nongken 58 S (Lu & Wang 1988). The mode of inheritance of sterility behaviour of CIS 28-10 S, a true photoperiod sensitive male sterile absolutely free from the influence of temperature, has been reported to be different by being governed by a single dominant gene, however, in association with minor genes that modify its effect (Huang & Zhang 1991, Huang 1991). It appears that only one major gene either in dominant or recessive state, is governing the photoperiod sensitive male sterility and dominance and recessiveness of the trait as reported by two different sources, could mean them to be non-allelic, though direct evidence on their allelic relationship is yet to be provided.

As for the temperature sensitive male sterility, studies carried out by Maruyama et al. (1990b) on the behaviour of H 89-1 show a single recessive gene to control it. Borkakati and Virmani (1996) reported that TGMS trait in the lines based Norin PL12 and IR 32364 is governed by a single recessive gene. Intensive study of the newly identified TGMS sources by Ali et al. (1995) along with the Chinese, Japanese and IRRI sources suggests all of them to be monogenic recessive. Their differential expression although depended on the genetic background to an extent, perceptible change in the expression appears to be characteristic to TGMS gene, as CSP/CFP differs depending on the level of influence of presumed '*Fd*' genes/ modifier complexes. Study of allelic relationships of all the available TGMS sources suggest four putative genes to confer male sterility. (Reddy 1997, Reddy et al. 1998). The nomenclature of thus identified genes and their sources are as under.

Gene symbol	TGMS source
tms 1	ID 24, JP1, 5460S (Annong S1)
tms 2	SA, 2 IR68945 (Norin PL 12)
tms 3	JP 8-8-1S, IC10, IR32364
tms 4	F61

Genetic investigations beyond suggesting that all the forms of environment sensitive genic male sterility are simply inherited in association with or without modifier gene complex and on the existence of non-allelic sources, have not as yet resolved (a) if PGMS and TGMS genes are different from genetic male sterility genes and nuclear fertility/ sterility (*rF*/*rf*) genes which in interaction with cytoplasmic gene (*ms*) induce male sterility, and (b) genetic basis as to how temperature sensitive sterility genes are influenced by photoperiod sensitive sterility genes and *vice versa*, warranting more systematic indepth study to precisely understand and exploit this apparently simple but really complex system.

Molecular Mapping of EGMS Genes

High-density molecular maps now available in some of the crop species, especially in rice have become handy to map and tag gene(s) of interest. In identification of desired genotypes/ recombinants in germplasm/segregating populations, possessing traits that offer difficulty in selection at phenotype level under field condition, tagging them with specific/ flanking molecular markers is being found increasingly rewarding. Identification of TGMS or PGMS lines through conventional means is quite laborious and time consuming. Through RFLP analysis of appropriate mapping

populations Zhang et al. (1993) could locate PGMS *loci pms 1* and *pms 2* respectively on chromosomes 7 and 3. Marker-based analysis shows that relatively the effect of *pms 1* to be higher than *pms 2* and dominance is nearly complete at both the *loci*. Wang et al. (1995) using bulked segregant analysis of the F_2 populations of the crosses involving the TGMS mutant 5460 S identified 1.2 kb fragment amplified by primer OPB 19 mapped on chromosome 8 to be linked with the TGMS gene *tms 1*. The marker was found to be 6.7 cM away from *tms1*. Subudhi et al. (1995, 1996) studying the mapping population of the cross IR 32364/IR68 employing RAPD technique have reported five markers generated by five primers viz., OFP18, OFB19, OPAC1, OPAC3 and OPAA7 to be putatively linked to the TGMS gene. Of these, all except fertility specific OPAC 3-640 have been found to be sterility specific. They also reported that *tms2* and *tms3* genes are located on different chromosomes. Reddy (1997) using appropriate mapping populations while confirming that 1.2 kb amplicon of primer OPB 19 to be closely linked to *tms 1* has shown it to be same as in the TGMS ID 24, JP 1 and IR 68945 meaning thereby their allelic nature. Also, he has reported that 0.74 kb amplicon of OPA 12 as well as 2.3 kb and 1.9 kb bands generated by OPS1 to be specific to the TGMS SA 2 (*tms 2*), which is non-allelic to the former. Fine mapping of all the 4 putative genes underway in India and China leading to the development of marker-assisted selection would greatly help as potential breeding/ selection tool for speedy development of diverse PGMS and TGMS lines for commercial use in hybrid rice seed production in the subtropical and tropical regions of the world.

