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Male Sterility and its Commercial Exploitation in Hybrid Seed Production of Vegetable Crops: A Review

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ABSTRACT

Male sterility is described as absence of functional pollen grains in hermaphrodite flowers facilitating large scale production of hybrid seeds in vegetable crops. It eases hybrid seed production at commercial level in crops like tomato, chilli, capsicum, carrot, onion, cabbage, cauliflower and cucurbits. Male sterility would reduce the cost of hybrid seed production by limiting the labour making it efficient and economical. Incorporation of biotechnological tools in conventional plant breeding techniques would aid the breeders in limiting the drawbacks surrounding exploitation of male sterility for development of new hybrids. The present review is an attempt to summarize and to know the commercial utilization of male sterile line in hybrid seed production of vegetables.

Key words: Functional pollen grains, Hybrid seeds, Vegetable crops.

Male sterility is described as absence of functional pollen grains in hermaphrodite flowers facilitating large scale production of hybrid seeds in vegetable crops. Male sterile plants occur rarely in nature as they are eliminated by natural selection forces. Through properly maintained domestication process, male sterility could be an important tool to plant breeding by providing natural and effective means for genetic emasculation of plants. It eases hybrid seed production at commercial level in crops like tomato, chilli, capsicum, carrot, onion, cabbage, cauliflower and cucurbits. Male sterility has been reported by different scientists in large number of crop plants as well as in vegetables. The plants having male sterility were either isolated in natural populations or artificially induced through mutagenesis. Now new approaches such as genetic engineering and protoplast fusion are also used to induce male sterility in crop plants.

MECHANISM OF MALE STERLITY

Male sterility arises due to disturbances in normal microsporogenesis leading to formation of non viable microspores as follows-

1) Mitochondrial mutation: Mitochondria is the power house and respiratory site within a cell. Any mutation in mitochondrial body results in loss of respiration and hence fertility.

2) Barrier of tapetal layer: Delayed degeneration of tapetal cells blocks availability of nutrients to microspore therefore affecting nourishment of pollen and leads to its abortion.

3) Proper timing of callase enzyme: Activity of enzyme at a particular time during microsporogenesis decides the fate of normal development of pollen. Early and delayed activity of callase in different plasma type leads to male sterility. pH within anther locules regulate callase activity.

4) Based on operon concept of gene action: Specific conditions added by nuclear genes are propagated in cytoplasm which imposes a permanent repression on genetic system that determines the pollen producing capacity of plant. This hypothesis fails to provide explanation for fertility in male sterile system.

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Male sterility is usually classified into following two groups (On the basis of genetic control of male sterility) (i) genetic and (ii) non-genetic male sterility. Genetic male sterility i.e male sterility under the control of genes while non genetic male sterility is induced by stresses. The utilization of non-genetic male sterility has not been found in vegetable crops. The phenotypic expression of male sterility is classified into three classes *i.e.* sporogenous, structural and functional. Similarly, non-genetic male sterility has been classified as chemical, physiological and ecological male sterility. Further, on genotypic basis; genetic male sterility is grouped as genetic, cytoplasmic and cytoplasmic-genetic male sterility (Table 1).

Detection of male sterility system

Under field conditions, the male sterile system is identified by observing the pollen at flowering stage of the plant. All the progenies in case of cross are observed and ratio derived by recording the total number of plants classified into pollensterile and pollen fertile. These are broadly classified into

GMS, CMS and CGMS lines and clubbed into the following types; Type I- All progenies in all the rows may be sterile- CMS, Type II- Some rows may consist all fertile, Some rows sterile and fertile in 1:1 ratio- GMS, Type III- Some rows fertile,some rows sterile and some rows sterile and fertile in 1:1 ratio – CGMS.

