2015, Horticulture for Nutrition Security *Pages* ***385–410***
Editor: **Prof. K.V. Peter**
Published by: **DAYA PUBLISHING HOUSE, NEW DELHI**

Chapter 18

Environment-Sensitive Male-Sterility in some Food Crops

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Flowering is an indispensable phenomenon of natural reproduction. To enter in the reproductive phase, most plant species get critical signals from different environmental factors which facilitate the conversion of their vegetative buds into reproductive tissues. The major factors that influence the appearance of flowers are moisture stress, temperature, photoperiod and irradiation. According to Bernier *et al.* (1993) these factors are perceived by different plant parts. For example, leaves are affected by photoperiod and irradiation; while temperature is perceived by all plant parts. Similarly, vernalization affects shoot apex and moisture availability is perceived by roots. These external determinants do not act independently and their interaction is inevitable and a particular factor may alter or substitute the direct effect of the other factors; and this may change the threshold level of individual factor in inducing flowering. Since at a given point of time,these environmental factors may act on different parts of the plant in their own way, the interaction among their gene products (proteins) may decide the transition of the vegetative buds into reproductive buds. Once flowering is induced in the plants, the further development of reproductive parts leads to differentiation into male/female gametes which participate in the natural reproduction. In this processes also, certain environmental factors play an active role and any departure from the normal process of microsporogenesis/ megasporogenesis leads to disorders such as male or female sterility.In this chapter the authors have not attempted a thorough review of male sterility systems of food cops but restricted to environment-sensitive male sterility with brief description of

their origin, possible variants, maintenance and utilization in two-parent hybrid breeding.

Basics of Male Sterility Systems in Plants

Any biological abnormality during microsporogenesis in plants,caused by either natural or artificial factors,leads to the appearance of male sterility in their flowers. Such events do not allow plants to reproduce through normal sexual mating. J. G. Kolreuter, a German scientist, is credited for recording the first ever incidence of male sterile plants while studying hybrid progenies of crosses involving different *Nicotiana* species in the middle of 18th century (Roberts, 1929). Darwin (1877) postulated that the loss of reproducing ability of plant helps in evolution and enhanced adaptation through gene transfer from diverse related and unrelated individuals through cross pollination.

The emergence of male sterile plants in nature is through spontaneousmutation (mostly recessive)of nuclear fertility alleles, it is popularly called as**'genetic male sterility'**. These abnormal plantsare exposed as homozygous recessive (*msms*) in the self-pollinated progenies of heterozygote (*Msms*) individuals.Generally, such mutants appear in low frequency and are often lost in the self-pollinated crops. However, in both cross- as well as partially cross-pollinated crops, therecessive mutant allele is protected by its dominant male fertile counterparts as heterozygotes.The other form of male sterility, commonly called as **'cytoplasmic male sterility' (CMS)** , is caused by certain deleterious interaction between nuclear and cytoplasmic genomes.In these genotypes the male sterility is conditioned by the factors housed in the cytoplasm and it is always inherited through maternal parent. These genotypes are maintained by the individuals carrying fertile or normal cytoplasm and identical nuclear genes. In certain cases the CMS genotypes with sterile cytoplasm,carry male fertility restoring dominant nuclear gene (*FrFr*) that makes the plant male fertile. The male sterile plants carry the corresponding recessive nuclear alleles (*frfr*) and sterile cytoplasm. These types are designated as '**cytoplasmic nuclear (or genetic) male sterility'**

In 20th century, the male sterility systems were reported in a number of plant species (Kaul, 1988) of economic importance. The evolution of the concept of hybrid vigour by Shull (1908) and subsequently by others, helped in understanding the potential benefits of male sterility in enhancing productivity of crops. Stephens (1937) for the first time utilized male sterility in hybrid seed production in sorghum. At the same time Jones and Emsweller (1937) also demonstrated its use in hybrid seed production of onion.These developments triggered male sterility based commercial hybrid breeding programmes in a number of food and horticultural crops. During this period massive research projects were undertaken globally at a number of plant breeding institutes to understand different types of male sterility systems with respect to their physiology, biochemistry, and genetics.

Male sterility in the plants can arise due to reasons such as defective growth anddifferentiation of anthers, impaired microsporogenesis, and failurein the release ofmature pollen grains and/or inability of pollen grains to germinate on stigma. In general these abnormalities do not adversely affect female reproductive system; and if such plants are pollinated they produce normal seeds.Based on the type of defects

in androecium, the male sterility systems have been classified by Kaul (1988) as structural (absence or deformity of anthers), sporogenous (defective microsporogenesis), and functional (failure of mature pollen to germinate). In addition, on the basis of genetic control mechanisms, it has also been classified as genetic, cytoplasmic, and cytoplasmic nuclear (or genetic) male sterility.

Genetic Male Sterility

It is the most common form of reproductive abnormality found in both monocots as well as dicots coveringover 300plant species (Kaul 1988). In this system the male sterility is controlled by nuclear genetic factors with no influence of cytoplasmic. According to Kaul (1988) in most cases genetic male sterility is controlled by one or two pairs of recessive alleles. However, there are a few exceptions where male sterility is controlled by dominant genes.Theserecessive mutant male sterile plants generally arise spontaneously, and are lost if not maintained as heterozygotes (*Msms*). In cases where male sterility is controlled by dominant allele, its maintenance through reproductive means is difficult.

Cytoplasmic Male Sterility

It arises due to the presence of abnormal (defective) mitochondrial genome. Such cytoplasmis designated as 'sterile' (S); and it can arise spontaneously or through wide hybridization. These male sterile plantsdo not produce pollen grains because their nucleus contains a pair of recessive non-restoring (*msms*) alleles. The cytoplasmic male sterility is maintained by the genotypes which carry 'fertile' (F) or 'normal' (N) cytoplasm and non-restoring recessive nuclear alleles. Over 150 plant species have been reported to carrythis type of male sterility (Kaul 1988).This male sterility system cannot be used for field crops due to difficulties in producing large quantities of hybrid seed; but it is suitable for horticultural crops where seeds are non-commercial entity and only fruits are consumed.

Cytoplasmic Nuclear Male Sterility

This male sterility system is the most popular as far as its utility in plant breeding is concerned. In this system the expression of male sterilityis conditioned by a strong interaction between cytoplasmic and nuclear genomes. The fertility restoration of this male sterility mechanisms is controlled by dominant nuclear genes and depending on the type of fertility restoring gene, the expression of male fertility restoration could be total or partial. The hybrid technology based on this male sterility is popularly called as "three-line hybrid system" and include 'A' line-the male sterile female line with 'S' cytoplasm and recessive fertility nuclear alleles (*frfr*),B-line (maintainer of the female parent) with fertile 'N' cytoplasm and recessive nuclear alleles (*frfr*). This line when crossed with 'A' line, the entire progeny is male sterile. The third parent(designated as 'R' - line) contains dominant fertility restoring gene (*FrFr*). This line has ability to restore the male fertility of the hybrid plants produced by crossing with 'A' line.

Environment–Induced Male Sterility

This male sterility system is unique and has a recent identification. In this case the expression of male sterility/fertility is determined by nuclear genes whose

expression is controlled by specific environmental factors; of these, temperature and photo-period are the major male sterility inducing agents. These two factors may or may not influence the plants independently. It is evident that every gene conferring male sterility in the plant kingdom is not prone to environmental changes and only few environment - sensitive gene(s) have so far been reported; and these are present in both genetic as well as cytoplasmic nuclear male sterility systems. In certain cases the 'converted fertile' plants revert back to male sterility under conducive environments.

Genetic Control of Male Sterility Systems

The molecular factors determiningCMS is still unclear. Studies have shown that its expression is linked to certain rearrangements of mitochondrial genome and consequently specific toxic proteins are produced that inhibit fertility restoration. In fact a number of explanations have been put forward from time to time, but still the genetic basis of this male sterility has not been properly understood in most crops. Recent studies have shown that the male sterility is associated with chimeric mitochondrial ORFs (open reading frames). Wang *et al.* (2006) demonstrated that in rice the ORF encodes a cytotoxin peptide which determines the expression of male sterility. Iwabuchi*et al*. (1993) showed that an abnormal copy of a mitochondrial gene produced aberrant mRNA transcripts containing an additional ORF. Hanson and Bentolila (2004) reported that male sterility may also be associated with alterations in promoter regions and portions of coding regions of mitochondrial ATP synthase. The genomic studies on mitochondria of A_4 cytoplasm in pigeonpea recognized 13 ORFs which can trigger male sterility (Tutej*aet al*. 2013). Further, Pallavi Sinha (unpublished) recorded10 bpdeletion in *nad7a* gene that was responsible for producing male sterile plants in pigeonpea.

