RESEARCH ARTICLE

Molecular and Genetic Basis of Male Sterility in Development of Hybrid Varieties: A Review

[1,]*V. K. Mishra, [2] D. K. Dwivedi, [3]D. K. Upadhyay and [1]D. K. Pandey

[1] Mahatma Gandhi Chitrakoot Gramodaya Vishwavidyalaya, Chitrakoot, Satna M.P.
[2] Department of Plant Molecular Biology and Genetic Engineering, Narendra Deva University of Agriculture and Technology, Narendra Nagar (Kumarganj), Faizabad-224 229 U.P.
[3] Crop Improvement division, Indian Institute of Vegetable Research, Varanasi U.P.

ABSTRACT

Male and female gametes are normal and functional, but can only produce offspring when correct parents are mated; etc. self-incompatibility, cross-incompatibility. Male sterility is the failure of plants to produce functional anthers, pollen, or male gametes. Phenotypic (structural male sterility, sporogenous male sterility, functional male sterility) and genotypic (genetic, cytoplasmic, cytoplasmic-genetic male sterility). Genetic male sterility system in which sterility expression is conditional by environmental factors. In environmental sensitive genic male sterility, a particular range or concentration of environmental factor at sensitive stage of the crop, which occurs during panicle development, result in complete male sterility *i.e.* photoperiod sensitive genic male sterility (PGMS), thermo sensitive genic male sterility (TGMS), photo-thermo sensitive genic male sterility (PTGMS), micronutrient sensitive genic male sterility (MGMS). Male sterility is also use in crop improvement for production of hybrids.

INTRODUCTION

Male sterility as the failure of plant to produce functional anthers, pollen, or male gametes. The agricultural exploitation of hybrid crop varieties has enabled enormous increases in food productivity through increased uniformity and hybrid vigour. In 1763, the first documentation of male sterility came in Joseph Gottlieb Kolreuter observed anther abortion within species and specific hybrids. Male sterility is easy to detect because a large number of pollen grains are produced and are easily studied. Male sterility is assayed through staining techniques (carmine, lactophenol or iodine). Genetically engineered male sterility provides tremendous opportunities to the breeders for enforcing pollination control in hybrid seed production systems. The first single cross grain hybrid "HB1based on this male sterile line released in India in 1965 out yielded improved local open pollinated varieties (OPVs) by as much as 100% (Athwal, 1965). Commercial exploitation of heterosis in pearl millet only became possible with the development of a cytoplasmic nuclear male sterile line *Tift 23A* in the USA (Burton, 1965). There are three breeding approach for hybridization.

1) Three-line breeding or Cytoplasmic genetic male sterility (CGMS)
2) Two line breeding
3) One line breeding
4) 1st documentation: 1763--Kolreuter observed anther abortion within species and species hybrids.
5) More prevalent than female sterility:

Corresponding author: vinay.mishra111@gmail.com

a) Male saprophyte and gametophyte less protected from environment than ovule and embryo sac.
b) Easy to detect male sterility, because a large number of pollen for study available.
c) Easy to assay male sterility: staining technique (caramine, lacto phenol or iodine); female sterility requires crossing.
d) Male sterility has propagation potential in nature (can still set seed) and is important for crop breeding, fst does not.
e) Arises spontaneously via mutations in nuclear and/or cytoplasmic genes

Classification of male sterility

A. Phenotypic

1. Structural male sterility (anomalies in male sex)
2. Sporogenous male sterility (stamens form but pollen absent)
3. Functional male sterility (viable pollen but barrier prevent fertilization)

B. Genotypic

1. Genetic male sterility
2. Cytoplasmic male sterility
3. Cytoplasmic-genetic male sterility

1. Genetic male sterility

Genetic male sterility is ordinarily governed by a single recessive gene, ms, but dominant genes governing male sterility are also known, e.g., in safflower. Male sterility alleles arise spontaneously or may be artificially induced. A male sterile line may be maintained by crossing

it with heterozygous male fertile plants. Such a mating produces 1:1 male sterile and male fertile plants.