Genetic male – sterility

Genetic male sterility is controlled by the gene(s) from the nuclear compartment. This male sterility is generally controlled by a single recessive gene (ms) and has been reported in tomato, brinjal, chilli, sweet pepper, pea, lima bean, pumpkin, squash, water melon, muskmelon, ridge gourd, cucumber, cauliflower, cabbage, broccoli and Brussels sprout. All the transgenic male sterile lines developed till date are GMS, since they have been developed through transformation of male sterility causing gene construct(s) inside the nuclear genome. Certain mutants, which although produce functional pollen, fail to self-fertilize, either due to non-dehiscence of pollen or their special flower morphology. These mutants are often termed as functionally sterile, for example genotypes with exerted stigma in tomato (Georgiev, 1991), brinjal and several other vegetables (Kaul, 1988). GMS has been commercially exploited in production of hybrids in some of the crops (Table 1).

Environment Sensitive Genetic Male sterility (EGMS)

Certain genetic male sterile lines are conditional mutants *i.e.,* male sterility is expressed only in certain set or range of environmental conditions, in absence of which the male sterile plants turn into male fertile. After determination of critical environment (usually temperature or photoperiod) for sterility and fertility expression, such GMS mutants are classified as Temperature sensitive Genic Male Sterile (TGMS) lines or Photoperiod sensitive Genic Male Sterile (PGMS) lines. EGMS lines (mostly temperature sensitive) have been reported in several vegetable crops like cabbage, Brussels sprout, broccoli, peppers (chilli and sweet pepper), tomato and carrot. A majority of these, however, were previously identified as normal genic male sterile lines. (Kumar *et al.,* 2000).

Due to the problem of instability, EGMS lines were considered to be of very less practical value initially, but presently they represent most efficient system for hybrid seed production. However, from practical viewpoint, it is necessary to identify critical temperature or photoperiod for the fertility/sterility expression in temperature and photoperiod sensitive genetic male sterility, respectively.

Utilization of GMS and EGMS

The GMS plants being recessive in nature are predominantly monogenic with the genetic constitution (msms) and seed set is difficult on selfing as the case observed in self-pollinated plants. These plants are to be maintained by crossing with male fertile plants with Msms gene type. However the resulting progenies obtained has both male sterile and male fertile plants in equal proportion. As such the progenies which are male fertile are to be rogued out. This process of removing the male fertile plants is to be done at pre-anthesis stage to avoid the chances of uncontrolled pollination. Normally being isogenic in nature both male sterile and male fertile plants are phenotypically

Classification of male sterility in plants (Kaul, 1988).

(i) Inherited (genetic) male sterility	
Phenotypic basis	**(i) Sporogenous male sterility.** Pollen formation is completely disrupted. Stamens are formed but there is absence of pollen due to abortion of microsporogenous cell which occurs before, during or after meiosis. **(ii) Structural sterility or positional sterility.** Floral organs are modified in such a way that selfing does not occur. Stamens are either absent, malformed or modified into other floral parts which lead to absence of microsporogenesis. e.g. tomato, lima bean and onion. **(iii) Functional sterility**. Viable pollens are produced but they are unable to self fertilize due to some barriers (non-dehiscent nature of anthers). Seed setting occurs when artificially selfed by its own pollen or crossed with other pollen.
Genetic basis	**(i) Genetic male sterility (GMS)** •Wide occurrence in plants. •Caused by the gene(s) from nuclear compartment (nuclear gene). •Mostly governed by a single recessive gene, *ms.* •ms alleles arise spontaneously or may be artificially induced. **(ii) Cytoplasmic male sterility (CMS)** •Determined by cytoplasm. •Result of mutation in the mitochondrial genome (mtDNA). •Can be easily transferred to a given strain. **(iii) Cytoplasmic-genic male sterility (CGMS)** •Also known as nucleoplasmic male sterility. •Male sterility is caused by mitochondrial genes and restored by the nuclear genes. •Fertility restorer gene *R* is required.
(ii) Non-inherited (non- genetic) male sterility	This kind of male sterility is temporarily induced by certain environmental stresses e.g., temperature, etc.**(i)Chemical, (ii) Physiological and (iii) Ecological**

Table 1: GMS based hybrids of important vegetable crops.