Fertility Restoration of Male Sterility

The restoration of male fertilityin the hybrids involving cytoplasmic nuclear male sterile lines is an integral part of hybrid breeding programmes. Once an 'R' line is crossed with 'A' line, the dominant *Fr* nuclear gene of 'R' line overcomes the ill-effects of defective mitochondrial genome in the hybrid plant. According to Kaul (1988) the *Fr* gene produces certain proteins which repair the damage and make the hybrid plant male fertile. In most crops the pollen fertility is controlled by one or two dominant genes (Kaul, 1988). Saxena *et al.* (2011) reported that in pigeonpea two dominant genes were responsible for fertility restoration and the hybrids with a single gene were also fertile but they produced small quantity of pollen and exhibited instability over diverse environments. Similarly in maize also, four fertility restoring genes are reported and of these, two are major genes, and the other two genes yieldonly partial restoration (Wise *et al.*, 1999). Further, it was also reported that one of the major fertility restoring gene reduced sterility causing protein by 80 per cent (Kennel *et al.*, 1987).The fertility restoration has also been associated with genes encoding pentatrico peptide repeat proteins (Hanson and Bantolila, 2004).

Origin of Male Sterility Systems

Natural Occurrence

Nature has provided unlimited variability and in the past it has yielded a number of economic traits in different crops. There are numerous examples of it and among these, various male sterility systems are unique and these have benefitted millions through the cultivation of high yielding hybrids. Genetic male sterility arises due to mutation of male fertility nuclear gene (*MsMs*) to its recessive form to produce heterozygote (*Msms*) individuals. Self-pollination of such plants reveals male sterile segregants. Under natural conditions in the self-pollinated crops the male sterile mutants are generally lost but, in cross pollinated or partially cross pollinated crops such mutants are preserved by natural hybridization. According to Kaul (1988) genetic male sterility arising due to spontaneous variation has been reported in over 175 plant species. The natural frequency of cytoplasmic and male sterile mutants in nature is relatively less, because it require natural mutation in mitochondrial genome to make its cytoplasm male sterile. Similarly, the natural occurrence of cytoplasmic nuclear male sterility system is also low since it requires simultaneous mutations both in the mitochondria and nucleus. According to Kaul (1988) so far only 46 plant species are credited to have produced cytoplasmic nuclear male sterility under natural conditions.

Chance Recombinants

In search of promising recombinants with useful traits, not available in the primary gene pool, the use of wild species representing secondary gene pool in breeding is quite common. There are numerous examples where duringevaluation of inter-specific F_1 or F_2 population some male sterile plants have emerged. This may be due to partial incompatibility of the two nuclear genomes or interaction between nuclear and cytoplasmic genomes. In this way male sterility systems have been successfully developed in some cereal, oil seeds, and various other groups of crops (Kaul, 1988).

Targeted Breeding

Cytoplasm Substitution with Related Wild Species

The development of male sterility through cytoplasm substitution is based on the concept of bringing cytoplasmic and nuclear genomes of diverse origins within a single genotype. This is achieved by crossing a wild relative of a crop as female parent with a cultivated line as male parent. This combination integrates the cultivated nucleus into the cytoplasm of wild species and brings together the two diverse entities in a new genotype; and in many cases it has yielded male sterile plants. The selection of an appropriate maintainer genotype is also important and it should be done with care. In most cases the fertility restorers can also be selected germplasm; but in case it is not available, then the breeders need to select fertile segregants originating from the same cross. Besides this, in crops like pearl millet, soybean, and cotton cytoplasmic nuclear male sterile recombinant segregants have also been selected from inter-varietal crosses (Kaul 1988). The frequency of such useful recombination, however, is very low.

Mutations

In addition to above, specific mutagenic agents can also be used to breed male sterility systems in a number of crops. According to Kaul (1988) over 35 plant species have been tried successfully to develop male sterility systems through mutagenesis. Among the mutagenic agents tried, gamma rays and Ethium Bromide have been found most effective. Soybean and pearl millet are good example where cytoplasmic nuclear male sterile lines have been developed through mutagenesis (Burton and Hanna 1976; Kaul 1988).

Chemical Hybridizing Agents

Some of the chemicals are also known to have gametocide properties, and these as a group, are called as 'chemical hybridizing agents' (CHA). Moore (1950) and Naylor and Davis (1950) were the first to induce male sterility by spraying maleic hydrazide in maize. Soon other chemicals (alpha naphthalene acetic acid and beta indole acetic acid) were reported to have induced female flowers in cucumber (Laibach and Kriben 1951). According to Colhoun and Steer (1982) the ideal CHA must be very specific and should not affect other parts of the plants; and at the same time should not be transmitted to the progeny in any form. The major advantage of this system is that it does not require any maintainer line. Tu and Banga (1998) reported that chemicals like 'Dalapan' can cause male sterility in cotton, pearl millet, wheat, linseed, sesame, capsicum and some other crops. 'Ethrel' is effective in barley, mustard, oat, pear millet, rice, and wheat. Similarly, 'gibberellic acid' has been found effective in inducing male sterility in rice, maize, barley, oats, sunflower, and onion. 'Maleic hydrazide' produces male sterility in capsicum, cotton, oats, sorghum, and onion.

Genetically Engineered Plants

Recent advances in DNA recombination technology has made it possible to synthesize male sterile lines and their restorers. Mariani*et al*. (1990) were the first to develop such a genotype. This was achieved by transferring tobacco and rapeseed plants with a chimeric dominant gene from *Bacillus amyloliquefacience.* This gene disrupts the normal process of pollen formation and causes male sterility. Besides this, some other technologies such as induction of modified glucanase gene (Worrall *et al.*, 1992) and hormone engineering (Schimulling*et al*., 1988) have been explored in the past. The cytoplasmic nuclear male sterility can also be produced through asexual recombination. Their use, however, has not found favor with plant breeders in any commercial hybrid crop.

Environment-Sensitive Male Sterility (ESMS)

In this form of male sterility changes in some environmental factors are responsible for the expression of male sterility. In fact all the reported male sterility genes do not respond to such changes. These environment-sensitive genes are spread across the species and genera and may be identified in both genetic as well as cytoplasmic nuclear male sterile genotypes. According to Kaul (1988) the major non-genetic factors which are known to cause male sterility in plants are temperature, photo-period, duration and qualityof light, and different soil-borne stresses. Of these, temperature and photo-period are the major male sterility inducing environmental agents. These two important factors may or may not function independently. There is

a lot of literature which shows a significant role of interaction between these factors in the expression of male sterility and its reversal to male fertility. It is also found that the genotypic differences within a species can also alter the expression of male sterility/fertility of the plants.Photo-periodism is a developmental response of plants to the length and frequency of dark period for specified durations. The plants in general use a photo-period receptor protein (phytochrome or cryptochrome) and provide signals to start the process of flowering or to remain vegetative. Recently, Jarillo*et al*. (2008) have published a detail model to explain how photoperiod controls flowering event in the plants. The first true ESMS was identified by Shi (1981), a Chinese researcher in rice and it was a spontaneous photo-sensitive male-sterile mutant. Similarly, the first case of temperature sensitivity was recorded in rice by Young and Wang (1990) and Sun *et al.* (1989).This line was male sterile under high temperature (28-33ºC) and male fertile under low temperature (22-27ºC). Subsequently, these types of male sterility systems were detected in a number of crops across the genera.The threshold for sex change and its reversal, however, may be different for different species or genotypes. It is also established fact that the expression of the sensitive genes may also be influenced by genetic background of the genotype. Besides these, there are reports in literature about the male sterility that is induced by micro-nutrient (*e.g.* copper, boron etc.) deficiency. This type of male sterility induction is of academic interest or for some specific glass house experiments. This aspect is not covered in this chapter.There is a huge literature on various aspects of environment related induction of flowering in dozens of crops representing different genera and species. The authors, therefore, have not made attempts to review the bulky literature and only important aspects of some basic contents have been covered with appropriate examples.

Cereal Crops

Rice

Among cereals,riceis the most researched and utilized crop with respect to environment induced male sterility system. The first rice ESMS mutant was reported by Shi (1981) in a late maturing japonica variety 'Nongken 58' in China in 1973; and it was found to be sensitive to photo-period changes; and after selection it was designated as 'Nongken 58S'.In this mutant the male sterility was induced by daylength of ≥14 h. On the contrary, the plants grown under the photo-periods of ≤13 h 45m restored their male fertility (Shi, 1981, 1985; Shi and Dong, 1986; Lu and Wang, 1988). Another photo-sensitive rice segregants was isolated from anF_1 population of a cross between *O. glaberrima* and *O. sativa* (Sano, 1993). Similarly, Satoh *et al.* (1992) identified yet another photo-sensitive male sterility system from an inter-variety cross. This material was also fertile under in the environmental conditions with photoperiod ≤ 13.5 h. Yuan *et al.* (1993) reported the existence of two photo-period reactions that govern growth and development of rice. The first reaction was responsible for acceleration (or delay) in panicle differentiation and heading; while the second photo-period reaction determinedthe formation of fertile (or sterile) pollen. They further mentioned that the second photo-period reaction required more critically timed daylength, greater light intensity, and relatively higher temperature than that of the first photo-period reaction.