Development and Use of EGMS on Commercial Hybrid Seed Production

Following the discovery and characterization of diverse sources of photoperiod and temperature sensitive genic male sterility systems, the research pursuit has been in developing commercially usable PGMS or TGMS lines using stable gene sources. Besides high combining ability for yield, vigour, high out-crossing potential and disease pest resistance, the following traits are considered important for making these lines ideal:

- Male sterile plants 100% in PGMS/TGMS lines
- Pollen sterility > 99.5%
- Well defined conditions for fertility-sterility alteration
- A minimum of 30 days in a year for inducing complete male sterility
- Seed setting not less than 30% during fertile phase
- Low critical fertility point and high critical sterility point and wide TRPS for safe hybrid seed production and sterile line multiplication.

In the Indian subcontinent, especially in south, where no distinct day length differences are available for making use of PGMS, it is preferable to take advantage of wide temperature range through TGMS sources. Unlike the PGMS, wherein the phenomenon of fertility-sterility transformation is greatly influenced by temperature, TGMS is less affected by day length. An ideal TGMS for field level exploitation would be one that combines the following:

- Low critical fertility point (20-24^{0}C) and low critical sterility point (30-32^{0}C)
- Synchronous and uniform flowering
- Seed setting exceeding 30% in fertile phase
- Favourable floral traits for high percentage out-crossing
- High combining ability for yield vigour.

Many of the TGMS lines now under development based on Norin PL12 of Japan/ IRRI, Annong S 1 of China and SA 2 of India combine most of the desired agrobotanic traits including ideal plant type, desirable maturity range (125-135d) and acceptable grain quality.

Having developed promising TGMS lines, efforts have been for identification of appropriate areas and seasons for large-scale commercial production of TGMS and hybrid seed. On the basis of analysis of about 50 year meteorological data Ali (1999 pers. comm)

could successfully identify areas and ideal periods for seed production in different parts of the country. Places generally located between 500 and 700m above MSL have been found to be highly suitable during the period May-September for hybrid as well as TGMS seed production. Through experimental validation the authors and their colleagues have identified the following as ideal locations for commercial seed production.

Hybrid seed production—	Aduthurai, Trichy, Killikulam, Madurai, Karnal, Delhi
TGMS seed multiplication —	Aduthurai, Gudalur, Samalkota, Karnal,
Hybrid seed production & TGMS seed multiplication —	Aduthurai, Samalkota

In the choice of place either in hills or coastal plains or interior plains and plateau regions, extra care needs to be taken to ensure that the temperature range is < 40^0C->16^0C, beyond which physiological sterility occurs.

Advantages and Pitfalls in the Use of TGMS System

Exploitation of hybrid vigour by TGMS based two-line breeding has several advantages over the CMS based three-line breeding and among them the following are important.

(i) Hybrid seed production is less cumbersome as TGMS system requires no maintainers

(ii) High probability of identifying heterotic hybrids as it readily facilitates use of any well-combining genotype as the pollinator parent and

(iii) Avoidance of negative effects associated with sterility-inducing cytoplasm.

