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Induction of Male Sterility: A Boon for Plant Breeding

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A B S T R A C T

Male sterility has played a pivotal role in increasing agricultural crop production. Temporary male sterility is induced through floral sprays and is used for making crosses in recombination breeding program. Heritable male sterility is induced through treatment with mutagenic agents, in general, and other chemicals that target cytoplasmic genome. And re-establishment of communication restores fertility. Common, chemical agents that target the mitochondrial genome are reported to induce cytoplasmic male sterility successfully. Genetic male sterility is a frequent happening comparatively. In this review, we present different approaches for induction of male sterility, including agents that are generally recognized as safe (GRAS).

Introduction

In general, the life cycle of plants consists of two phases, one prolonged vegetative or sporophytic generation and a short sexual or gametophytic generation. During the entire period a seed germinate, differentiate into root and shoot, undergoes vegetative growth and completes its life cycle once the flower is converted into fruit. Male sterility is a common phenomenon seen in higher plant species. Inability of a living organism for sexual reproduction is known as sterility and failure to produce functional pollen is called male sterility. In agriculture, male sterility has been proven as highly beneficial to produce hybrid seeds which are often superior in terms of quality and yield compared to their parents. Plants exhibits diverse forms of male sterility, like absence of normal anthers or reduced anther size, difference in petaloidy, abnormal meiosis which leads to formation of empty, shriveled microspores or normal meiosis, but with abnormal microspore development and failure of anthesis that doesn't allow pollen shed (Kaul, 1988). In all the above cases of

male sterility, the female gametes are fertile with normal function. Male sterility in plants was first reported within species and species-specific hybrids as anther abortion in *Verbascum phoeniceum* by Koelreuter (1763). These male sterile plants are either selected from natural populations or may be induced artificially through mutagenesis. Anther and pollen development pathway consist of several stages involving formation of stamen, anther, pollen and anther dehiscence at maturity followed by pollen shed. Development of anther and pollen has been well studied in *Arabidopsis* (Wilson and Zhang, 2009), and many of the key genes involved in this pathway have been identified. During the process, the tapetum plays a central role in supplying nutrients, proteins, lipids and polysaccharides that are associated with pollen-wall formation and release of microspore (Parish and Li, 2010). Thus, most often, tapetal cells are targeted for inducing male sterility, this indicates very crucial role of mitochondria in male organ development.

Hybrid seed production

It is an important tool for improving the productivity of any crop for which occurrence of male sterility is critical. Crops, where large-scale emasculation is a tedious job, male sterile lines permit hybrid seed production as well as commercial exploitation of heterosis. Presence of genetic male sterility or genetic cytoplasmic male sterility, which are used for commercial purposes are not available in many crops. To enhance the production of hybrid seed at large-scale, to reduce the cost of hybrid seed production for commercial purpose, to minimize huge manpower required for emasculation and crossing and to hasten up the breeding programs, many efforts are made to induce male sterility in plant species. Heterosis, or hybrid vigor, has been shown to increase yield and successfully commercialized in many crops. Different methods such as Cytoplasmic Male Sterility (CMS), Chemical Hybridizing Sterility (CHS) etc are in use that ensure cross-pollination rather than selfing for hybrid production to, are diverse and species specific (Cheng *et al.*, 2007; Longin *et al.*, 2012). In the modern agriculture sector production of hybrid plants plays critical role in terms of increasing the production and in improving the product quality (hybrid vigor). But, hermaphrodite nature of most of the plants is the bottleneck in the cross between two different plants. Due to which fertilization occurs within the plants (self-pollination), so inhibiting self-pollination by any means (genetically, chemically or physically), which can disturb the male organs or the male gametes, facilitating cross pollination of plants and hybrid production in a large- scale is practiced. Thus, development of CMS lines for hybrid seeds is important as it evades self-pollination.

Types of male sterility and its use in plant breeding

Male sterility in simple terms refers to failure of a plant to produce functional anthers or male gametes. Once the concept and importance of hybrid vigor was realized, this trait was utilized to incorporate into many crop species for crop improvement (Sage, 1976). The term heterosis or hybrid vigor means superiority of progeny to its parents in terms of yield, followed by other traits like biotic or abiotic stresses, its adaptability to different environmental conditions, its vegetation etc based on the type of crop involved. Hybrid vigor is considered for progenies (hybrid) that are developed by crossing two parents in either to incorporate desired traits of one parent into another or for development of new hybrid. Development of hybrids is a challenging task in self-pollinated crops as it requires avoidance of self-pollination, which is conventionally carried

out by emasculation method. It is a tedious job involving time, manpower and skill. Here male sterile lines come into picture with a leading role that permits hybrid seed production as well as commercial exploitation of heterosis. Male sterility is either phenotypic or genotypic in nature and the genotypic male sterility can be genetic or cytoplasmic.