A thermo-sensitive male sterile natural mutant was identified in rice by Tan *et al.* (1989). This mutant expressed male sterility under high temperature and male fertility under low temperature. Subsequently, temperature sensitive rice genotypes were also reported by Zhou *et al.* (1988), Sun *et al.* (1989), Young and Wang (1990), Virmani and Voc (1991). These genotypes were male sterile under high temperature (28-33°C) and male fertile under low temperature (22-27°C).Maruyama *et al.* (1991) identified a rice mutantthat was derived through irradiation using 20 kr gamma rays. This mutant was completely male sterile at 31/24° C; partial male-fertile at 28/15° C; and complete male fertile at 25/15° C. On the contrary, Jiang (1988) and Zhang *et al*.(1991)reported temperature-sensitive mutants with a reverse response to variations in temperature; and with male sterility at 24° C and male fertility at 27° C. Satake and Yoshida (1977) reported that high temperature (35-41°C) during the period of anthesis in rice produced sterile pollen. According to Sun *et al.* (1989) and Maruyama *et al.* (1991) the genes that control the response to temperature were simply inherited. On the contrary, Zhang*et al*. (1991) and Siddiq *et al*.(1995) reported that the male sterility gene in ricewastightly linked to temperature sensitive nuclear gene. In summary, rice crop has both types of responses in expressing male sterility/fertility*i.e.* some genotypes produce male sterility reaction under high temperature or long photo-period regime; and male fertility under low temperature or short photo-period; while the other group of materials responses in the reverse way.

The discovery of Nongken 58S, a natural recessive photoperiod sensitive male sterile line, served as a starting point for development of two-line hybrid rice in China. The original PGMS rice Nongken 58S is a spontaneous mutant, and many studies have shown that fertility segregation in crosses between 58S and its wild-type progenitor is conditioned by a single Mendelian locus. It is thus interesting that a second locus has become involved in this system, and homozygosity of recessive alleles at both loci is required for expression of male sterility. This implies that the cultivar Nongken 58 was already homozygous for the recessive allele at the second locus before it mutated to become PGMS rice. In this connection it should be noted that, in a previous study of a cross between two japonica lines in which the fertility also displayed typical two-locus segregation, Zhang *et al.* (1990) reported a linkage between a PGMS gene and a locus for dwarfism located on chromosome 5. A major difficulty presently encountered in the utilization of PGMS rice in two-line hybrid breeding is the temperature-mediated fertility variation observed in many newly developed PGMS lines. The substantial amount of self-pollinated seeds produced by these male sterile lines under some environmental conditions prevented their use in hybrid seed production. The seed-setting rates of these male sterile lines under long-day conditions varied from zero under favourable growing conditions, to low in average growing conditions, and to 30-40 per cent in cooler-than-usual conditions. The extent of such fertility variations depends on the genetic background of the genotypes and, in general, it is more serious in *Indica* than in *Japonica* genetic backgrounds. There may be several reasons for such temperature-mediated fluctuations in the male sterility. These may include (i) a possibility of the gene at the "second locus" may differ from the one in PGMS line (ii) the possibility that there may be a presence of multiple alleles at the second locus conditioning male sterility,

as is the case in soybean and pea (21) and for a locus governing fertility restoration in corn (22), so that different lines may carry different alleles with varying degrees of temperature sensitivity; and (iii) the possibility that additional modifying genes with thermo-sensitive expression may be involved in the system.

Wheat

Among winter cereals relatively more research has been carried out in wheatand both GMS and CMS genotypes have been reported to be influenced by environmental factors. Fisher (1972) recorded amazing results of variable photo-periods on reproductive organs in wheat. A short day (10 h) treatment at initial reproductive stage converted stamen into ovaries and ovules that were formed on the anther lobes. In another report the early and late formed tillers in wheat were found to have different pollen fertility levels and thus, signified the role of temperature on the male fertility (Jan, 1974). Luo *et al.* (1998) also reported the selection of photo-sensitive wheat lines. Murai and Tsunewaki (1993) reported identification of true photo-sensitive wheat genotypes which produced male fertility reaction during the daylength of≤14.5 h and male sterility during photo-periods of ≥ 15 h.

Utilization of a two-line breeding system via photoperiod-thermo sensitive male sterility has a great potential for hybrid production in wheat (*Triticum aestivum* L.). 337S is a novel wheat male sterile line sensitive to both short daylength/low temperature and long daylength/high temperature.The first long daylength-sensitive D2 type CMS wheat line was discovered by Sasakuma and Ohtsuka (1979), and thereafter a series of photoperiod-thermo sensitive male sterile lines have been identified in wheat (Tan *et al.,* 1992; Murai and Tsunewaki, 1993; Luo *et al.,* 1998; Murai, 1998; Xu and Yan, 1998). Most of these sterile lines are difficult to use for hybrid wheat production due to their requirements for extreme daylength or temperature. Recently, a novel wheat male sterile line 337S was identified, which shows good male sterility under both short daylength/low temperature and long daylength/high temperature (Guo*et al*., 2006a). The photoperiod-thermo sensitive male sterility in 337S is governed by two recessive genes located on chromosomes 2B and 5B, respectively, under long daylength/high temperature (Guo*et al.*, 2006b). There are two sowing windows for this line to be used as a male sterile line. Under an appropriate sowing time, it becomes fertile with the self-fertility rate >50 per cent, and thus it can also be used as a maintainer line. 337S is the first wheat male sterile line sensitive to both short daylength/low temperature and long daylength/high temperature, providing two sowing time windows for the expression of male sterility. This male sterile line has no harmful cytoplasmic effect and is controlled by recessive genes (Guo*et al.*, 2006a). The inheritance of male sterility under short daylength/low temperature was detected to be monogenic.

Other Cereals

In **maize** only the lines with S-cytoplasm exhibit thermo-sensitivity. Duvick (1966) while studying the stability of male sterility system in maize observed that some genotypes were male sterile in hot and dry environments, while other genotypes expressed partial male fertility in cool and humid environment. He *et al.* (1997) selected a thermo-sensitive male sterile maize mutant in 1992 in China and it was designated

as 'Qiong 6 Qms'. This mutant was insensitive to change in the lengths of photoperiod and expressed male sterility in summer and male fertility in winter sowings. Qiong 6 Qms is being used to develop two-parent maize hybrids in China.Tang *et al.* (1997) reported a male sterility system in **sorghum** that was controlled by some specific interaction between temperature and photo-period. Kidd (1961), perhaps, was the first to report the induction of male fertility in the sterile population by exposing sorghum plants to high (40° C) temperature regime. These observations were later confirmed by Kontian and Hongyi (1981) and Zhang and Fu (1982). On the contrary, Downes and Marshall (1971) reported development of male sterility under cool (13° C) nights during meiosis. Murty (1986) reported male sterility during short days and low temperature in A_2 cytoplasm of sorghum. In the warm weather, however, the expression of male sterility was not complete with existence of partial fertile flowers. The exposure of **barley** plants to short days for a period of two weeks significantly reduces pollen fertility. Sharma and Reinbergs (1976) reported the identification of male sterile mutants in barley that were male sterile at high (≥30° C) temperature and male fertile at low (≤15° C) temperatures. By evaluating barley genotypes at two diverse altitudes, Ahokas and Hockett (1977) reported differential photo-period sensitivity for flowering.

Legume Crops

In **faba bean** only limitedsources of heritable male sterility have been reported. Berthelem and Le Guen (1975) and Duc (1980) reported significant effects of light intensity and temperature on the expression of male sterility. With plants converting to male fertility when the temperature ranged between 17-27°C. In **soybean**, Caviness and Fagala (1973) documented the effect of both photo-period and temperature on pollen fertility, with no pod set recorded under high temperature. Wei *et al.* (1994, 1997) reported the first true photo-period sensitive male sterile mutant from a local soybean cultivar.

In **pigeonpea** (*Cajanus cajan*) boththemperature and photo-period are known to influence the initiation and appearance of floral buds, but their role in determining the male fertility/sterility has not been established. In the studies conducted by Saxena (2014),temperature was found to influence the fertility status of plants. Under the temperature regime of ≥25° C the plants were completely male-sterile. In contrast when daily mean temperatures dropped down to < 24° C, the male-sterile plants turned fully fertile and produced self-pollinated pods (Table 18.1). In early generations of breeding this material, Saxena *et al.* (2004)observed that some male sterile pigeonpea plants converted to male fertility much earlier than the rest, and these male sterile plants were classified as 'early' and 'late' converters. This suggested the presence of more than one gene with different temperature thresholds to produce fertile plants. All the 'converted male fertile' plants reverted back to male sterility when these plants encountered high temperatures (Table 18.1).

Vegetable Crops

In **cabbage**, Rundfeldt (1960) identified a natural mutant that was male fertile under low temperature and expressed male sterility under warmer environments.