Among the pitfalls, the most important is the adverse effect of temperature fluctuations due to sudden/unforeseen local weather changes. During hybrid seed production at high temperature locations sudden drop in temperature (less than CSP) can prove disastrous, due to reversion to fertile phase resulting in selfing in the female parent i.e., TGMS line. Hence care must be taken to use TGMS lines of low CSP and low CFP. Equally important is identification of temperature stable locations based on several years of meterological data. Further, the high temperature regime be prolonged for a minimum of four weeks during the sensitive phase. Likewise higher temperature (more than CSP) during TGMS seed multiplication at locations of low temperature can result in reduced percentage seed set affecting seriously seed yield. Adverse effect of low temperature during hybrid seed production can be partially minimised by application of gametocides like Ethyl 4'fluorooxanilate, and sodium methyl aresenate on the female parents. Similarly effect of high temperature can be reduced considerably by use of fertility reverting chemicals (FRC) like IAA (100ppm) Brassinolide (1ppm) (BR) during TGMS seed multiplication. Aside chemical remedies, real solution to these problems lie in developing stable TGMS lines adapted to reasonable fluctuations in temperature during hybrid seed production and TGMS seed multiplication. Rather than using strictly temperature sensitive sterility system, it is desirable to prefer slightly photoperiod-influenced TGMS lines, as they are found to be flexible to temperature fluctuations with least effect on fertility-sterility expression.

Breeding Approaches for Development and Use of TGMS/PGMS Lines

An effective breeding methodology involving hybridization and selection under appropriate natural and controlled temperature/day length conditions is being practiced in China for development of commercially usable TGMS and PGMS lines (Yuan 1992 Lu et al. 1992, Mou et al. 1998). The stepwise approach is as given in table 3. The method consists of intensive selection in the F_2 and F_3 generations of crosses of TGMS or PGMS with target parent under long day/low temperature and short day/high temperature conditions, planting of breeding material in summer at higher altitudes where, mean temperature is low ($\simeq$ 24°C) at the sensitive stage for fertility alteration, selection at flowering stage of plants with desirable

Table 3 *Selection procedure for TGMS and PGMS lines*

Step	Year	Environment	Method
1.	1st	Any	PGMS or TGMS Target Parent
2.	—	Any	F_1
3.	2nd	Long day/low temp.	F_2-selection for male steriles
4.	—	Short day/high temp.	Ratooning of selected F_2 sterile plants *
5.	3rd	Long day/low temp.	F_3-selection under climate-room for sterile plants
6.	—	Short day/high temp.	Ratooning of selected F_3 sterile & fertile plants
7.	4th	Short day/ low temp.	F_4
8.	—	Long day/high temp.	F_5-study of steriles for genetic stability
9.	5th	Short day/low temp.	F_6- Multiplication
10.	—	Varied photoperiod-temp. combinations in phytotron	F_7-Formal identification of true breeding PGMS/TGMS

* Anther culture and study of anther derived lines from this till final identification at the 10th stage.

Source: Mou et al. 1998.

agronomic features and transplanting the ratoons of the selected plants under short day/ high temperature conditions at a low altitude region. The sterile plants selected under long day/low temperature when ratooned, under short day/higher temperature conditions are found to comprise two types one reverting back to fertility and the other remaining as sterile. The former could be PGMS and the latter TGMS.

Several commercially utilizable PGMS and TGMS lines have been developed adopting the above described breeding model in both *indica* and *japonica* backgrounds in China (table 4). Among them W 91607S, W 9461S, W 9593S etc., characterized by lower critical temperature point are being used in the development of two-line hybrids. Similar efforts underway at IRRI and India have led to the identification of several promising Norin PL12 source-based TGMS lines (table 5). By and large the lines now in the advanced experimental stage at IRRI and in India showing high percentage spikelet fertility during fertile phase are capable of giving seed yields in the range of 2 to 4 t/ha. Unlike PGMS, TGMS lines are highly sensitive to temperature fluctuations and they require special treatments such as "cool-water continual irrigation device" in some parts of China (Zhou & Liu 1993).

PGMS system has been used for production of experimental and commercial rice bybrids in China, where day length differences are quite distinct while prospects of TGMS system are being explored in typical tropical situations as in India and the Philippines, where day length differences are marginal, but temperature variations are distinct. The male parents characterised by synchronous flowering with TGMS/PGMS lines, high combining ability for yield, acceptable quality and disease-pest resistance have been chosen among both promising restorers identified for 3-line breeding and elite breeding lines in the conventional breeding programme. Commercial hybrid seed production is done at locations/ periods, where/when temperature/day length ideally facilitate expression of complete male sterility in TGMS/PGMS lines. Under ideal management conditions as high as 3.0-4.5 t/ha of seed yield has been obtained in China. Some of the commercialised two-line hybrids have been