Cytoplasmic male sterility (CMS)

Cytoplasmic male sterility is not a common type of male sterility in nature (Vinod, 2005) and arises due to spontaneous mutation in organelle (especially in mitochondrial) involving rearrangements of the mitochondrial genome. Mutations in CMS are due to different recombination events that occur between homologous and non-homologous genome of the two individuals resulting in new Open Reading Frames (ORFs) (Dufay *et al.,* 2007).The CMS trait is maternally inherited, due to which transfer of the CMS character from the female parent to the progeny plant is carried out and the progenies are completely male sterile. During crossing a CMS plant is used as female partner or recipient, while a fertile plant is used as male partner (pollen donor). The contribution of the male parent is limited mainly to nuclear traits (Laughnan and Gabay-Laughnan, 1983). In CMS system cytoplasm plays critical role for the development of male sterile or fertile lines. So, there are two types of cytoplasm *Viz.,* S type (sterile) and N type (normal). Such male sterility is stable and can be seen in a large number of crops, however cannot be used were seed is an economic part.

Use of CMS lines for generating hybrids was first employed in maize, thereafter it has been continuously expanded in major food crops like wheat and rice (cereals), onion, carrot, sugar beet, pepper (vegetables), brassicaceae, sunflower, soybean (oilseeds), sorghum, pear millet (millets), legumes etc (Mackenzie, 1988; Singh *et al.,* 2015; Bohra *et al.,* 2016; Kalia *et al.,* 2019). CMS provides an expedient mechanism to produce large populations of male-sterile plants for commercial F_1 hybrid seed production. Apart from hybrid seed production, CMS system can also be utilized for basic studies. It can be used as a model system to study the interaction between the genetic material of both mitochondria and nucleus, different genes responsible for induction of male sterility (Hanson and Bentolila, 2004) and development of male gametophytes (Fujii *et al.,* 2011). It also helps us to study role of mitochondria in the development of reproductive organs in flowering plants (Hanson and Bentolila, 2004) and to develop seedless citrus varieties (Fang *et al.,* 2016).

Cytoplasmic-genetic male sterility (CGMS)

Cytoplasmic genetic male sterility (CGMS) is very common and available throughout the plant kingdom (Vinod, 2005). It mainly arises due to the compatible interaction and double mutation in both mitochondrial and nuclear genomes. Here also there are two types of cytoplasm as in case of CMS, however restorers with fertility (Rf) genes are also present. These genes don't express unless they come in contact of S cytoplasm. So, a combination of S cytoplasm with rfrf produces only sterile lines, whereas N cytoplasm with RfRf combined with S cytoplasm and rfrf leads to fertile lines. The restore genes in CGMS differ from Rf genes present in genetic male sterility. During this interaction male sterility is induced by mitochondrial genes, whereas nuclear genes restore the male fertility (Rf genes) traits. This type of male sterility system was first identified in onion (Jones and Davis, 1944), later it was seen in other crops such as jowar, bajra, maize, cotton, sunflower, rice and

wheat. It is highly reliable and stable system and can be used in both seed and vegetative propagated crops. The difference between CMS and CGMS is due to their fertility restoration mechanisms. In CMS male fertility is regulated by N type cytoplasm of the maintainer line, whereas in CGMS, dominant, fertility restoring genes are located in the nucleus of restorer line, leads to fertility restoration. For breeding and CGMS based hybrid seed production involves three different lines as used in CMS system**.**

Genetics and Mechanism behind CMS

The sterility factor (S) is present on mitochondrial DNA, whereas the fertility restorer allele (rf) is present on nucleus genome. For breeding and hybrid seed production CMS and CGMS system involves three different lines. A line (sterile) having both recessive allele (rfrf) and (S) factor (Sterile cytoplasm), B line denoted as maintainer of the female line (male sterile) and has fertile (N) cytoplasm with recessive restorer allele in nucleus (rfrf). When an identified A line (male sterile) is crossed with male fertile B line (maintainer) having recessive 'rfrf' factor and cytoplasm (N), all the resultant progenies are male sterile (with S cytoplasm) with same nuclear constitution (rfrf) as both A and B line has recessive allele for fertility restoration. The B line has an ability to make the A line set seed in the progeny, but the plant remains sterile, thus the CMS line is maintained (Figure 1a). If R line (restorer), which contains dominant allele for fertility restoration (RfRf) is identified for CMS then it can be used for hybrid seed production. This line has the ability to restore the male fertility of the hybrid plants (F_1). These restorer lines are identified and are used for commercial hybrid seed production. So, when a CMS with S cytoplasm and rfrf is crossed with restorer line having N cytoplasm and dominant fertility restorer gene (RfRf), thus sterility inducing effect of CMS cytoplasm is reversed and leads to fertile hybrids. For large scale seed production, the progenies are selfed (Figure 1b). In the next phase, if a new genotype is intended to make CMS, then the pollen from the intended line will be used on the identified CMS line. This will constitute male sterile cytoplasm, but nucleus is composed 50 % original and 50 % intended line. Resultant CMS plant will be repeatedly back crossed with the pollen from the intended plant to restore nucleus of the intended line, but the cytoplasm will be male sterile (Figure 1c).