Crop	Male sterile line	Male sterile allele	Cultivar/ Hybrids developed	Features	Reference
Chilli	MS-10(mc-509**)**	*Msms*	MS-12, MS-13 and MS-41	recessive male sterile allele (*msms*)	
	MS-12	*Msms*	Punjab Lal	recessive male sterile allele (*msms*). Male st	Singh and Kaur (1986)
	MS-12	*Msms*	CH-1-(MS-12× LS), CH-3-(MS-12×2025) and CH-27(MS-12x S-343).	Released by PAU	Dhall, (2011)
	ACMS-2	acms2acms2	-	monogenic recessive gene	Patel *et al.,* (1998)
Tomato	-	55 male sterile (*ms*) alleles			Kaul (1988) Georgiev (1991)
	-	mutants, *viz.,* stamenless-1 (*sl-1)* and stamenless-2 (*sl-2*)ms-13, ms-15	-	Produces flowers without stamens when grown at higher temperature, while those grown at low temperature produce flowers with abnormal stamens and often with viable pollen. Similarly in *ms-15* and *ms- 33* mutants, low temperature (30^{o}C) is reported to be associated with fertility restoration.	Sawhney, (1983)
Okra	MS-1	ms1ms1	-	Male sterility in is controlled by a pair of single, recessive genes when present in the homozygous(*ms1ms1*) condition and can be utilized by hybrid seed produ--ction. Male sterility has not been obser-ved in nature, but, has beeninduced by gamma radiation.	Dutta (1971)

alike except for the male sterile gene. Though practically on close observation the male sterile plants possess smaller flowers as seen in tomato (Sawhney, 1997) less nectarines, shriveled anthers without pollen as in capsicum and radish, pettaloid stamens as seen in carrot and are less preferred by pollinators *viz*., Honey bees, house fly, bumble bees etc., The labour factor applicable on removal of male fertile plants can be reduced by proper identification of the male sterile plants at seedling stage by employing phenotypic marker gene *viz*., stem colour, seed colour etc., associated with male sterile plants. Utilization of suitable marker genes will lead to cost reduction in hybrid seed production.

Advantages of EGMS

Hybrid seed production through EGMS line is more attractive because of the ease in seed multiplication of male sterile line. Seeds of EGMS line can be multiplied in an environment where it expresses male fertility trait while hybrid seeds can be produced in other environment, where it expresses male sterility. Male (pollen) fertility in hybrid crop is not affected, as male parent contributes normal (wild) allele of the environmental sensitive mutant gene. Since only two parental lines are involved, breeding method involving EGMS is more popularly termed as "Two Line Hybrid Breeding".

Utilization of marker gene

Marker genes facilitate identification of male sterile lines at seedling stage saving time and labour by roguing out male fertile lines in F1 hybrids. In watermelon the non-lobbing character characterized by recessive gene is exploited for hybrid seed production in watermelon (Whitaker and Davis, 1962). Alternate row planting of inbred lines with lobed and non-lobed plants (identified at seedling stage) is done and hybrid seeds collected only from non-lobed lines. As only one third of the sown seeds are hybrid, sowing seeds up to 6-8 per hill is practiced. In tomato marker genes have been reported in two male sterile and one male fertile line (Radkova *et al*., 2009). One of the male sterile line possessed marker gene potato leaf (c) and fruits without green shoulders resulting in uniform ripening (u) while another male sterile line employed the marker gene, anthocyaninless with green shoulders on fruit. The male fertile line possessed the marker gene anthocyaninless and fruits without green shoulders (u) for easy identification. Similarly in brussels sprout, glossy foliage is the marker gene for the production of FI hybrid seeds (North and Priestley, 1962). Partial chlorosis in addition to glossy foliage was suggested as the marker gene criteria for identification in both the A and B lines (Johnson (1966). Use of brown seed and seed coat colour was suggested for identification of male sterile lines and black seed colour for identification of male fertile plants for hybrid seed production (Davis (1966) whereas light green anthers was used for detection of male sterile lines in variety Alfa São Francisco' (Santos *et al*., 2010).