Utilization in crop improvement

Genetic male sterility may be used in hybrid seed production. The progeny from msms x Msms crosses are used as female, and are interplanted with a homozygous male fertile (MsMs) pollinator. The genotypes of the ms ms and Ms ms lines are identical except for the ms locus, i.e., they are isogenic and are known as male sterile (A) and maintainer (B) lines, respectively. The female line would, therefore, contain both male sterile and male fertile plants; the latter must be identified and removed before fertile plants; the latter must be identified and removed before pollen shedding. Pollen dispersal from the male (pollinator) line should be good for a satisfactory seed set in the female line. In India, it is being used for hybrid seed production of arhar (*C.cajan*) by some private seed companies. It is use in several other crops, e.g., cotton, soybean, etc. In two line system, EGMS (Environmental Sensitive genic male sterility) system or chemical hybridizing agent (CHA)

Environmental sensitive genic male sterility (EGMS)

This is a genetic male sterility system in which sterility expression is conditional by environmental factors. In Environmental sensitive genic male sterility, a particular range or concentration of environmental factor at sensitive stage of the crop, which occurs during panicle development, result in complete male sterility. Classification of Environmental senestve genic male sterility

A. Photoperiod sensitive genic male sterility (PGMS)
B. Thermo sensitive genic male sterility (TGMS)
C. Photo-thermo sensitive genic male sterility (PTGMS)
D. Micronutrient sensitive genic male sterility (MGMS)

A. Photoperiod sensitive genic male sterility (PGMS)

This type of male sterility was discovered in rice by Professor Shi Ming Song in Hubei province of China in 1973. Several male sterile plants were noticed in late japonica cutvar Nongken 58, when exposed to photoperiod of less than 13 hrs. and 45 minutes, turned to fertility (Shi-1981 and 1985, shi and deng- 1986, Lu and Wang-1988). PGMS trait from Nongen 58 S has been transferred to several elite japonica and indica cultivars through back crossing in china.

B. Thermosenestive genic male sterility (TGMS)

This type of male sterility which is controlled by temperature prevailing at sensitive stage of crop was discovered in china (Zhu *et al* 1991; Sun *et al* 1989). Subsequently it was reported from Japan (Maruyama *et al* 1991), Philippines (Virmani and Voc 1991), India (Ali 1993, Ali *et al* 1995, Satyanarayana *et al* 1995) and Vietnam (Du *et al* 1997). The most TGMS mutants reported so far, such as Annog-1s from China, Norin PL-12 from Japan, IR 32364 TGMS from Philippines and several mutant reported from India and Vietnam, the sterility caused by higher temperature (above 30^0C) at the sensitive stage where as at lower temperature (below 24^0C) fertility is observed. In few cases, sterility is observed at lower temperature and fertility is observed at higher temperature, such sterility referred as Reverse TGMS type. Reverse TGMS type reported are mutant Diaxin 1A and 4A and a mutant variety 26 Zhaizao from China, JP-38S from India. A shuttle breeding programe between China and IRRI was conducted and develop and evaluate TGMS line and two line hybrid rice. Three TGMS line are TM 104S, TM 105S (China) and IR73287-23S (IRRI) (Tongmin *et al.*).

S.No.	Rice varieties	Sterile (^{0}C)	Partial Sterile (^{0}C)	CSP (^{0}C)
1.	TM 104S	24.9^0C	23.9^0C	24-25^{0}C
2.	TM 105S	23.9^0C to 24.9^0C	22.9^0C	23-24^{0}C
3.	IR73827-23S	24.9^0C	23.9^0C	24-25^{0}C

CSP: Critical sterility point

C. Photo-thermo sensitive genic male sterility (PTGMS)

Several japonica and indica male sterile line have been developed, utilizing the Nongken 58S mutant. Intraction both with photoperiod as well as temperature. Such type of male sterility is called Photo-thermo sensitive genic male sterility (PTGMS). Critical light length for Nongken 58S, at 26^0C is 14.00 to 14.33 hrs, at 28^0C it is 13.67 to 14.00 hrs and at 30^0C critical light length is 13.67 hrs. at the same short day of 13 hrs, the spikelet fertility of Nongken 58S is 61.8% at 24^0C, 54.6% at 26^0C, 42.8% at 28^0C and only 21.5% at 30^0C.

D. Micronutrient sensitive genic male sterility (MGMS)

Deficiencies of copper, Boron and same other micronutrient are reported to cause male sterility in wheat and some other crops. High genetic variability has been reported in sensitivity to deficiency of these micronutrients.

Chemical induced male sterility

This non-genetic method of inducing male sterility involves the use of chemical called hybridizing agents (CHA) or gametocides. This method is very useful for plant with bisexual flowers in which it is difficult to obtain genetic or cytoplasmic genetic male sterility
The ideal gametocyte should be.