Timin and Dobrutskaya (1981) reported significant effects of environment on male sterility in **carrots**. In **watermelon** and **muskmelon** among cucurbits,the sex expression of the plants is greatly influenced by prevailing environmental factors (Rudich and Peles, 1976; Kaul, 1988).

Table 18.1: Field Observations Recorded in Three Months on Male-Sterility and Fertility in Four Temperature-Sensitive Selections

Year	*Selection*	*September*		*November*		*February*	
		Sterile Plants	*Fertile Plants*	*Sterile Plants*	*Fertile Plants*	*Sterile Plants*	*Fertile Plants*
2007	Envs S-1	13	1	2	12	11	0
	Envs S-2	11	2	1	12	11	2
	Envs S-3	9	1	2	8	8	0
	Envs S-5	13	3	2	14	12	2
	Total	46	7 (13.2 per cent)	7	46 (86.8 per cent)	42	4 (8.7 per cent)
2008	Envs S-1	22	0	1	21	22	0
	Envs S-2	8	0	1	7	8	0
	Envs S-3	10	0	0	10	7	0
	Envs S-5	18	0	3	15	16	0
	Total	58	0 (0.0 per cent)	5	53 (91.4 per cent)	53	0 (0.0 per cent)
2009	Envs S-1	37	0	0	37	37	0
	Envs S-2	32	0	0	32	32	0
	Envs S-3	27	0	0	27	25	0
	Envs S-5	23	0	0	22	21	0
	Total	119	0 (0.0 per cent)	0	118 (100.0 per cent)	115	0 (0.0 per cent)

() per cent fertile plants

Source: Saxena, 2014.

Among vegetables relatively good work has been done in onion and tomato. Temperature variability has been reported to influence pollen fertility/sterility in **onion.** Barham and Munger (1950) reported that no viable pollen was produced at the temperature <21ºC. van der Meer and van Bennekom (1978) reported genotypic variability for response to variable temperatures. In one population the pollen sterility was found to be controlled by temperatures of 14ºC and below; while the other population did not show any response. In **tomato** the influence of temperature on genetic male sterility system has been reported with no influence of photoperiod. Rick and Boynton (1967) reported a tomato mutant that was male sterile at 30-32ºC. Stevens and Rudich (1978) reported a mutant where temperature of 38º/27ºC resulted

in the reduction in pollen production. Sawhney (1983) also reported temperature control of male sterility in tomato. An exposure of the mutant line at 23°/18°C produced sterility and under low temperature regime of 15-18° C the plants produced fertile pollen grains.

Other Crops

Brar (1982) reported a mutant in **sesame**, that was male sterile under glass house and fertile under field conditions. In **sugarbeet** also, low temperature was found to induce male sterility (Kinoshita, 1971). Xi *et al.* (1997) reported selection of a thermo-sensitive mutant in **brassica**. Fan and Stefensson (1986) found that the expression of male sterility was controlled by low (22°/16°C) temperature in brassica; and the full pollen fertility was expressed at 30°/24° C. In the middle temperature range the plants exhibited variable response to pollen fertility. According to Myer and Myer (1965) the male sterility in **cotton** is expressed at high (32-38° C).

Besides male sterility,fertility restoration of some F_1 hybrid combinations is also adversely affected by environmental factors, mainly temperature and humidity; and in different environments produces different levels of pollen fertility in the plants. In pigeonpea also, this has been a problem in A_2(*C. scarabeoides*) cytoplasm based hybrids. Saxena *et al.* (2011) attributedthe variability in fertility restoration due to low temperature stress in A_4 cytoplasm (*C. cajanifolius*) based hybrids.

Breeding Elite ESMS Lines

The environment-sensitive male sterility system is unique and if explored seriously, then it can be used for enhancing crop productivity through its use in hybrid breeding programmes. Besides its low cost, it is easy to implement and large seed quantities of female parent and hybrid can be produced with good quality control. Although this type of male sterility has been found in a number of crops (see section 4) but China is the sole leader in this field with ESMS-based hybrids commercialized in rice, sorghum, maize and brassica. In rice this technology has been exploited on a very large scale with high adoption to benefit the Chinese farmers. Some other countries such as India, Vietnam, Malaysia, Philippines, and Bangladesh etc. have also started using ESMS hybrid rice technology; but China is the real champion.

For a sustainable ESMS hybrid breeding programme it is essential that new parent material is generated on a regular basis. In this endeavour, it is essentialthat the sensitive male sterility gene is not lost during breeding process and each selected plant/progeny should carry the sensitive gene. To achieve this, it is important that in each generation the breeding materials should be planted under inductive environment that would allow the expression of male sterility gene. The seed from the selection should be harvested (as a ratoon crop) when the selected male sterile plants convert to full fertility and produce normal seed set. To launch a breeding programme for hybrid breeding using EGMS lines, the first important activity is to characterize the line with respect to its behavior under different temperature/

photoperiod regimes to find out the critical fertility and sterility points. Once these threshold points are confirmed, then the next important activity will be to create or identify locations with specific requirements of the environments. There should two specific sites, one site should be male fertility inducing (environment I), while the other should be able to induce male sterility (environment II). These distinct sites/ environments should be used in breeding new EGMS cultivars. For rice breeding in China, two sites with specific temperature and photo-period requirements are being used on a large scale (Virmani *et al.,* 1997). For a typical TGMS crop breeding/selection, a programme has been outlined in Table 18.2 using the summer (hot) and winter (cool) environments.

Table 18.2: A Procedure for Breeding New Thermo-Sensitive A-lines

Year	*Generation*	*Season*	*Activity*
1	–	Rainy (warm)	Plant parents, select male sterile plants, make crosses with elite male line
2	F1	Rainy (warm)	Grow F1, examine sterility of each plant, harvest F2 seed
3	F2	Rainy (warm)	Grow 2000 plants, examine each plant for sterility, reject the fertile plants, number the sterile plants. Carry them to winter season.
		Winter (cool)	Observe each plant for fertility and pod set. Reject poor pod setting plants. Harvest about 200 single converted plants for evaluation in F3 progeny rows.
4	F3	Rainy (warm)	Grow F3 rows, evaluate them for male sterility. Reject off- type progenies. Take them to the cool season.
		Winter (cool)	Select progenies on the basis of conversion to male fertility. Record data on the rate of conversion. Bulk or single plant harvest after rouging.
5	F4	Rainy (warm)	Grow F4 rows, evaluate them for male sterility. Reject off- type progenies. Take them to the cool season.
		Winter (cool)	Select progenies on the basis of conversion to male fertility. Record data on the rate of conversion. Bulk or single plant harvest after rouging.
6	F5	Rainy (warm)	Grow F5 rows, evaluate them for male sterility. Reject off- type progenies. Take them to the cool season.
		Winter (cool)	Select progenies on the basis of conversion to male fertility. Record data on the rate of conversion. Bulk harvest and record yield.
7	F6 (New A- lines ready; test them for combining ability before using in hybrid program		Grow F6 rows, evaluate them for male sterility and other traits. Reject inferior progenies. Take them to the cool season. Select progenies on the basis of conversion to male fertility. Record data on the rate of conversion andyield. Bulk harvest for use in hybrid breeding program.

Molecular Aspects of ESMS Systems

Wheat

To date, only a few temperature, photoperiod, or photoperiod-thermo sensitive male sterile genes inwheat have been mapped (Xing *et al.,* 2003; Cao *et al.,* 2004). The mapping analysis indicated that the male sterile gene *wptms3* located on chromosome 1B, flanked by *Xgwm413* and *Xgwm182*, differed from those reported by Guo*et al.* (2006b) and thus is a new gene. To date, several fertility restoring genes against CMS in wheat have been mapped on chromosome 1B (Ahmed *et al.,* 2001; Li*et al.*, 2005; Zhou *et al.,* 2005). Therefore, there are regions on chromosome 1B related to fertility performance. The SSR marker *Xgwm413* was identified to be closely linked to the male sterile gene and was found to be linked to yellow rust resistance genes in earlier reports (Peng *et al.,* 1999; 2000a; 2000b; Ma *et al.,* 2001). These studies indicated that the genes are not randomly distributed over the genome of a species, but they are rather frequently clustered on particular chromosomes (Peng *et al.,* 1999). The clustering of genes coding a trait may be the result of the co-evolution of plant species and their adaptation to environments (Peng *et al.,* 1999). With the identification of the molecular markers linked to the male sterile genes *wptms3, wptms1* and *wptms2*, they could be very useful for developing and improving new male sterile lines via marker-assisted selection.