Genetic male sterility arises due to spontaneous variation in male fertility nuclear genes (independent of cytoplasm influence) and follows Mendelian gene inheritance pattern. This is one of the most common forms of male sterility found in many plants (Kaul, 1988) both in monocots and dicots. It is governed by recessive gene (ms), which targets the entire process of male organ development leading to male sterility. It involves an extra- step such as identification and removal of heterozygotes from the group, for hybrid seed production. When male sterile line (msms) is crossed with male fertile parent (MsMs), all F_1 progenies are male fertile (Msms) due to the presence of dominant fertility controlling allele (Figure 2). Inheritance of dominant male sterility is suitable for hybrid seed production as it represents 75% of the population sterile (Figure 3a), whereas inheritance of recessive male sterility represents only 25 % population sterile (Figure 3b). These sterile lines may be lost if they are not maintained as heterozygotes (Msms), thus they need to be pollinated with either a fertile hetero (Msms) or homozygous (MsMs) maintainer line. But if the expression is controlled by a dominant allele (MsMS) it is difficult to maintain the lines through reproductive means. Utilization of GMS in hybrid seed production involves

two lines and its use was limited for hybrid seed production due to improper maintenance of male sterile lines. These limitations were overcome with development of Environmental Genetic Male Sterility, (EGMS), which doesn't need any maintainers.

Environmental dependent genetic male sterility

In this system male sterility and fertility trait are expressed and controlled by specific environment conditions like temperature (low or high), variable light intensity (short or long photoperiod), different soil borne stresses (Kaul, 1988). This system is heritable in nature. Sterility trait is controlled only by single recessive nuclear gene without the involvement of cytoplasm, so there is no need for fertility restorer lines to convert the sterile lines into fertile. Hybrid rice has been successfully developed and applied widely in agriculture based on two-line system using photosensitive genetic male sterility (PGMS) and thermosensitive genetic male sterility (TGMS) lines (Zhou *et al.,* 2012; Chen and Liu, 2014). PGMS was discovered by Shi (1981), where sterility in plant depends upon the length of photoperiods. Male sterile mutant rice lines were induced using either sterile or fertile pollen depending on the changes in temperature (Yang and Wang, 1989). TGMS is controlled by a single recessive nuclear gene (msms), where plant remains sterile or fertile at a particular temperature or photoperiod (Virmani and Ilyas-Ahmed 2001).

Confirmation of type of male sterility generated

Identification of type of male sterile system induced by any of the above-mentioned method can be carried out by detecting its progeny on crossing with normal or control genotype. In the first case if all the progenies in a row are sterile then the system is cytoplasmic male sterility (CMS). In the second case if some rows are fertile and some rows show both fertile and sterile individuals in 1:1 ratio then the system belong to genetic male sterility (GMS). In the third case if some rows are completely sterile, some rows are completely fertile and some rows containing both sterile and fertile lines in the ration 1: 1 then the male sterility belongs to cytoplasmic genetic male sterility (CGMS).

How male sterility is introduced

Under traditional breeding, the plant breeders emasculate the anthers by hand to prevent self-pollination for crossing, which is a labour intensive as well as cost expensive procedure, however, with the availability and use of male sterile lines hybrid seeds can be produced easily for research or cultivation. Sterility can be genetic (nuclear) termed as genetic male sterility (GMS) or can be due to cytoplasmic termed as (CMS) due to the genome of mitochondria. These sterile genes can be recessive or dominant. The recessive inheritance of GMS made it difficult to fully utilize its potential, because the resultant progeny is 50% sterile and 50% fertile, thus attention of breeders was inclined more towards CMS where 100% sterility is achieved. CMS is widespread in occurrence in higher plants and is reported in more than three hundred species (Kaul 1988). The majority of CMS are due to wide hybridization, spontaneous mutation, using mutagenic and chemicals hybridizing agents (CHA) as gametocides, using frontier tools of biotechnology such as genetic engineering. Many efforts have been made in this endeavor.

Natural variation

Nature is a collection for unlimited variations and some of the economically important traits

has been collected, characterized and exploited in the past in different crops by the researchers. A revolution in seed sector and in hybrid seed programme was seen with the identification of male sterility trait that made us self-sufficient. Generation of genetic variations is a continuous process by the nature, which need to be carefully observed and selected by the researchers in the near future.