- Selectively induce male sterility without adversely affecting the female fertility.
- Have systematic effects so as to sterilize both early and late.
- Have a broad range of effectively in order to withstand adverse environmental conditions.
- Have minimum side effects on plant growth and panicle development

S. No.	Chemical	Concentration	Growth stage for application	Remark
1	Ethrel	800-1000 ppm	Prior to anthesis	
2	Monosodium methyl or Arsenate (MGI)	0.02 % or 2000 ppm	Uni-nucleate pollen stage	
3	Sodium methyl arsenate	0.02 % or 2000 ppm	Five days before heading	

For developing hybrids by using gametocides

a. The female line should have a synchronous flowering habit.
b. The line should respond to chemical treatment.
c. The parent should possess good out crossing trait.

Important factors that decide the efficiency of chemical gametocides are:

a. The correct dosage of the chemical.
b. Appropriate stage of treatment

2. Cytoplasmic male sterility

Cytoplasmic male sterility is the total or partial male sterility associated with plant biology as the result of specific nuclear and mitochondrial interactions. Male sterility is the failure of plants to produce functional anthers, pollen, or male gametes. They show non-Mendelian inheritance and are under the regulation of cytoplasmic factors. In this type, male sterility inherited maternally. In general there are two types of cytoplasm *viz.*, N (normal) and the aberrant S (sterile) cytoplasms.

Utilization in crop improvement

Cytoplasmic male sterility may be utilized for producing hybrid seed in certain ornamental species, or in species where a vegetative part is of economic value. But in those crop plants where seed is the

economic part, it is of no use because the hybrid progeny would be male sterile. The first time in rice, four cytoplasmic genetic male sterile (CMS) lines with different cytoplasmic backgrounds, namely, Wild abortive, *Oryza perennis* (through conversion), Kalinga I, and Lalruma (through indica/indica hybridization) were developed by repeated backcrossing with a common isonuclear maintainer, cultivar (cv.) Krishna. These four new CMS lines with a common nuclear background were confirmed to be cytoplasmically different from one another

3. Cytoplasmic genetic male sterility (CGMS)

It is caused by an interaction between genetic factor (s) present in cytoplasm and the nucleus. Absence of a sterility inducing factor either in the cytoplasm or in the nucleus makes a line male fertile.

The cytoplasmic genetic male sterility system involves

- A CMS (A) line
- A maintainer (B) line
- A restorer (R) line

A-line and B-line are exactly same genetically but they differ only in respect of cytoplasm content. A-line has sterility inducing where as, B-line has normal cytoplasm. The R-line possesses fertility restorer gene in dominant condition. The R-line will be always fertile with respect to cytoplasm either it is sterile or fertile.

CMS source can be identified in-

- Intervarietal reciprocal crosses
- Interspecific crosses

Utilization in crop improvement

The cytoplasmic-genetic male sterility is used commercially to produce hybrid seed in maize, bajra and jowar. A triple cross may be produced by crossing single cross with a fertility restoring inbred so that all the plants in the triple cross would be male fertile In this case, the sterility is transmitted only through the female and all progeny will be sterile. This is not a problem for crops such as onions or carrots where the commodity harvested from the F_1 generation is produced during vegetative growth. These CMS lines must be maintained by repeated crossing to a sister line (known as the maintainer line) that is genetically identical except that it possesses normal cytoplasm and is therefore male fertile. In genetic cytoplasmic male sterility restoration of fertility is done using restorer lines carrying nuclear restorer genes in crops. The male sterile line is maintained by crossing with a maintainer line which has the same genome as that of the MS line but carrying normal fertile cytoplasm.

Origin of Male Sterile Cytoplasm

a. Spontaneous mutation

Mutant male sterile cytoplasms arise spontaneously in low frequencies. Mutant cytoplasms have been isolated in maize, bajra and sunflower.

b. Interspecific hybridization

Transfer of the full somatic chromo-some complement of a crop species, through repeated backcrossing into the cytoplasm of a related wild species often leads to cytoplasmic male sterility. In cross-pollinated crop species, the male sterile cytoplasms have generally originated through mutation, while in self-pollinated crops they have been transferred from related species.

c. Induction through ethidium bromide

Ethidium bromide is a potent mutagen for cytoplasmic genes or plasmagenes. Male sterile cytoplasm may be induced by seed treatment with ethidium bromide e.g., Petunia

Limitation of Cytoplasmic-Genetic Male Sterility

The limitation of cytoplasmic-genetic male sterility is given below.