In wheat breeding programmes, we can use the linked markers to distinguish the sterile genotypes earlier, which can help to shorten the breeding time and facilitate the whole procedure. As the long daylength/high temperature induced male sterility in 337S is controlled by two complementary genes (Guo*et al.*, 2006a; 2006b), and only the plants with genotype *aabb* are sterile and the ratio of sterile plants is low. Thus, mapping of *wptms3* provides a more feasible and reliable method for identifying male sterile plants in practical breeding. Therefore, it is also helpful for breeders to use these linked markers to identify those male sterile recessive genotypes in early generation and period with high accuracy. It is clear that the fertility of photoperiod sensitive sterile lines is determined by the interaction of genes and environments and the mechanism is complex. Isolation, cloning, and characterization of the male sterile gene can promote molecular study of the genetic male sterile trait and improve its application in hybrid breeding. Even though three male sterile genes (*wptms1*, *wptms2*, and *wptms3*) have been identified in the novel wheat male sterile line 337S, the linkage between markers and target genes is not tight enough for gene cloning. Therefore, fine mapping and cloning the male sterile genes in 337S under different environments are underway and will further improve understanding of the genetic mechanism of the male sterile system in utilization of wheat heterosis.

Rice

ESMS line is useful in hybrid rice seed production. It is not possible to identify the locus of sterility gene using morphological marker even if it is single gene, because the evaluation of fertility which is easily influenced by the environmental factor and is expressed as quantitative trait is difficult (Yoshiahi and Yamaguchi, 1997). Zhang *et al.* (1994) reported that expression of PGMS is more stable in *japonica* than in *indica* genetic background leading to the assumption that TGMS expression is also variable according to the genetic background.Although two-line hybrids developed using

this EGMS germplasm have made great impact in improving rice yield in China during the past two decades, people knew less about the molecular mechanism of how the daylength and temperature co-ordinately regulate the fertility transition of EGMS in rice.

In the genetic analysis it was reported that *pms3*waslocated on chromosome12. This was the original mutation which converted Nongken58 to become the PGMS rice NK58S. Recently, *pms3* was cloned and shown to encode a long non-coding RNA (lncRNA) named LDMAR. A sufficient amount of LDMAR is required for male fertility under long-day conditions. A spontaneous G-C mutation causing a SNP between NK58 and NK58S, eventually brings about heritable increased methylation in the promoter region of LDMAR, which reduces the level of LDMAR expression. This then results in premature programmed cell death (PCD) in the anther development under long days, and hence express male sterility(Ding *et al.,* 2012a). In addition, Ding*et al*. also reported that RNA-dependent DNA methylation (RdDM) is involved in the regulation of PGMS. Promoter siRNA of LDMAR derived from *AK11270* is associated with the DNA methylation level of LDMAR, which reduces the expression level of LDMAR, and therefore male sterility in Nonken58S under long-day conditions (Ding *et al.,* 2012b). *P/TMS12-1,* which confers PGMS in the japonica rice line NK58S and TGMS in the indica rice line PA64S, encodes a unique non-coding RNA, which produces a 21-nucleotide small RNA named *osa-smR5864w*. This RNA shares identity with the product of *pms3* at the nucleotide level, which is responsible for the fertility of the pollen of NK58S and PA64S (Zhou *et al.,* 2012). Taken together, these findings suggest that this non-coding small RNA gene is an important regulator of male development controlled by cross-talk between the genetic networks and the environmental conditions.

The studies by Zhou (2012) suggest that a non-coding small RNA gene *p/tms* 12-1 is an important regulator of male development controlled by cross talk between the genetic networks and environmental conditions. They further indicated that a point mutation in this gene probably leads to loss-of-function for a small RNA namely *osa-smR5864m* constituting a common cause for PGMS and TGMS in the *japonica* and *indica* lines, respectively. To date a number of loci that control PGMS or TGMS in different lines have been mapped in different chromosomes. Photoperiod-sensitive genic male sterile (PSGMS) rice has a number of desirable characteristics for hybrid rice production.

Great efforts have to be invested to understand the biological functions of long non- coding RNAs (lncRNAs). In PGMS, how the lncRNAs (pms3) sense the different photoperiod and alter their expression is also very interesting and awaits much deeper research. The studies have shown that an lncRNA of 1,236 bases in length, referred to as long-day specific male-fertility–associated RNA (LDMAR), regulates PSMS in rice. The sufficient amount of the LDMAR transcript was required for normal pollen development of plants grown under long-day conditions. A spontaneous mutation causing a single nucleotide polymorphism (SNP) between the wild-type and mutant altered the secondary structure of LDMAR. This change brought about increased methylation in the putative promoter region of LDMAR, which reduced the transcription of LDMAR specifically under long-day conditions, resulting in

premature programmed cell death (PCD) in developing anthers, thus causing PSMS (Ding *et al.*, 2011). Thus, an lncRNA could directly exert a major effect on a trait like a structure gene, and a SNP could alter the function of an lncRNA similar to amino acid substitution in structural genes. Molecular elucidating of PSMS has important implications for understanding molecular mechanisms of photoperiod regulation of many biological processes and also for developing male sterile germplasm for hybrid crop breeding.

According to Denmang(2012)the C-to-G mutationin a non-coding RNAis responsible for the temperaturesensitivemutation in *indica* background, suggesting thatit is the genetic background, but not thencRNA locus *per se*, that is responsiblefor the photoperiod to temperature sensitivemale sterility change.

Elucidation of the genetic and molecular bases of PSMS, although still far from completion at this stage, provides important implications for the studies of other photoperiod-regulated processes. It indicates that each of the processes may have a distinct genetic and molecular control at least at the lower level of regulatory hierarchy of photo-periodism. Therefore, the processes have to be investigated individually to understand them. We also speculate that at higher levels, such as the perception of day length, time keeping, and photoreceptors, among other things, these processes should be subjected to the same regulatory machineries as photoperiod flowering.

Characterization of genes and proteins related to male sterility aims to understand how and why the male sterility occurs, and which proteins are the key players for microspores abortion. Recently, a series of genes and proteins related to cytoplasmic male sterility (CMS), photoperiod-sensitive male sterility, self-incompatibility, and other types of microspores deterioration have been characterized through genetics or proteomics. Especially the latter, offers us a powerful and high throughput approach to discern the novel proteins involving in male-sterile pathways which may help us to breed artificial male-sterile system. This represents an alternative tool to meet the critical challenge of further development of hybrid rice. Taken together, concerted efforts from multiple angles would pave the road to rapid progress in understanding the complex regulatory networks and finally enable us to attain a holistic concept for the development of rice male reproductive system. All the data harvested in these studies will definitely help us to freely manipulate fertility in rice and other crop plants to facilitate hybrid breeding in the future.

Use of Environment-Sensitivity in Hybrid Technology

To make practical use of the environment-sensitive genotypes in hybrid seed production programmes, the selection of two production sites with strict temperature regimes and least fluctuations is essential. For the multiplication of quality seed of female parent and hybrid, the maximum safe mean temperature during crop growth, particularly reproductive phase, should not exceed the limits prescribed through field and/or laboratory experiments. These temperature/photoperiod bars would allow complete expression of the gene(s) responsible for the unique behavior of the genotypes and maintain pollen sterility/fertility status of the plants for quality seed production.

The salient features of ESMS-based hybrid seed production of rice, as used in China, is summarized in Table 18.3. This methodology can also be explored in pigeonpea as described by (Saxena, 2014) and given in Table 18.4. For sowing of the crop the months of June (high temperature) and September (low temperature) were selected. The data (Table 18.4) indicated that the two crops behaved differently with respect to their pollen fertility. In the June sown crop, over 92 per cent plants were male-sterile and this population can be used for hybrid seed production. In contrast, the September-sown crop appeared like a normal pure line variety with 98 per cent plants being male-fertile, and it can be used for the multiplication of A-line without involving B-line. Therefore, the seed system strategy involving temperature-sensitive pigeonpea genotypes would require two distinct sites, each with characteristically different temperature regime. These will allow complete expression of the gene(s) responsible for the unique behavior of the genotypes. For multiplication of female parent, the maximum safe temperature during crop growth, particularly reproductive phase, should be in the range that does not allow conversion of male fertile plants to sterility. This temperature bar will maintain pollen fertility status of the plants and allow production of fertile flowers and normal pod set. The seed produced from such isolated plots will remain genetically pure. For hybrid (A x R) seed production involving temperature-sensitive the male-sterile lines should be grown a season when the temperatures are high.

Table 18.3:Techniques Involved in Hybrid Seed Production of Rice in China Using TGMS System

Technique	*Number/Quantity*
Row ratio	2: 14-16
Width of female parent (m)	2.5
Plants/hill	2
Hills/ha	
A-line	530,000
R-line	27,000
Plants/ha	
A-line	1060,000
R-line	54,000
GA_3 (g/ha)	120-180
GA_3 Concentration	100
Seed set (per cent)	45-50
Hybrid yield (kg/ha)	2,600

Source: SS Virmani, IRRI, Philippines.

The pigeonpea experiment (Table 18.4) also showed that in some special environments the seed production of both the female parent and hybrid can be done at a single location. In this system the hybrid seed production plot is sown in isolation in early rainy season (June) to produce male sterile flowers for hybridization.The

multiplication of female parent can be taken up in another isolation in September sowing; and this will produce male fertile flowers and a good harvest of female parent (A-line) can be taken without the use of B-line and pollinating insects.