Wide hybridization

Intergeneric hybridization involves cross between two different genera. In this type, crosses may not be successful due to highly diverse nature of the two parents. In such cases, the embryos are rescued by *in vitro* culture. Resultant hybrid may be sterile due to some abnormalities during meiosis. On the other hand, interspecific hybridization involves cross between species within the genera. It may be either between wild relative with a cultivated parent or within the cultivars, thus development of a new genotype (integration of cultivated nucleus into the cytoplasm of wild species). Breeding populations developed from different inter-varietal crosses sometimes generate new genetic variation that may arise due to rare recombination of recessive alleles or transgressive segregation. In many crops like pearl millet, soybean, and cotton, cytoplasmic nuclear male sterility has been derived from recombinant populations in the past (Kaul, 1988). The frequency of such useful recombination is, however, very low.

This is being used as the most common and successful approach for breeding cytoplasmic nuclear male- sterile genotypes in many crop species of cereals, legumes. Wide crosses in development of CMS lines have been reported in oilseed crops. In soybean, Sun *et al.,* (1997) crossed wild-type Chinese *Glycine max* and wild annual *G. Soja* and in the progeny the pollen grains showed mitochondria with degraded plastids, puffy inner mitochondrial spaces, undeveloped intine, and absence of starch and lipid reserves. Thus, mentioned degeneration of mitochondria and energy deficiency leads to male sterility (Smith *et al.,* 2002). Similarly, Ding *et al.,* (2002) crossed two cultivated *Glycin max* cultivars and reported male sterile progenies followed by backcrosses that resulted in CMS, no pollen germination was observed and pollen abortion was recorded at binucleate stage. Wide crosses between *Sesamum indicum* and *S. malabaricum* revealed male sterile lines due to cytoplasmic differences in the two parents (interaction of the cytoplasm of *S. malabaricum* with the nuclear genome of S. *indicum*) rather than chromosomal abnormalities (Prabhakaran *et al.,* 1995). Cross between another oil producing crop, *B. juncea* var. 'Pusa Bold' with its wild species, *Diplotaxissii folia* followed by repeated backcrosses with *B. juncea* lead to CMS plants having no effect on female fertility however, shrivelled anthers were observed, which failed to dehisce (Rao *et al.,* 1994). Intergeneric hybridization between *Festuca pratensis* (female parent) and *Lolium perenne* induced male sterility (cytoplasm/genetic) (Connolly and Wright-Turner, 1984). Similarly, cross between Sorghum × Saccharum showed complete pollen sterility. They mentioned presence of fragmented chromatin material in the anther sac due to abnormalities that occurred during meiosis, degradation of chromatin content in uninucleate cells (Sobhakumari and Nair, 2013).

Based on the amount of genetic material contributed by the two parents involved in the somatic hybridization process recombination of genetic material of the two mitochondria can be used for the development of CMS either by symmetric or asymmetric fusion of protoplast (Garcia *et al.,* 2019). Very recently

Chen *et al.,* (2020) developed CMS line in a cash crop, tobacco (Nta(gla)S) by asymmetric somatic hybridization between protoplasts of K326 and *N. glauca* followed by backcrossing with K326. These lines were represented by short filaments and shrivelled stamens. Similarly, a novel CMS with carpelloid stamens was induced by protoplast fusion of *Chinese Woad* and *Brassica napus* (Kang *et al.,* 2017).

Chemical hybridizing agents (CHAs)

Though different male sterility systems (GMS, CMS and CGMS) are available, still researchers felt the need for chemical induced male sterility in order to overcome the tedious process of emasculation, which involves tremendous manpower and money demanding for hybrid seed production. Chemical based induction does not involve identification, maintenance of male sterile and restorer lines. They bypass the issues like identification and restoration of male sterile line associated with GMS (McRae, 1985) and is alternative method to generate male sterility in plants. They act as gametocide and target the male gamete by creating abnormalities, thus effecting development of pollen, its viability and its dehiscence (Cross and Ladyman, 1991) without affecting the female fertility.

The mode of action involves disruption of meiosis, interruption of anther development, degeneration of microspores, formation of thin walled exine, non-viable and irregular microspores development. They decrease starch deposition and lead to abnormal growth of tapetal layers. They either prevent or delay the process of dehiscence of normal anthers with viable pollen or inhibit viable pollen germination on stigma or elongation of pollen tube to egg for fertilization, thus leading to induction of sterility (Sharma and Sharma, 2005).