1. Undesirable - Effects of the Cytoplasm for e.g., the Texas cytoplasm in maize, by far the most successful cytoplasm commercially, slightly retards growth, yield (2.4%) plant height and leaf number; induces earlier silking and delayed pollen shedding; and makes the plants highly susceptible to *Helminthosporium* leaf blight. Restorer genes only restore male fertility; they are unable to remove the side effects of the male sterile cytoplasms.
2. Unsatisfactory fertility restoration, in many cases, restoration of fertility is not satisfactory and cannot be used in the production of hybrid seed.
3. Unsatisfactory pollination Natural pollination is often not satisfactory, except in wind-pollinated crops like maize. This reduces the production of hybrid seed, and thereby increases its cost. In some species e.g., *Capsicum*, this has prevented the use of male sterility in hybrid seed production. Poor pollination would always be a major problem in self-pollinators e.g. wheat.
4. Modifier genes may reduce the effectiveness of cytoplasmic male sterility.
5. Sometimes, cytoplasm may also be contributed by the sperm which, in the long run, may lead to a breakdown of the male sterility mechanism.
6. Male sterility mechanisms may break down partially under certain environmental conditions resulting in some pollen production by the male sterile lines.

In crops like wheat, polyploid nature of the crop and undesirable linkages with the restorer gene make it very difficult to develop a suitable restorer (*R*) line.

MOLECULAR APPROACH FOR MALE STERILITY

Marker assisted selection or marker aided selection (MAS) is a process whereby a marker (morphological, biochemical or one based on DNA/RNA variation) is used for indirect selection of a genetic determinant or determinants of a trait of interest (i.e. productivity, disease resistance, abiotic stress tolerance, and/or quality). This process is used in plant and animal breeding.

Restoring pollen fertility in transgenic male-sterile plant by Cre/loxp-mediated site-specific recombination system

This study was designed to control plant fertility by cell lethal gene Barnase expressing at specific developmental stage and in specific tissue of male organ under the control of Cre/loxP system, for heterosis breeding, producing hybrid seed of eggplant. The Barnase-coding region was flanked by loxP recognition sites for Cre-recombinase. The egg plant inbred/pure line ('E-38') was transformed with Cre gene and the inbred/pure line ('E-8') was transformed with the Barnase gene situated between loxp. The experiments were done separately, by means of Agrobacterium co-culture. Four T0-plants with the Barnase gene were obtained, all proved to be male-sterile and incapable of producing viable pollen. Flowers stamens were shorter, but the vegetative phenotype was similar to wild-type.

Barnase/barstar system for engineered male sterility (pp. 1226-1227 in Goldberg *et al*., 1993)

Barnase is extracellular RNase; barstar is inhibitor of barnase (both from bacterium *Bacillus amyloliquefaciens* which uses barnase for protection from microbial predators and barstar to protect itself from barnase. Fuse the barnase and barstar genes to TA29 promoter–TA29 are a plant gene that has tapetum specific expression–good for a wide range of dicot and monocot crop plants. Plants containing the TA29–barnase construct are male sterile; those with TA29–barstar are not affected by the transgene. Cross male sterile (barnase) with male fertile (barstar) to get hybrid seed, which now has both barnase and barstar expressed in tapetum and, hence, is fully fertile. Barstar is dominant over barnase.
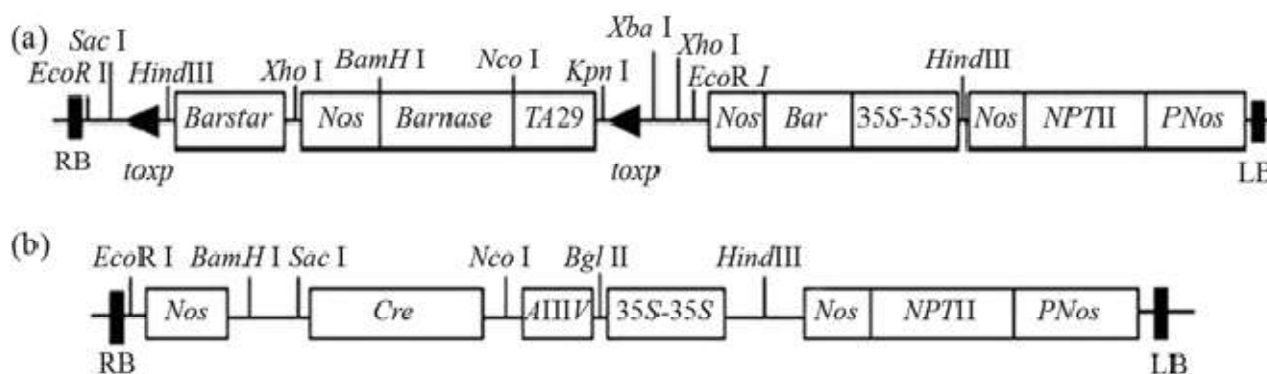
Fig. Sketch map of plant expression vector of Barnase and Cre gene. a: pCABARTABn. b: pBINPLUSCre