Table 18.4: Segregation for Male-Sterility and Fertility as Affected by Date of Planting at Patancheru during 2008

Date	*June 15 Sowing*				*September 30 Sowing*			
	Sterile Plants	*Fertile Plants*	*Per cent Fertile Plants*	*Yield/ Plant (g)*	*Sterile Plants*	*Fertile Plants*	*Per cent Fertile Plants*	*Yield/ Plant (g)*
August 28-29	164	13	7.9	–	–	–	–	–
November 25-30	3	148	–	68-113	8	204	–	12-53
March 21-24	113	8	7.1	–	168	13	7.7	–

Source: Saxena, 2014.

General Discussion

Exploitation of two basic genetic phenomenon in commercial crops, identified as cytoplasmic nuclear male sterility and hybrid vigour, have not only saved the earth from hunger but also spared space for the cultivation of food crops. In the cultivation of hybrids seed is the most expensive input because every year a new seed stock is required to realize the maximum benefit from the technology. To limit the seed cost, the cytoplasmic nuclear male sterility based three-parent hybrid technology is always preferred over genetic male sterility based two-parent hybrids. To further ease the situation the hybrid technology based on environment-sensitive male sterility system is being advocated, particularly for rice in China. This system not only eliminates the use of B-line but also makes the selection of heterotic hybrid combinations much easier and faster.

The environment-sensitive male sterile mutants have been recovered in about three dozens of crops but its use in commercial hybrid breeding is limited to rice only. Yuan (1987) proposed its use in rice hybrid breeding programme. Since it eliminates the requirement of maintainer 'B' line, this hybrid system, is popularly called as 'two-parent hybrid breeding'. The hybrids rice based on this technology cover over 300,000 ha areas with yields as high as 8-9 t/ha. Now considering the pressure of providing food to ever increasing population, it would be appropriate that this technology be extended to other crops also. For example in a new crop like pigeonpea, this male sterility system is now available (Saxena, 2014) and breeding programmes to develop hybrids can be launched.This will a collaborative effort that would involve identification ofideal seed production sites each for female parent and hybrids. The temperatures at Patancheru during the months of August, November, and March arevastly different; and thesecan be used to provide the required fertility status of pigeonpea hybrid parents (Table 18.5). Based on prevailing temperatures one canalso identify the large scale seed production sites and then the process of commercialization of hybrids can begin. The future research in this endeavour should now be concentrated on the issues such as identification of high yielding hybrids with known threshold

Table 18.5: Mean Temperatures and Photo-periods during Critical Standard Weeks Recorded at Patancheru

Std. Week	*Period*	*Day Length (h)*	*Average Air Temperature (°C)*		
			2007-08	*2008-09*	*2009-10*
34.0	20-26 Aug	12.6	26.0	26.0	25.8
35.0	27 Aug-02 Sep	12.5	26.0	26.7	25.2
Mean	12.6	26.0	26.4	25.5	
47.0	19-25 Nov	11.2	19.1	23.9	22.9
48.0	26 Nov-02 Dec	11.2	20.3	22.3	20.4
Mean	11.2	19.7	23.1	21.7	
12.0	19-25 Mar	12.1	25.8	27.0	29.0
13.0	26 Mar-01 Apr	12.2	26.5	28.3	30.3
Mean	12.2	26.2	27.7	29.7	

Source: Saxena, 2014.

points of fertility conversion of different A- lines with respect to temperature and photo-period. Simultaneously, attempt should also be made to understand the molecular basis of sex reversion under different environments. Finally, it will be important to identify genes/QTLs responsible for controlling this trait that will facilitate quick transfer of these genes into heterotic hybrid parents.

References

1. Ahmed, T.A., Tsujimoto, H. and Sasakuma, T. (2001). QTL analysis of fertility-restoration against cytoplasmic male sterility in wheat. *Genes Genet. Syst.*, 76(1): 33-38. [doi: 10.1266/ggs.76.33].
2. Ahokas, H. and Hockett E. A. (1977). Male sterile mutant of barley. IV. Different fertility levels of *msgqci* (cv. Vantage), an ecoclinal response. *Barley Genet. Newsl.*, 7: 10-11.
3. Barham, W.S. and Munger, H.M.(1950).The stability of male sterility in onions. *Proc Am SocHorticSci*, 56: 401-409.
4. Bernier, G., Havelange, A., Claude, H., Petitjean, A. and Lejeune, P. (1993). Physiological signals that induce flowering. *The Plant Cell*,5: 1147- 1155.
5. Berthelem, P. and Le Guen J. (1975). Rapport d'activite-station d'amelioration des plantes. INRA Rennes (France): 1971-74. (As reported in Kaul, 1988.).
6. Brar, D. S. 1982. Male sterility in sesame. *Indian J. Genet.*,42: 23-27.
7. Burton, G.W. and Hanna, W.W.(1976). Ethidium bromide induced cytoplasmic male sterility in pearlmillet. *Crop Sci.*, 16: 731-732.
8. Cao, S.H., Guo, X.L., Liu, D.C., Zhang, X.Q. and Zhang, A.M. (2004). Preliminary gene-mapping of photoperiod-thermo sensitive genic male sterility in wheat (*Triticum aestivum* L.). *Acta Genet. Sin.*, 31(3): 293-298 (in Chinese).

9. Caviness, C.E. and Fagala, B.L.(1973). Influence of temperature on a partially male sterile soybean strain. *Crop Sci*., 11: 564-566.

10. Colhoun, C.W. and Steer, M. W. (1981). Microsporogenesis and the mechanism of Cytoplasmic Male Sterility in maize. *Annual Botanic*, 48: 417-424.

11. Danmeng Zhu and Xing Wang Deng. (2012).A non-coding RNA locus mediates environment-conditioned male sterility in rice. *Cell Research*, 1: 1-2.

12. Darwin, C. (1877). The different forms of flowers on plants of the same species, Murray, London.

13. Ding, J., Lu, Q., Ouyang, Y., Maoa, H., Zhanga, P., Yaob, J., Xua, C. and Lia, X. (2012a).A long noncoding RNA regulates photoperiod- sensitive male sterility, an essential component of hybrid rice. *Proc Natl Acad Sci USA*,109: 2654-2659.

14. Ding, J., Shen, J., Mao, H., Xie, W., Li, X. and Zhang, Q. (2012b). RNA-directed DNA methylation is involved in regulating photoperiod-sensitive male sterility in rice. *Mol. Plant* 5, 1210–1216.

15. Downes, R.W. and Marshall, D.R. (1971). Low temperature induced male sterility in Sorghum bicolor. *Australia J. Expt. Agr. Anim. Husb.*, 11: 352–356.Duc, G.1980. Effect of environment on the instability of two sources of cytoplasmic male sterility in faba beans. *FEBS Lett.*, 2: 29-30.

16. Duvick, D.N. (1966). Influence of morphology and sterility on breeding methodology. (In) Frey KJ, editor. *Plant breeding*. Vol. I. Ames, Iowa (USA): Iowa State University Publications. p 85-138.

17. Emsweller, S.L. and Jones, H.A.(1935). An interspecific hybrid in *Allium.Hilgardia,* 9: 265–273.

18. Fan, Z. and Stefansson, B. R. (1986). Influence of temperature on sterility of two cytoplasmic male sterility systems in rape (*Brassica napus* L.) *Can. J. Plant Sci*., 66: 221-227.

19. Fisher, J.E. (1972). The transformation of stamens to ovaries and of ovaries to inflorescences in *L. aestivum* L. under short day treatment. *Bot. Gaz*., 133: 78-85.

20. Guo, R.X., Sun, D.F., Cheng, X.D., Rong, D.F. and Li, C.D. (2006a). Inheritance of photoperiod-sensitive male sterility in wheat. *Aust. J. Agric. Res.*, 57(2): 187-192.

21. Guo, R.X., Sun, D.F., Tan, Z.B., Rong, D.F. and Li, C.D. (2006 b).Two recessive genes controlling thermophotoperiod-sensitive male sterility in wheat. *Theor Appl Genet.*,112(7): 1271–1276.

22. Zhou, H., Liu, Q., Li, J., Jiang, D., Zhou, L., Wu, P., Lu, S., Li, F., Zhu, L., Liu, Z., Chen, L., Liu, Y. and Zhuang, C. (2012). Photoperiod- and thermo-sensitive genic male sterility in rice are caused by a point mutation in a novel noncoding RNA that produces a small RNA.*Cell Research,* 22: 649-660.

23. Hanson, M.R. and Bentolila, S. (2004). Interactions of mitochondrial and nuclear genes that affect male gametophyte development. *Plant Cell,* 16: S154–S169.

24. Iwabuchi, M., Kyozuka, J. and Shimamoto K. (1993). Processing followed by complete editing of an altered mitochondrial atp6 RNA restores fertility of cytoplasmic male sterile rice. *EMBO J.,* 12: 1437-1446.