Effect of different gametocides on pollen germination and sterility percentage has been reported in different crops. Induction of male sterility was reported by Moore (1950) in *Zea mays* L. with maleic hydrazide (MH). Foliar application of 50, 200, 800 µg/ml MH affected the pollen germination percentage of *Phaseolus mungo*, *P. aureus*, *Cyamopsistetra gonolob*, respectively (Salgare, 2004). Induction of male sterility by ethyl 4-fluorooxanilate (E_4FO) has been reported as highest pollen and spikelet sterility on application of 1500 ppm when applied at stamen-pistil primordia stage or at a stage between pollen mother cell formation and meiosis (Jahuar *et al.,* 1999) and at a rate of 1.5mg/l (Ali *et al.,* 1999) in rice. In wheat 99.7 % sterility was reported without any chemical residues in the plants (Chakraborty and Devakumar, 2006). About 96–99% male sterility was reported in tef by E_4FO @ 1–1.5 mg/l without affecting female fertility (Ghebrehiwot *et al.,* 2015). Application of either 2 mg/l E_4FO or 3 ml/l ethrel was found effective in inducing male sterility in sorghum (Amelework *et al.,* 2016). Other chemicals like ethrel @ 800 ppm and salicylic acid @ 600 ppm induced high percentage of male sterility in rice (Praba and Thangaraj, 2005). Detergents also have proven effective in induction of sterility of the pollen in crops like mustard, rice (Chauhan and Vandana Singh, 2002), and niger (Gangaprasad *et al.,* 2004) at different concentrations (1 to 6 %). Complete and long-lasting sterility (91-99 %) was reported by foliar application of surf excel in lentil (Singh, 2017) and 99.87% pollen sterility in sunflower without effecting the production in sunflower, the treatment led to elongation of the style in floral buds (Tripathi and Singh, 2008). Plant growth regulators (PGR) has also been reported to target the male organ. Application of Gibberellic acid (GA) @ 150 ppm in sunflower resulted in maximum pollen sterility (Garcia Torres, 1979). Duca *et al.,*

(2014) reported exogenous application of gibberellic acid (GA_3) induces the expression of CMS specific *orfH522* gene in sunflower when applied at apex of inflorescence in the early reproductive stages.

Jinyang *et al.,* (2018) induced male sterility in rapeseed (*Brassica napus* L.) by Tribenuron-methyl (TBM), herbicide, which inhibits the activity of acetohydroxyacid synthase (AHAS), an enzyme in the first step of the amino acids biosynthesis pathway (branched-chain). Liu *et al.,* (2007), reported high sterility in *Brassica napus* with novel CHA (EN) @ 0.5- 0.8 µg/mL at uni-nucleic stage, however some effects on female fertility and agronomic traits were also reported. George and Rooney *et al.,* (2018) reported application of TFMSA, trifluoromethanesulfonamide, 2 mg at 2-6 d (time) before the emergence of flag leaf for inducing male sterile plants in sorghum. Similarly, application of 30 mg TFMSA at 34 d before the flag leaf emerged also induced complete sterility of the panicle without changes to female fertility and no phytotoxic effects were observed at higher dosage (40 mg). However, genotype dependent response to TFMSA was observed. Clofencet is a pyridazinone, which induced insufficient levels of male sterility in wheat cultivars (Parodi and Gaju, 2009).

Similarly, Benzotriazole, a copper chelating compound that inhibits microspore development (Cross and Schulz, 1997) was found successful in inducing male sterility in a wide range of plants (Shivanna and Sawahney, 1997; Castro *et al.,* 2001). In sunflower complete pollen sterility by Benzotriazole was reported without affecting the seed yield (Tripathi and Singh, 2013). Other mutagenic agents such as acriflavine (1500 ppm), and ethidium bromide (2000 ppm) produced male sterility in sugar beet (Kinoshita *et al.,* 1980, Mikami *et al.,* 1980), CMS lines in Tift $23DB_1$ (pearl millet) using ethidium bromide (250 and 1,000 ppm) at 5 °C for 40 hours (Burton and Hanna, 1976). SQ-1 is an eco-friendly and approved gametocide that hinders pollen development after meiosis (Cheng *et al.,* 2004). It is generally used as spray for production of male sterile lines at commercial level. SQ-1 has been used to induced male sterility in wheat (Liu *et al.,* 2018), common millet (Cui, 2008), 95–100 % male sterility by spraying at an optimum dosage in foxtail (Song *et al.,* 2011; Zhang *et al.,* 2017), > 90 % male sterility at 5.0 kg ha-1 in Maize (Wei *et al.,* 2012).

Induction of male sterility using antibiotics in crops

Antibiotics are powerful drugs used as anti-bacterial agents to combat diseases mainly caused by bacteria either destroying or by slowing down the growth of bacteria by targeting the extracellular organelle, which leads to mutation. The endosymbiotic (bacterial origin) nature of mitochondria and chloroplast makes this organelle vulnerable to antibiotics. Both of them have bacterial type ribosome in plants (chloroplasts: 70S and mitochondria: 70–80S) that differs with cytoplasm (80S) (Tiller *et al.,* 2012). Some antibiotics (Streptozoocin) are potent mutagen and carcinogenic and leads to point mutations, while other antibiotics have chromosome-breaking properties. Though the mechanism of action of these antibiotics on extracellular organelle is unclear, but it is believed that they induce the formation of toxic reactive oxygen species (ROS) in bacteria and imbalance mito-nuclear protein by effecting mitochondrial translation. Tetracycline at low concentrations reported to induce proteotoxic stress in mitochondria that effects the expression of nuclear genes and hampers the function by modifying fission and fusion process in different organisms including plants (Moullan *et al.,* 2015).