Engineering Male Sterility

The expression of a chimeric RNAase gene from *Bacillus amyloliquifaciens* in tobacco and rape heraldedthe beginning of engineering male sterility in crop plants. Mariani *et al.* (1990) constructed a transgenic rape line carrying the barnase gene from *B. amyloliquifaciens* in which barnase was selectively expressed in anther using anther-specific promoter. The expression of the barnase gene caused tapetal cell destruction causing pollen abortion. Restoration of sterility was achieved by crossing sterile plants to transgenic plants containing the barstar gene from *B. amyloliquifaciens*, which is a barnase inhibitor gene. The F1 expressed both barnase and barstar genes and was fertile due to inhibition of cytotoxic activity of barnase. Similarly, in tobacco transgenic lines expressing *rol C* gense of T-DNA of *Agrobacterium rhizogens* were found to be male sterile (Schmulling *et al.*, 1988). Over-expression of *rol C* genes also caused male sterility in other crops as well (Fladung, 1990). The restoration of *rol C* governed male sterility was achieved by crossing it with a transgenic line containing the role C-antisense gene (Schimulling *et al.*, 1993). Goetz *et al.* (2001) used antisense repression of extracellular invertase gene Nin 88, which caused sterility. Nin 88 is an invertase that catalyses cleavage of sucrose in tapetal cells and developing pollen and maintains a carbohydrate supply for energy demands. They were able to achieve highly cell specific antisense repression of Nin 88 using Nin 88 promoter, thereby resulting in normal plant growth and development except for the failure to produce functional pollen.

Even though male sterility is strongly associated with gene dysfunction in mitochondria, chloroplasts have emerged as a more than ideal choice for engineering male sterility through the transgenic approach. Such an approach offers a number of advantages such as high levels of transgene expression, multi-gene cassette engineering, transgene containment viz., maternal inheritance, lack of gene silencing, position effect due to site-specific transgene integration, and lack of pleiotropic effects (Daniell, 2005). Moreover, such engineering strategy can enable the transfer of male sterility systems in certain recalcitrant crops such as cotton and soybean (Grevich and Daniell, 2005). It also overcomes the disadvantages of nuclear transformation, wherein the transformants segregate for fertility and sterility (Ruiz and Daniell, 2005). Polyhydroxybutyrate (PHB) has been identified as a potential candidate gene system for engineering male sterility in crop plants. The synthesis of PHB is the result of three genes, namely PhaA (a-ketothiolase), PhaB (Acetoacetyl-coA-reductase) and PhaC (PHB synthase). The PHB causes abnormal development of the epidermis and endothecium with a broken tapetal layer. The first report of expression of Phab and PhaC genes *vis-a-vis* nuclear transformation came from Poirier *et al.* (1992), which yielded low levels of PHB. Nawrath *et al.* (1994) transformed Arabidopsis to reconstruct whole pathways by expression of all three genes by targeting their expression to chloroplasts and reported up to 14% PHB of leaf dry weight with no pleiotropic effects. In order to overcome pleiotropic effects of PHB, specifically due to PhaA gene encoding a-

ketothiolase, Bohmert *et al.* (2002) analyzed transgene expression via nuclear transformation and reported decreased levels of a-ketothiolase but reduced transformation efficiency. A recent study on chloroplast engineering showed no pleiotropic effects and complete male sterility (Ruiz and Daniell, 2005). They used the PhaA gene from *Acinetobacter* driven by constitutive 16S rRNA promoter (*Prrn*) and transferred through the biolistic method. The important finding was that the expression of PhaA caused 100 % male sterility; the light illumination reverted the sterile lines back to fertile lines. They proposed that the PHB biosynthesis system was a viable system for engineering male sterility in crop plants. In crops like maize where hybrid seed production is based on nuclear restoration factors, PHB-mediated CMS and its subsequent restoration by continuous illumination offers a novel approach for creating transgenic male sterile plants. This will help save the time and resources required to backcross a male sterility-inducing cytoplasm into elite genetic backgrounds (Havey, 2004). Another major advantage of chloroplast engineering is the scope for diversification of CMS sources by introduction of different sterility-inducing factors into elite parental lines. This, therefore, prevents the risk of genetic uniformity of CMS sources as was encountered in CMS -T in maize.