Table 4 *Elite PGMS and TGMS lines developed in China*

Name	Subspecies	EGMS	Donor Source
N 50889	*Japonica*	PGMS	HPGMR
7001S	*Japonica*	PGMS	HPGMR
Peiai 64S	*Indica*	P(T)GMS	HPGMR
GD 2S	*Indica*	TGMS	HPGMR
KS 1S	*Indica*	TGMS	HPGMR
6442S	*Indica*	TGMS	HPGMR
Shuguang 612S	*Indica*	TGMS	HPGMR
W91607S	*Indica*	TGMS	HPGMR
W9451S	*Indica*	PGMS	HPGMR
3418S	*Indica*	PGMS	HPGMR
1103S	*Indica*	PGMS	HPGMR
Anxiang S	*Indica*	TGMS	Annong S
Xiang 125 S	*Indica*	TGMS	Annong S
9201	*Indica*	TGMS	560 S
HS-1	*Indica*	PGMS	HPGMR

Source: Lu et al. 1998

Table 5 *Some of the promising TGMS lines developed at IRRI, the Philippines*

TGMS	Parentage	Days to 50% flowering	Plant height (cm)	Spikelet fertility%	
				Sterile phase	Fertile phase
IR 68945	Norin PL12/IR 36	78	112	0	72.8
IR 68949	Norin PL12/BG 90-2	96	108	0	81.7
IR 71018	Norin PL12/IR 4683013	88	98	0	80.6
IR 68294	Norin PL12/IR 62829B	83	125	0	67.8
IR 68297	Norin PL12/IR 9761-61-1R	88	123	0	85.8
IR 68948	Norin PL12/IR 47686-17-2	82	102	0	77.3

Source: Lu Xinggui et al. 1998

Table 6 *Some of the commercialised two-line hybrids in China*

Two-line hybrid	Parentage	Subspecies
70 You 9	7001S/Wanhui	*Japonica*
E-Jing-Za No.1	N 50885/R187	*Japonica*
Hua-Jing-Za No.1	N 50885/1514	*Japonica*
Liang-You-Pei-Te	Peiai-64S/Teqing	*Indica*
Pei-Za-Shan-Qing	Peiai 64S/Shanqing	*Indica*
Pei-Liang-You 288	Peiai 64S/288	*Indica*

Source: Lu et al. 1998

reported to yield 5-10% more than the popular three line hybrids (table 6) (Wang & Li 1992, Luo et al. 1994, Wang et al. 1995, Chen et al. 1996).

One of the advantages of EGMS based two-line breeding is its amenability to exploit inter-subspecific (*indica/japonica*) hybrids, which have distinct yield edge over the intra-subspecific (*indica/indica and japonica/japonica*) hybrids by introducing into one of the parents the wide compatibility (WC) gene, which neutralizes intersubspecific F_1 sterility, a serious hurdle being experienced by breeders all along. Breeders in China and elsewhere have successfully recombined the WC genes ($S\text{-}5^n$) including a non-allelic one from the Indian source Dular into many of the restorer and advanced breeding lines, which can be readily used in the development of commercial inter sub-specific hybrids.

Conclusion

By virtue of its several advantages over the traditional cytoplasmic-genetic male sterility system, the environment sensitive genic male sterility would be an ideal alternative for effective exploitation of hybrid vigour in rice and other crops. Nevertheless, sustainable use of this system on a commercial scale would require further refinement of the system, keeping in view the impending global warming, the effect of temperature-light interaction and the influence of not very well understood *Fd* and other minor gene complexes on the stability of fertility-sterility reversion. The following are some of the areas, warranting more indepth study.

-Streamlining of breeding methodology for development of PGMS and TGMS lines.

-Precise characterization of all the usable PGMS and TGMS lines derived from different sources for the benefit of users in the seed industry.

-Development and use of molecular markers for improving breeding/selection efficiency.

-Development of EGMS system for developing more productive inter-subspecific hybrids.

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