Limitations of GMS

Because of more tedious maintenance process and non-availability of suitable marker gene among the vegetable crops, GMS are utilized commercially only in chilli and muskmelon in India.

Transgenic genetic male sterility system (Barnase-Barstar system)

Transgene refers to the transfer of gene into the genome of an organism and this manipulation is achieved by genetic engineering technology. Genetic engineering of male sterility can selectively and specifically destroy or interfere with the normal development of anthers or pollen. Barnase (bacterial cytotoxic ribonuclease) a bacterial protein is secreted by the bacterium *Bacillus amyloliquefaciens*. The lethal action of the Barnase is inhibited by Barstar preventing barnase from damaging the cell's RNA. The barnase/barstar system has the specific property of tight protein binding. Though commercial exploitation of genetic engineered male sterility is rarely used except the case of barnase barstar system in case of tobacco which plays a significant role in hybrid seed production.

The genetic modification technique involves development of male sterile transgenic plants by inserting the gene sequences that are active only in the male reproductive organs and destroy or interfere with pollen or anther development (microsporogenesis). This results in purely female ("male-sterile") plants that can then be used for cultivation purposes and for the production of hybrid seeds. The development of transgenic male sterility system has been made possible because of isolation, cloning and characterization of anther or pollen specific genes and promoter sequences. These genes are expressed in pollen themselves (gametophytic expression) or cells and tissues (sporophytic expression) that directly or indirectly support pollen development, such as tapetum, filament, anther wall etc. (Kumar *et al.,* 2000). However, the sterile source does not exist in all vegetable crops, which may be obtained by searching in nature, artificial mutation and protoplast fusion. The rapid development of plant genetic engineering offers a shortcut for creating male sterile materials.

Barnase-Barstar system (Abolition-restoration system)

As the transgenes are reported to produce genetic male sterility, it is essential to evolve a mechanism for fertility restoration so that hybrid seed production is achieved. This fertility restoration is seen in the barnase- barstar system. In this system the process of interference of pollen development is achieved by the transgene constructs. These constructs link for the cytotoxic compounds *viz*. RNAase, lipase, protease etc., which are disruptive to the cellular integrity. Promoters regulate gene expression specifically in the tapetum cells (innermost wall of microsporangia) present in the pollen tissues and cause male sterility by abolition of the gametophytic and sporophytic cells. Pollen fertility is restored by another transgene inhibiting action of the disruptive gene.

Mariani *et al.,* (1990; 1992) reported the Barnase-Barstar system, an example of the first transgenic male sterility system in tobacco and rapeseed. These transformed plants were developed encompassing the RNAase gene (called Barnase) containing tapetum specific promoter (TA29). The barnase was expressed in the tapetum cells having the tapetum specific promoter (TA29) resulting in transgenic male sterility. Homozygosity in such male sterile plant is difficult and maintenance remains a disadvantage. Crossing of transgenic male sterile plants (hemizygous;

Barnase) with normal plants results in 50% hemizygous (Barnase -) male sterile F1 progeny. This poses a challenge wherein fruits and seeds are of economic value and as such these male sterile plants are of no value.

These problems (seed increase of male sterile plants and restoration of fertility in F1 have been addressed (Mariani *et al.,* 1992). It was demonstrated that fertility in F1 derived from transgenic male sterile plants (dominant) could be restored by developing F1 from the pollen of another transgenic plants possessing Barstar gene coupled with TA29 promoter. In the F1 plants, product of Barstar gene is dominant suppressor of cytotoxic products of chimeric RNAase. Transcript of Barstar gene formed complexes with chimeric RNAase transcribed from TA29-Barstar gene in tapetum cells (Mariani *et al.,* 1992). To overcome maintenance problem transgenic with Barnase:herbicide resistant gene (linked) construct has been developed. Since male sterility gene (Barnase) is linked with the herbicide resistant gene, in such cases hemizygous male sterile plants can be crossed with normal sister plants and spraying of herbicide on the progenies, will ensure survival of 50% sterile segregants and elimination of 50% fertile segregants.