Identification of potential candidate genes is imperative for engineering male sterility in crop plants. In this context, a broad group of proteins called PPR (Pentatricopeptide repeat) proteins hold great promise. The PPR protein family is a large group of proteins frequently present in higher plants. They are characterized by tandem repeats of 35 amino acids, often arranged as a tandem array of 2-26 repeats per peptide (Small and Peeters, 2000), and are mainly targeted to mitochondria or chloroplasts. In Arabidopsis (450) and rice (600) such proteins have been identified and their closest homologues have been found in petunia (PPRS 92), maize (CRP1), radish (P67), and rice (PPR-8-1) (Fisk *et al.*, 1999; Lahmy *et al.*, 2000; Koizuka *et al.*, 2003; Kazama and Toriyama, 2003; Wang *et al.*, 2006) PPR proteins consist of two alpha-helices with the tandem repeats forming a supercoil with a central groove lined with hydrophilic side chains and positively charged bottom residues. Kotera *et al.* (2005) proposed that PPR proteins are basically involved in RNA editing in chloroplasts. In fact, the petunia Rf gene, which was the first restorer gene to be cloned (Bentolila and Hanson, 2001), was found to encode a PPR protein (PPR 592) that functions as an mRNA editing factor. Fertility restoration was followed by characterization of PPR proteins in other crops as well. An interesting observation in petunia was that non-restoring alleles carried a deletion in the promoter region of PPRs92 that reduced its tissue specificity. While the restoring allele is expressed in floral buds, the non-restoring allele encoding transcript is found in root tissues only. Kazama and Toriyama (2003) proposed that the Rf-1 restorer of BT type or ms-boro type in rice (Kadowaki *et al.*, 1990) was a PPR gene designated as PPR-8-1, encoding PPR motifs consisting of 18 repeats of 35 amino acids. This gene restored fertility by processing mRNA of the aberrant *atp 6* gene. The non-restoring allele had deletions in coding region. The sequence comparison of PPR-8-1 and petunia PPR-592 revealed 68% similarity

between the first and thirteenth PPR. In Kosena radish, *orf 687* restored fertility by reducing the level of *Orf 125* protein that causes sterility. Genetic characterization of *Orf 687* (Koizuka *et al.,* 2003) revealed that *Orf 687* had 16 repeated PPR motifs comprising 81.5 % of the protein sequence. Even though *Orf 687* shared only 27 % similarity, this was an important feature of the activity of PPR genes in regulating CMS-associated gene expression. Since most of the restorer genes seem to function by reducing the expression of CMS-inducing transcripts or proteins in mitochondria, PPR proteins are ideal candidate genes for use in engineering of male sterility in crop plants (Desioire *et al.,* 2003). Another PPR protein with 16 repeats of 35 amino acids encoded by RF-1 restorer gene in insboro rice that is widely used in seed production of Japonica hybrids was cloned by Komori *et al.* (2004). The RF-1 PPR protein is a mitochondrial-targeted protein that reduces expression of CMS-associated mitochondrial genes. Sequences analysis revealed that the recessive allele had a deletion in coding region. Most of the PPR proteins characterized to date have striking similarity among themselves and with 20 odd Arabidopsis PPR proteins. It is very intriguing to speculate about the function of these PPR genes in Arabidopsis, which are similar to Rf-PPR genes, as CMS has never been described in this plant. However, a likely proposition may be that these genes have evolved as RNA processing genes and have been recruited for restoration of sterility arising out of aberrant mitochondrial genes.

Marker-assisted breeding of photoperiod-sensitive male sterile japonica rice with high cross-compatibility with *indica* rice (Liangming, *et al.* 2010)

The incomplete fertility of japonica × indica rice hybrids has inhibited breeders' access to the substantial heterotic potential of these hybrids. As hybrid sterility is caused by an allelic interaction at a small number of loci, it is possible to overcome it by simple introgression at the major sterility loci. The use of marker-assisted backcrossing to transfer into the elite japonica cv. Zhendao 88 a photoperiod-sensitive male sterility gene from cv. Lunhui 422S (*indica*) and the yellow leaf gene from line Yellow249 (*indica*). The microsatellite markers RM276, RM455, RM141 and RM185 were used to tag the fertility genes S5, S8, S7 and S9, respectively. Line 509S is a true-breeding photoperiod-sensitive male sterile plant, which morphologically closely resembles the japonica type. Genotypic analysis showed that the genome of line 509S comprises about 92% japonica DNA. Hybrids between line 509S and japonica varieties suffer from a level of hybrid sterility, although the line is highly cross compatible with indica types, with the resulting hybrids expressing a significant degree of heterosis. Together, these results suggest that segment substitution on fertility loci based on known information and Marker-assisted selection are an effective approach for utilizing the heterosis of rice inter-subspecies

Apomixis for hybrid seed production

Apomixis is a form of asexual reproduction that occurs via seeds in which embryos develop without fertilization. Apomixis is known in some 300 species belonging to about 40 families; it occurs most frequently in compositae, Rosaceae and Gramineae. But there appears to be no genous composed entirely of apomictic species species, and there is no evidence that apomicts, even those with a facultative breeding system, have ever given rise to a new genus or subgenus.