Fig.1 Cytoplasmic male sterility models: a) Maintenance of CMS line; b) Hybrid seed production using CMS or CGMS system; c) Conversion of breeding line to new male sterile

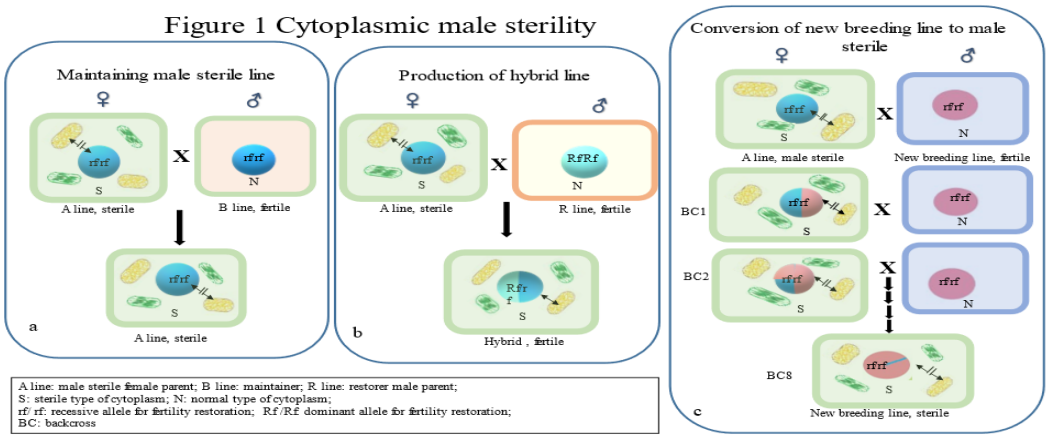


Fig.2 Genetic male sterility, Commercial hybrid seed production

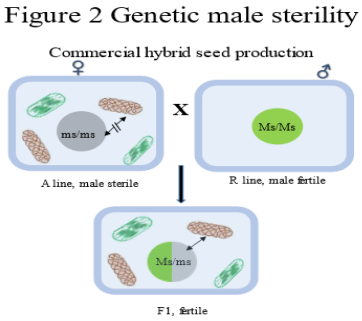
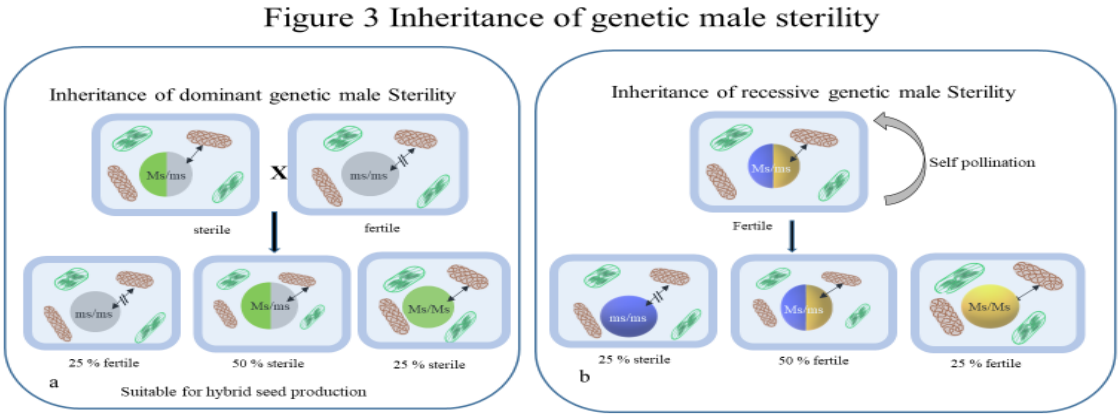


Fig.3 Inheritance of genetic male sterility a) Inheritance of dominant genetic male Sterility, b) Inheritance of recessive genetic male Sterility



Induction of CMS with chemicals targeting organelle DNA has been explored in some crops. Streptomycin acts as a mutagen by targeting protein translation of non-chromosomal elements (Sager, 1962). These organelles are sensitive to streptomycin and it has been successfully used to induce male sterility in several plant species including rice, sorghum, pearl millet, sugar beet, and sunflower (Elkonin and Tsvetova, 2008). Streptomycin generated mutation of extra chromosomal genes and developed CMS in maize (Petrov *et al.,* 1971), both CMS and GMS in rice at a rate of 250 and 1000 ppm (Sarma and Patnaik, 1932).