Cytoplasmic male sterility

Male sterility determined by the sterile cytoplasm is known as Cytoplasmic male sterility (CMS). CMS in plants is the result of mitochondrial dysfunction due to mutation of the mitochondrial genome (mtDNA). CMS a maternally inherited trait is the result of the recombination events resulting in formation of novel protein coding sequences or the open reading frames (ORFs) in mitochondrial genome (Chase and Babay-Laughnan, 2004; Hanson and Bentolila, 2004). As a result the CMS plants exhibit mutations, novel genes reorganization or rearrangements in the mt-DNA sequences. The most classic examples of CMS are the cms-T of maize, Ogura CMS and Anand CMS of cole crops. The ogura cms system in brassicas was researched extensively and coworkers have reported that ORF138 protein was strongly associated with the inner mitochondrial membrane of male-sterile plants (Duroc *et al.,* 2005). The CMS is found in cole crops *viz*., cauliflower, cabbage, broccoli, Chinese cabbage, root crops *viz*., carrot, radish, turnip and in crops such as sweet pepper and cucumber. The Cytoplasmic male sterility is a maternally inherited trait, because mt-genome is responsible for the expression of male sterility and the mitochondria are usually excluded from the pollen during fertilization. The progeny of male sterile plants are hundred percent male sterile as the cytoplasm of a zygote is derived from egg cell. A desired variety can be converted to CMS variety by using the pollen of that variety and crossing with the female CMS line. Repeated backcrossing of the progeny and further generations (5-6) with the pollen of the recurrent parent will result in male sterile line identical to the desired variety.

In Cole crops *viz*., Cabbage, Cauliflower and Broccoli, CMS is introduced through introgression from different sources like sterile cytoplasm of radish and black mustard. Ogura based hybrids in cauliflower have been reported to cause chlorosis at low temperature, reduced nectarines causing less visits by honeybees, pettaloid stamen, split anthers etc., causing hindrances in hybrid seed production. Ogura based improved CMS lines of cauliflower were developed following seven generations of backcrossing with snowball group ideal for cultivation in the hilly areas.

Utilization of CMS

Cytoplasmic male sterility can be maintained by crossing a male sterile line (A line) with the pollinator strain (B-maintainer line) used as a recurrent parent in the back cross programme since the nuclear genotype of the pollinator is identical with that of the new male sterile line. (Table 2).

Cytoplasmic-genic male sterility

It differs from the CMS wherein the nuclear gene for restoring fertility in male sterile line is known. This is commonly known as cytoplasmic- genic male sterility (CGMS). It was first reported by Jones and Davis in 1944 in Onion. The fertility restorer gene R is dominant and found is certain species of crops. The CGMS is the result of interaction of the nuclear gene and sterile cytoplasm but not due to genetic or cytoplasmic factor alone. Thus sterility or fertility in such plants is decided by the contribution of both the nuclear genes and cytoplasm.

Along with the sterile cytoplasm, the sterility is determined by a single recessive gene (ms ms) in onion, radish, cabbage, cauliflower, chilli and sweet pepper; by two recessive genes (xx zz) in beet and by a dominant gene (Ms ms or Ms Ms) in carrot. However, the sterility in carrot and beet is found to be unstable due to the presence of modifying genes. Cyto-nuclear male sterility has been extensively exploited for hybrid seed production in onion and carrot. However, this system is sensitive to environmental factors and change in environment leads to restoration of fertility and contamination of hybrid seeds.