Mechanism of CMS

The occurrence of male sterility in wild species is revealed by the coexistence of hermaphrodite and female (male sterile) plants in natural populations (gynodioecy). Most of the studies on such populations have led to the emergence of theoretical models based on the premise that CMS is a result of interaction between nuclear and cytoplasmic genomes. Gynodioecy is supposed to appear and be maintained in populations either due to female advantage or counter selection of nuclear fertility restorers (Budar *et al*., 2003). The genomic conflict theory (Cosmides and Tooby, 1981) provides for the

interaction of cytoplasmic determinants (that prevent pollen production) and nuclear restorers (that restore fertility). The modes of inheritance of cytoplasmic (uniparental) and nuclear (biparental) factors generate a situation where selective interests are conflicting, resulting in differential resource allocation for pollen and seed production. Most of the CMS's were a result of an alloplasmic state, wherein an impaired mitochondrial activity occurred due to a conflict at genomic, proteomic or transcriptomic levels (Kaul, 1988). Studies on the genetic and physiological factors underlying male sterility have revealed that significant differences exist between normal and sterile anthers (Nair, 1993). The tapetum, the innermost cell layer surrounding pollen grain, is a physiologically active site and nourishes the developing gametes. However, it degenerates later on and is not found as an organized tissue in mature anthers. The tapetum and other surrounding cell layers suffer early degeneration in sterile pollen as compared to fertile ones. An important question was: how does a mitochondrial dysfunction at the genetic and physiological level specifically alter pollen development (Levings, 1993)? Since mitochondria harbor genes governing vital biochemical pathways such as the electron transport system and encode enzyme systems and structural RNA's, how do such mutations specifically block pollen development, as mutations to any of them could be lethal?

One explanation could be that mitochondrial genes are expressed differently during anther development. Such differential gene expression may occur either by the interaction of CMS genes with some unknown factors in target tissue leading to localized developmental changes or interference of CMS gene products in the physiological machinery resulting in reduced respiration and ATP synthesis (Levings, 1993). Wallace (1989) suggested that minor impairments in mitochondrial function might selectively affect degeneration of specific cell types. Such deviations in expression were observed in all cell and tissue types and have also been reported in case of human cardiac dysrythmia (Wallace *et al*., 1988). Lee and Warmke (1979) observed a 10-fold increase in mitochondria/cell count in the tapetum during anther development. Such amplification indicates greater demand for energy. Sarria *et al.* (1998) observed that in *Phaseolus*, the CMS-associated gene product was degraded by proteases in mitochondria of vegetative cells. In CMS-T, the first sign of sterility is the rapid disorganization of tapetal cells soon after meiosis, which could be due to a mutant gene. Interestingly, such mitochondrial amplification has not been found in other maize tissues including the developing ear. *Urf 13* was proposed to be pivotal for affecting sterility in T-cytoplasm sources. Flavell (1974) proposed that some anther-specific substances might interact with *urf 13* proteins to cause sterile phenotypes, because *urf 13* is expressed in other organs as well.

The *urf 13* protein as such has also been found to be detrimental to cell viability, but such toxicity seems to be specifically mediated by membrane bound proteins for pollen abortion. Specific toxicity of *urf 13* protein to anther is not clearly understood. Levings (1993) proposed that it may be over-expressed in tapetal cells, a notion which gets boost from the fact that there are increased levels of mitochondrial biogenesis in tapetal cells during pollen formation. Alternately, the tapetal cells may be more sensitive to *urf 13* proteins. Iwabuchi *et al.* (1993) reported that CMS-Boro II is due to an abnormal version of the mitochondrial *atp 6* gene encoding aberrant mRNA transcripts in rice. Similarly, alterations in promoter regions of mitochondrial ATP synthase genes also cause sterility. Almost 12 mt-DNA regions associated with CMS have been found to involve ATP synthase subunit promoter regions and portions of coding sequences. Even if the chimeric gene itself does not involve an ATP synthase sequence, the ORF is often located in close proximity to an atp gene. In CMS-T maize, *orf 221* that is present downstream to *urf 13* encodes the Fo protein of ATP synthase (*atp 4*). Similarly, in CMS -S the *orf 77* is a chimeric gene downstream to *orf 355* and contains portions of coding sequences of *atp 9*. Cytotoxic gene products have been found to be associated with male sterility in sunflower (Nakai *et al.* 1995) and *Brasicaceae* (Duroc *et al.,* 2005). In radish, Ogura CMS