Stable CMS mutant were induced with both Mitomycin (50 ppm) and Streptomycin (200 and 500 ppm) at low temperature for 40 hours in pearl millet (Burton and Hanna, 1982). Both CMS and GMS were induced in cultivated sunflower using Mitomycin C and Streptomycin (Jan and Rutger, 1988).

In sugar beet 1500 ppm streptomycin induced CMS (Kinoshita *et al.,* 1980). In wheat both stable and complete male-sterile mutants were generated using streptomycin. In-complete male-sterile mutant with low seedset (less than 5%) was also reported (Zhiyong *et al.,* 1997). Dang *et al.,* (2000) treated oilseed flax with five antibiotics, streptomycin, penicillin, rifampicin, erythromycin and tetracycline @ 0, 500, 1500 and 2000 mg/l for 24 h prior sowing and induced male sterile mutants with very low (0.25%-0.8%) frequency.

We initiated seed treatment of Indian foxtail millet with different doses of streptomycin solution at two different time intervals (24 and 48h) by seed soaking method for induction of heritable male sterility. It resulted in partial sterile inflorescence with sterile pollen grain, however more efforts need to be made in this endeavour.

Transgenic approach

CMS system suffers with disease susceptibility, poor genetic diversity, and unstable restoration, which can be overcome by GMS system, however, generating male-sterile female lines on large-scale by self-pollination is a challenging task. Development of frontier tools and technologies involving rapid methods to identify a MS gene, its isolation, transformation and its introgression into a desired genotype by genetic engineering method for development of a new male sterility systems in plants (Wu *et al.,* 2016, Zhang *et al.,* 2018) has been possible. Male sterility/ restoration system was reported in *Brassica juncea* using barstar gene for heterosis breeding (Jagannath *et al.,* 2002). Recently, Liu and Yang (2020) transformed *B. nivea atp9* RNAi vectors into tobacco plants and reported 50% pollens sterile, thus utilization of *atp9* gene for developing male sterile lines in plants. Similarly, sunflower CMS associated *orf*H522 gene was transformed into tobacco plants and generated transgenic male sterile lines (Nizampatnam *et al.,* 2009). Whereas, wheat mitochondrial *atp9* was transformed to develop transgenic tobacco male sterile lines (Hernould *et al.,* 1993). Roque *et al.,* (2019) reported development of transgenic male sterile using pea anther specific promoter PsEND1 fused with ribonuclease gene in both model and crop plants by aborting anthers in the early stages of development.

Very recently, a new technology CRISPR/Cas9 system has emerged as an effective tool for genome editing (site-specific) that generates sufficient amounts of mutation and is simple to operate. This, technique has been widely used to develop male sterile lines in different crops. A novel male-sterile line was developed by targeting stamen-specific gene *SlSTR1* by CRISPR-

Cas9 and developed a transgenic maintainer by transforming male-sterile plants with a fertility-restoration gene linked to a seedling-colour gene in tomato (Du *et al.,* 2020). Artificially synthesized *Cas9* gene with biased codons targeted *MS8* gene (Chen *et al.,* 2018), and *MS26* gene (Djukanovic *et al.,* 2013) in maize to develop male sterile lines. The technology was used to mutate *ZmTMS5* gene in maize and generated *tms5* male sterile thermosensitive mutants (Li *et al.,* 2017), similarly, *TMS5*gene was targeted in rice to generate new thermo-sensitive genic male sterile (Barman *et al.,* 2019).

Mitochondria a powerful organelle for male sterility

Mitochondria and chloroplast are the extracellular organelles endowed with their own genome, and are maternally inherited. Experiments with protoplast fusion showed that CMS phenotype is not associated with chloroplasts. Significant differences on comparison of mtDNA from fertile and CMS plants were reported (Belliard *et al.,* 1979). The trait CMS is associated with morphological and functional damage of mitochondrial and was supported by somatic hybridization and other genetic approaches in tobacco (Belliard *et al.,* 1978). Linkage between CMS and the mitochondrial was strongly supported by reversion of CMS phenotype (Hanson *et al.,* 1989). Mitochondria are the most active organelle that has a tendency to undergo repeated fusion and fission (Tilokani *et al.,* 2018). They are the power house for energy generation and plays crucial role in respiratory energy production via tricarboxylic acid cycle (TCA) and oxidative phosphorylation in eukaryotes. Reduced levels of ATP are associated with increased concentrations of reactive oxygen species (ROS), especially when mitochondria are disfunction, which play a major role in the pollen production (Horn *et al.,* 2014). All the events associated with anther development (microsporogenesis) are tremendous energy demanding, which is mainly fulfilled by the power house of the cell- mitochondria. So, any abnormalities hamper the function of mitochondria, which in turn targets the normal pollen development process leading to cytoplasmic male sterility.