25. Jan, C.C. (1974). Genetic male sterility in wheat (*Triticum aestivum* L.): expression, stability, inheritanceand practical use. Ph.D. thesis. University of California, Davis, Calif., USA.

26. Jarillo, J.A., Pineiro, M., Cubas, P. and Martinez-Zapater, J.M. (2009). Chromatin remodeling in plant development. *Inter J Dev Biol* 53: 1581–1596.

27. Jiang, Y. M. (1988). Studies on the effect of high temperature on fertility of the female sterile line in Dain-type hybrid rice.*J.Yunnah. Agric. Univ*., 3 (2): 99-107.

28. Kaul, M.L.H. (1988) Male sterility in higher plants. Springer Verlag Berlin, Heidelberg. New York,1805 pp.

29. Kennell, J.C., Wise, R.P. and Pring, D.R. (1987). Influence of nuclear background on transcription of a maize mitochondrial region associated with Texas male sterile cytoplasm. *Mol. Gen. Genet.,* 210: 399–406.

30. Kinoshita, T. (1971).Genetical studies on the male sterility of sugarbeets (*Beta vulgaris*) and its related species. *J Fac Agric Hokkaido Univ.,* 56: 435-541.

31. Kongtian, Z. and Hongyi, F.(1981). Effect of high temperature on fertility of the male sterile lines in Sorghum. *Annu Rep Inst Genet Acad Sin*, p. 120.

32. Laibach, F. and Kribben, F.J. (1951). Der Einfluss von Wuchstoff auf das Geschlecht der Bliiten bei einer monoezischen Pflanze (*Cucumis sativus* L.). *Beitr Bioi Pflanz*., 28: 64-67.

33. Li, X.L., Liu, L.K., Hou, N., Liu, G.Q. and Liu, C.G. (2005). SSR and SCAR markers linked to the fertility-restoring gene for a D2-type cytoplasmic male-sterile line in wheat. *Plant Breeding*, 124(4): 413-415.

34. Lu, Q., Li, X.H., Guo, D., Xu, C.G. and Zhang, Q.(2005). Localization of *pms3*, a gene for photoperiod-sensitive genic male sterility, to a 28.4-kb DNA fragment. *Mol Genet Genomics.,*273: 507- 511.

35. Lu, X. and Wang, J. (1988). Fertility transformation and genetic behavior of Hubei photoperiod-sensitive genic male sterile rice. In: Hybrid rice. Los Baños (Philippines): International Rice Research Institute. p 129-138.

36. Luo, H.B., He, J.M., Dai, J.T., Liu, X.L. and Yang, Y.C. (1998). Studies on the characteristics of seed production of two ecological male sterile lines in wheat. *J Hunan Agric Univ.,*24(2): 83–89. (in Chinese).

37. Luo, Z., Zhong, A. and Zhou, Q. (1988). Effect of a new non-toxic chemical gametocide in rice. In: Hybrid rice. Manila (Philippines): International Rice Research Institute.p. 147-156.

38. Ma, J.X., Zhou, R.H., Dong, Y.S., Wang, L.F., Wang, X.M. and Jia, J.Z. (2001). Molecular mapping and detection of the yellow rust resistance gene *Yr26* in wheat transferred from *Triticum turgidum* L. using microsatellite markers. *Euphytica*, 120(2): 219-226.

39. Mariani, C., De Beuckeleer, M., Truettner, J., Leemans, J. and Goldberg R.B. (1990). Induction of male sterility in plants by a chimeric ribonuclease gene. *Nature*, 347: 737-741.

40. Maruyama, K., Araki, H. and Kato, H. (1991). Thermosensitive genetic male sterility induced by irradiation. In: Rice genetics II. Manila (Philippines): International Rice Research Institute. p. 227-235.

41. Meyer, V.G. and Meyer, J.R.(1965). Cytoplasmically controlled male sterility in cotton. *Crop Sci*., 5: 444-448.

42. Moore, R.H. (1950). Several effects of maleic hydrazide on plants. *Science.,* 112: 52-53.

43. Murai, K. (1998). Two-Line System for Hybrid Wheat Production Using Photoperiod-Sensitive Cytoplasmic Male Sterility. *In*: Zhang, A., Huang, T.C. (Eds.), Proceedings of the First International Workshop on Hybrid Wheat. China Agricultural University Press, Beijing, p.37-39 (in Chinese).

44. Murai, K. and Tsunewaki, K.(1993). Photoperiod-sensitive cytoplasmic male sterility in wheat with *Aegilops crassa* cytoplasm. *Euphytica*, 67(1-2): 41-48. [doi: 10.1007/BF000 22723].

45. Murty, U.R. (1986). Effect of A_2 cytoplasm on the inheritance of plant height in temperate × tropical sorghum crosses. *Sorghum Newsletter,* 29: 77.

46. Naylor, A.W. and Davis, E.A.(1950). Maleic hydrazide as a plant growth inhibitor. *Bot Gaz*., 112: 112-116.

47. Pallavi Sinha., Saxena, K.B.,Rachit Kumar Saxena., Vikas Kumar Singh.,Suryanarayana, V., Sameer Kumar, C.V., Mohan Katta, A.V.S., AamirKhan, W. and Varshney. R.K. (2014). *nad7a*: a candidate gene associated with cytoplasmic male sterility in pigeonpea (*Cajanas cajan*). Unpublished.

48. Peng, J.H., Fahima, T., Röder, M.S., Li, Y.C., Dahan, A., Grama, A., Ronin, Y.I., Korol, A.B. and Nevo, E. (1999). Microsatellite tagging of the stripe-rust resistance gene *YrH52* derived from wild emmer wheat, *Triticum dicoccoides*, and suggestive negative crossover interference in chromosome 1B. *Theor. Appl. Genet*., 98(6-7): 862-872. [doi: 10.1007/s001220051145].

49. Peng, J.H., Fahima, T., Röder, M.S., Huang, Q.Y., Dahan, A., Li, Y.C., Grama, A. and Nevo, E. (2000a). High-density molecular map of chromosome region harboring stripe-rust resistance genes *YrH52* and *Yr15* derived from wild emmer wheat, *Triticum dicoccoides*. *Genetica*, 109(3): 199-210. [doi: 10.1023/A: 1017573726512].

50. Peng, J.H., Fahima, T., Röder, M.S., Li, Y.C., Grama, A. and Nevo, E. (2000b). Microsatellite high-density mapping of the stripe rust resistance gene *YrH52* region on chromosome 1B and evaluation of its marker-assisted selection in the F2 generation in wild emmer wheat. *New Phytol.*, **146**(1): 141-154. [doi: 10.1046/ j.1469-8137.2000.00617.x].

51. Rick, C.M. and Boynton, J.E. (1967). A temperature sensitive male sterile mutant of the tomato. *Am. J. Bot.*, 54: 601-611.

52. Roberts, H.F. (1929). Plant Hybridization Before Mendel, New Jersey: Princeton University Press.

53. Rudich, J. and Peles, A. (1976) Sex expression in watermelon as affected by photoperiod and temperature. *Sci. Hort.*,5: 339-344.

54. Rundfeldt H. (1960). Untersuchungen zur zuchtung des Kopfkohls (*B. olerecea* L. var. *Capitata*). *Z. Pflanzenzuchtung* 44: 30-62. (As reported in Kaul, 1988.).

55. Sano, Y. (1983). A new controlling gene sterility in F_1 hybrids of two cultivated rice species: Its association with photoperiodic sensitivity. *J Hered*., 74: 435-439.

56. Sasakuma, T. and Ohtsuka, I. (1979). Cytoplasmic effects of *Aegilops* species having D genome in wheat. I. Cytoplasmic differentiation among five species regarding pistilody induction.*Seiken Ziho*, 27: 59–65.

57. Satake, T. and Yoshida, S. (1977). Mechanism of sterility caused by high temperature at flowering time in *indica* rice. *JARQ Japan*, 11: 127-128.

58. Satoh, K. (1992). Influence of different seeding times on fertility performance of rice strain X 88 and Chunkanbohan nou 12in Okinava. Jpn. J. breeding (Suppl.1).

59. Sawhney, V.K. (1983). Temperature control of male sterility in a tomato mutant. *J. Hered*., 74: 51-54.

60. Saxena, K.B., Sultana, R., Saxena, R.K., Kumar, R.V., Sandhu, J.S., Rathore, A. and Varshney, R.K. (2011). Genetics of fertility restoration in A_4 based diverse maturing hybrids in pigeonpea [*Cajanus cajan* (L.) Millsp.].*Crop Science,* 51: 1 - 5.

61. Saxena, K.B. (2014). Temperature sensitive male-sterility system in pigeonpea. *Current Science*, 7 (2): 277-281.

62. Saxena, K.B., Tikka, S.B.S. and Mazumdar, N.D. (2004). Cytoplasmic genic male-sterility in pigeonpea and its utilization in hybrid breeding programme.(*In*) Pulses in New Perspective. Ali M, Singh, B.B., Kumar, S., and Dhar, V. (eds.). Indian Institute of Pulses Research, Kanpur 208024, UP, India, p 132-146.