is controlled by two contranscribed reading frames *orf 138* and *Orf B*. While *Orf 138* is similar to most mitochondrial sterility-inducing genes, *orf B* codes subunit 8 of ATP- synthase complex (Gray *et al.,* 1998). In a recent study, Wang *et al.* (2006) characterized the role of mitochondrial open reading frames (ORF's) in male sterility in rice. They found that E. coli transformed with *Orf 79* established cytotoxicity of the *Orf 79* gene product. Later on, *Orf 79* was over-expressed in a normal, fertile rice line using the CaMV35S promoter and resulted in 750% pollen abortion, whereas female fertility was unaffected by such transgenic expression. They concluded that Qrf 79 encoded a cytotoxic peptide that caused CMS in rice. These and other studies have implicated different mechanisms of mitochondrial impairment in CMS phenotypes, but ultimately the programmed cell death of sporogenous tissue, especially the tapetum, causes pollen sterility. Mitochondria have been found to be involved in interpretation of cell death signals in both animals and plants. Recently Balk and Leaver (2001) showed that in CMS sunflower, the tapetal cells exhibited characteristic features of programmed cell death including the release of cytochrome c, cell condensation, and separation of chromatin into delineated masses and oligonucleosomal cleavage of nuclear DNA. Such characteristics are similar to apoptosis in mammals, except the condensation of nuclei and disintegration of cells into apoptotic bodies. In the case of maize, it was proposed that the *urf 13* gene caused male sterility by PCD, characterized by formation of pores in the mitochondrial membrane (Wu and Cheung, 2000). Holden and Sze (1987) proposed that in the presence of the Bm toxin of *Bipolais maydis*, the mitochondria carrying the *urf 13* gene experienced leakage of small ions and, consequently, loss of membrane potential. Tapetal degeneration has been found to be the common feature of such phenomenon in plants like petunia, wheat, sorghum, sunflower and maize (Laser and Lersten, 1972; Schnable and Wise, 1998). Frank and Barr (2003) generalized the term PCD for triggered and orderly death of cells with apoptosis as its specific subtype in animals.

SUMMARY

Male sterility is easy to detect because a large number of pollen grains are produced and are easily studied. Male sterility is assayed through staining techniques (carmine, lactophenol or iodine).Cytoplasmic male sterility show non-mendelian inheritance and are under the regulation of cytoplasmic factors. Cytoplasmic male sterility inherited maternally. In India, Genetic male sterility is used for hybrid seed production of arhar (*C. cajan*) by some private seed companies. It is use in several other crops, e.g., cotton, soybean, etc.,.The cytoplasmic genetic male sterile line is maintained by crossing with a maintainer line which has the same genome as that of the MS line but carrying normal fertile cytoplasm and commercially to produce hybrid seed in maize, bajra and jowar. The sterility caused by higher temperature (above 30^0C) at the sensitive stage where as at lower temperature (below 24^0C) fertility is observed at TGMS and PTGMS exposed to photoperiod of less than 13 hrs and 45 minutes, turned to fertility. Plastid transformation, DNA to specific region of gene transfer, MF gene can be transfer into the plastid genome of male sterile plant and expression male sterility. Use of transiently active promoters the combined lethal gene RNase or ribosomal inhibitor protein, then splice with the male fertility restoration gene and transfer into male sterile plants Barnase is a extracellular RNase; barstar is inhibitor of barnase (both bacterium *Bacillus amyloliquefaciens*) which use barnase for protection from microbial predators and barstar to itself for barnase. In Cre/lox system, the male fertility gene will be spliced into a DNA sequence to each end, recognized by Cre recombinase and transfer into the ms line. The assumption of MAS that linked allele associates with the gene and/or quantitative trait locus (QTL) of interest and also useful for traits that are difficult to measure, exhibit low heritability. In QTL analysis, Significance p value ($P<0.05$) obtained from differences between phenotypic means of groups due to recombination, use of marker system and the type of population linked to a QTL controlling trait.

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