Mechanism lying behind the CMS development is not yet clear, as every male sterile system varies depending upon the mitochondrial gene that is responsible of sterility. However, role of energy deficiency (Heng *et al.,* 2018), reactive oxygen species (ROS) (Liu *et al.,* 2018), programme cell death (PCD) (Qiu *et al.,* 2018) and signals from mitochondria affecting the nuclear pathway (Chakraboraty *et al.,* 2015) are evident. Variation in electron transport of Petunia and toxin-mediated membrane disruption due to toxin in maize plants, bacteria, provide some information about mechanisms for disruption of pollen development (Hanson, 1991). Role of different mitochondrial genes in induction of male sterility in different crops has been reported, Succinate dehydrogenase, (SDH) is a part of mitochondrial complex II, and is involved in TCA cycle and respiratory electron transport chain. Leon *et al.,* (2007) reported pollen abortion and reduced seed set by down-regulating *SDH1-1* by RNA interference, which is important for gametophyte development in *Arabidopsis.* Similarly, Chen *et al.,* (2019) studied role of mitochondria fission in pollen development in *Arabidopsis*. Dynamic related proteins play major role in mitochondria fission. 3D analysis of single tapetum cell from *drp3a*, *drp3b*, and *elm1* mutant showed change in the volume and shape of tapetal cells, variation in morphology and number of mitochondria in the tapetum during pollen development. These abnormalities in mitochondrial fission led to reductions in pollen development due to

mitochondrial disruption in the tapetum and pollen (Chen *et al.,* 2019). Tapetum degrades to nourish the pollen grains during their development. Degradation of tapetal cells at right time is prerequisite for normal pollen development. Xie *et al.,* (2020) reported mitochondrial aldehyde dehydrogenase *OsALDH2b* as a key regulator of tapetum degeneration in rice (*Oryza sativa*). Mutation in *OsALDH2b* gene accumulates extra malonaldehyde and early PCD in tapetum, which lead to premature degradation of tapetum and abnormal development of microspores. Recently in wild rice a mitochondrial gene, *WA352* was identified that interacts with COX11, a mitochondrial protein encoded by nucleus. Accumulation of WA352 protein in the tapetum hinders peroxidase metabolism activity of COX11 and leads to PCD followed by pollen abortion and development of CMS (Luo *et al.,* 2013). Another example comes from cotton, where expression of *GhLETM1* gene at higher or lower levels lead to defective stamen development with shortened filaments and indehiscent anthers and abortion of pollen (Zhang *et al.,* 2020). *RAFTIN* gene in rice and wheat (Wang *et al.,* 2003), OsDEX1, a Ca^{2+} binding protein in rice (Yu *et al.,* 2016) also been reported to associate with male sterility.

Palumbo *et al.,* (2020) reported role of two *atp6* gene sequences for CMS in Fennel (*Foeniculum vulgare)* by mitochondrial genome assembly of male sterile and fertile accessions. Similarly, the mechanism of abortion in CMS was studied and reported by transcriptome analysis in CMS tobacco and fertile lines (Liu *et al.,* 2020). Wang *et al.,* (2020) identified *orf463a* gene as the causal factor associated with CMS in radish by next generation sequencing of CMS and fertile mitochondria genome. Another report is from male sterile somatic hybrids obtained from *Brassica juncea* and *Moricardia arvensis*, where mitochondrial *orf108*, was found co-transcribed with *atp1* gene and reported possibly *orf108* either prevents the *atp1* translation or it translates into a cytotoxic protein (Ashutosh *et al.,* 2008). Similarly, in *B. juncea* expression assays at protein level in CMS (hua lines) showed association of mitochondrial *orf288* that aborts pollen development by cytotoxicity (Jing *et al.,* 2012). Presence of *Orf220* and *atp-1* in CMS lines of tuber mustard was reported as the reason for CMS in their study (Ming-Fang *et al.,* 2009). Condensed cytoplasm, irregular exine with abnormal or degraded tapetum in TGMS-*Brassica napus* was correlated with pollen abortion (Sun *et al.,* 2020). CMS lines obtained by protoplast culture of *Nicotiana sylvestris* showed deletion of mitochondrial DNA and deletion of gene or regulatory effects of deletions that changes the expression of mitochondrial gene was the reasons for CMS trait (Chetrit *et al.,* 1992).

In conclusion the contribution of male sterility in agriculture is unequivocal. Introduction of CMS is crop species is always interesting and useful. On one hand male sterility can be used in commercial seed production, while it can be used in recombination breeding programs for crop improvement. Enumerating the efforts and success in induction of male sterility is useful in understanding the basic mechanism as well as increasing the productivity of crops.

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