63. Schimalling, C., Schell, J. and Spena, A. (1988). Single genes from Agrobacterium rhizogenes influence plant development. *EMBO J.,* 7: 2621-2629.

64. Sharma, R.K. and Reinbergs, E. (1976) Male sterility genes in barley and their sensitivity to light and temperature intensity. *Ind. J. Genet. Plant Breed.*, 3 2: 408-410.

65. Shi, M.S. and Deng, J.Y. (1986). The discovery, determination and utilization of Hubei photosensitive genic male sterile rice (*Oryza sativa* L. subsp. *japonica*). *Acta Genet. Sin*., 13(2): 107-112.

66. Shi, M.S. (1981). Preliminary report of breeding and utilization of late japonica natural double purpose line. *J. Hubei Agric. Sci.,* 7: 1-3.

67. Shi, M.S. (1985). The discovery and the study of the photosensitive recessive male sterile rice (*Oryza sativa* L. subsp. *japonica*). *Sci. Agric. Sin*., 2: 44-48.

68. Shull, G. H. (1908). The composition of a field of maize. *Am. Breeders Assoc. Rep.*, 4: 296–301.

69. Siddiq, E.A., Ahmad, I., Viraktmath, B.C., Jauhar, Ali. and Hoan, T. (1995). Status of hybrid rice research in India. *In*: Rai,M Mauria S (eds). Hybrid research and development. Indian Society of Seed Technology. New Delhi, India., Pp 139 - 158.

70. Stevens, M.A. and Rudich, J. (1978). Genetic potential for overcoming physiological limitations on adaptability, yield and quality in tomato. *Hort. Sci.*, 13: 673- 678.

71. Sun ZX, Min SK and Xiong ZM. (1989). A temperature sensitive male sterile line found in rice. Rice Genet. Newsl. 6: 116-117.

72. Tan, C.H., Yu, G.D., Yang, P.F., Zhang, Z.H., Pan, Y. and Zheng, J. (1992). Preliminary study on sterility of thermo-photosensitive genetic male sterile wheat in Chongqing. *Southwest China J. Agric. Sci.*, **5**(4): 1-6 (in Chinese).

73. Tan, Z. C., Li, Y.,Chen,B. and Zhou,G. Q. (1990). Studies on ecological and adaptability of dual purpose line Annong 1S. *Hybrid Rice*, 3: 35-38.

74. Tang, H.V., Pring, D.R., Shaw, L.C., Salazar, F.A. and Muza, F.R. (1996). Transcript processing internal to a mitochondrial open reading frame is correlated with fertility restoration in male-sterile Sorghum. *Plant J.*, 10: 123-133.

75. Timin, N. I. and Dobrutskaya, *E.G.* (1981). Cytoplasmic male sterility under different environmental conditions. *Ekol Genet Moldavian USSR*, 147: 1-17.

76. Tu, Z.P. and Banga, S.K. (1998). Chemical hybridizing agents. Narosa Publishing House, New Delhi. *In*: Hybrid cultivar development p.160.

77. Tuteja, R., Saxena, R.K., Davila, J., Shah, T., Chen, W., Xiao, Y.L., Fan, G., Saxena, K. B., Alverson, A. J., Spillane, C., Town, C. andVarshney, R. K. (2013). *Cytoplasmic male sterility-associated chimeric open reading frames identified by mitochondrial genome sequencing of four Cajanus genotypes.DNA Research*, 20 (5): 485-495.

78. van der Meer, Q.P. and van Bennekom, J.L. (1978). Improving the onion crop (*Allium cepa* L.) by transfer of characters from *A. fistulosum*. *Biuletyn Warzywniczy*, 22: 87-91.

79. Virmani, S.S., Viraktamath, B.C. and Lopez, M.T. (1997). Nucleus and breeder seed production of thermosensitive genic male sterile lines. *Int. Rice Res. Newsl.* 22(3): 26-27.

80. Virmani, S.S. and Voc, P.C. (1991). Induction of photo- and thermo-sensitive male sterility in *indica* rice. *Agron. Abstr.* 119.

81. Wang, Z., Zuo, Y., Li, X., Zhang, Q., Chen, L., Wu, H., Su, D., Chen, Y., Guo, J., Luo, D., Long Y.,Zhong, Y. and Liu, Y.G. (2006). Cytoplasmic male sterility of rice with Boro II cytoplasm is caused by a cytotoxic peptide and is restored by two related PPR motif genes via distinct modes of mRNA silencing. *Plant Cell* 18, 676–687.

82. Wei, B.G., Sun, G.C., Chang, J.W. and Jiang, C.X. (1994). The determination of photoperiod-temperature sensitive male sterile soybean. Proceedings of Third National Youth Symposium on Crop Genetics and Breeding. Edited by China Agricultural Scientech Press. p 185-189.

83. Wei, G., Zhang, Y., Zhang, D. and Liu, J.(1997). Mt DNA heterogeneity of cytoplasmic male sterility in maize. *Acta Genet Sin.,* 24: 66-77.

84. Wise R.P., Gobelman-Werner K., Pel D., Dill C.L. and Schnable P.S. (1999). Mitochondrial transcript processing and restoration of male fertility in T-cytoplasm maize *J. Heredity* 90: 380-385.

85. Worrall, D., Hlrd, D.L., Hodge, R., Paul, W., Draper, J. and Scott, R. (1992). Premature dissolution of the microsporocyte callose wall causes male sterility in transgenic tobacco. *Plant Cell*, 4: 759-771.

86. Xi, D.W., Cheng, W.J., Yi, D.L., Yu, D.R., Ning, Z.L., Deng, X.X. and Li, M. (1997). Genetic analysis on the TGMS line in rape of *B. napus* Xiangyou 91S. In: Lu RL, Cao XB, Liao FM, Xin YY, editors. Proceedings of the International Symposium on Two-Line System of Heterosis Breeding in Crops, 6-8 September 1997, Changsha, China. p 215-220.

87. Xing, Q.H., Ru, Z.G., Zhou, C.J., Xue, X., Liang, C.Y., Yang, D.E., Jin, D.M. and Wang, B. (2003). Genetic analysis, molecular tagging and mapping of the thermo-sensitive genic male-sterile gene (*wtms1*) in wheat. *Theor Appl Genet*., 107(8): 1500–1504.

88. Xu, N.Y. and Yan, J.Q. (1998). Studies on photoperiod-sensitive cytoplasmic male sterility in wheat. *J Wuhan Bot Res.*,16(2): 97–105. (in Chinese).

89. Yamaguchi, Y., Ikeda, R., Hirasawa, H., Minami, M. and Ujihara, A. (1997). Linkage analysis of thermosensitive genic male sterility gene, *tms-2* in rice (*Oryza sativa* L.) *Breed. Sci.,* 47: 371-373.

90. Yang, D. and Wang, N.Y. (1990). The breeding of themosensitive male sterile rice R 59TS. *Scientia Agric. Sinica*, 23: 90.

91. Yuan, L. (1986). Hybrid rice in China. *Chin J Rice Sci.,* 1(1): 8–18.

92. Yuan, S.C., Zhang, Z.G., He, H.H., Zen, H.L., Lu, K.Y., Lian, J.H. and Wang, B.X. (1993). Two photoperiodic reactions in photoperiod-sensitive genic male sterile rice. *Crop Sci*., 33: 651-660.

93. Zhang, K.T. and Fu, H.Y. (1982). Effect of high temperature treatment on male sterility in sorghum. *Acta Genet*. (China) 9: 71-77.

94. Zhang, D., Deng, X., Yu, G., Lin, X., Xie, Y. and Li, Z. (1990). Chromosomal location of the photoperiod sensitive male genic sterile gene in Nongken 58S. *J HuazhongAgric Univ*.,9: 407–419.

95. Zhang, M., Liang, C., Huang, Y. and Chen, B. (1994). Fertility expression of photoperiod temperature sensitive genic male sterile rice in Guangzhan and the response to photoperiod and temperature treatments. *J. Trop. Subtrop. Bot.,* 2(4): 100-107.

96. Zhang, Z.G., Yuan, S.C., Zen, H.L., Li, Y.Z., Li, Z.G. and Wei, C.L. (1991). Preliminary observations of fertility changes in a new type temperature sensitive male sterile rice IVA. *Hybrid Rice*, 1: 31-34.

97. Zhou, T.B., Xiao, H.C., Lei, D.Y. and Duan, Q.Z. (1988). The breeding of *indica* photoperiod male sterile lines. *J. Hunan Agric. Sci.*, 6: 16-18.

98. Zhou, W.C., Kolb, F.L., Domier, L.L. and Wang, S.W. (2005). SSR markers associated with fertility restoration genes against *Triticum timopheevii* cytoplasm in *Triticum aestivum*. *Euphytica*,141(1-2): 33–40. doi: 10.1007/s10681-005-5067-5.