Utilization of CGMS

CGMS system has three lines A- male sterile, B- male fertile and R-restorer Lines. In this system the B- line is also called the maintainer line as it maintains fertility of the A-line and the R-line is called the restorer line as it restores fertility in the F1 hybrid. The Cytoplasmic male sterile with the genotype {(ms/ms)S} is crossed with the male fertile with the genotyope {(ms/ms) F} to obtain the male sterile with genotype {(ms/ms)S}. Fertility restoration is done by crossing the male sterile line (ms/ms) f with male fertile line {(Ms/Ms) F/S} to obtain the male fertile with the genotype {(Msms) S}.

Limitations of CGMS

The limitations of CGMS are non availability of CGMS in many crops and their wild relatives, need of fertility restorer allele in fruit producing vegetables, undesirable pleiotropic effect of sterile cytoplasm on horticultural qualities and breakdown of male sterility in particular environments.

Chemical Hybridizing Agents (CHA)

Chemical hybridizing agents (CHA) are the chemicals that induce male sterility in plants. They are also known as male gametocides. Basically two types of CHA are used, one is the mutagen based and other non-mutagen or male gametocides however they are broadly classified as CHAs

Table 2: Use of Cytoplasmic male sterility/ Cytoplasmic-Genetic male sterility in commercial vegetable crops.

Crop species	Cytoplasm	Restorer genes	Remarks	Reference
Capsicum	*C. annuum* (PI-164835)	*C.annum*	Not commercially used due to instability under fluctuating conditions, particularly temperatures and a low rate of natural cross pollination in cultivated hot pepper.	Peterson (1958) Kumar *et al.,* (2007)
	CCA-4759 and CCA-4757	Known	The World Vegetable Centre, Taiwan. Reliably sterile under conditions of night temperatures less than 15°C	Liu and Gniffke (2004)
	CCA-4261	Known	Hybrids developed- Kashi Surkh (IIVR), Arka Meghana Arka Harita and Arka Sweta (IIHR)	https://www.iivr.org.in https://www.iihr.res.in
	CMS line 181A	98199	Hybrid developed Jingla no 2	Geng *et al*. (2005)
	S200243A	S200244C	F1 hybrid -Nongda 082	Shen and Shi (2005)
	CCA-4261	SL-461, SL-462, SD-463	Ten male sterile lines were completely sterile across environments	Meena *et al*. (2018)
Onion	CMS-S (alloplasmic)			
	CMS-T	- Two independent systems Gene A, Gene A and C and complementary action.	Thermolabile, commercially used. May be thermolabile e.g. 93 % sterile plant at 14°C and 10 % at 23°C	Pelletier *et al*., (1995).
	"13-53"	Two recessive genes *ms1* and *ms2*	First male sterile plant reported in Italian Red variety.	Jones and Emsweller, (1936)
	Nashik white Globe Pusa Red	Known	Commercially useable	Pathak and Gowda (1994); Hazra and Som, (1999).
Carrot	(*ba*) Brown anther type- CMS *sa* cytoplasm	Two independent recessive nuclear genes	First discovered in Tendersweet and first type used for developing hybrid carrot varieties. formed but unrolled, shriveled filaments and brownish anthers which are a result of tapetum degeneration.	Welch and Grimbal, (1947) Michalik and Sleczek (1997)
	Petalloid (*pt*) type Sp cytoplasm Nantes variety	Two independent dominant genes (*M1* and *M2*).	Widely employed in carrot breeding programs. Replacement of stamens with whorl of petals or petal-like, bract-like or carpelloid structures. Hybrids- Pusa Nayanjyothi and Pusa Vasudha released.	Thompson, (1961) Davey, (1999); Bach *et al*. (2002). Erickson *et al*. (1982); Kitagawa *et al*. (1994).
	S_p-cytoplasm	*atp9* genes	Transformation of stamens into petal-like organs. *atp9*-1 version dominates over *atp9*-3, while in N-cytoplasmic plants this proportion is reversed.	Szklarczyk *et al.* (2014)
Musk melon	Punjab Hybrid and Punjab Anmol	ms-1, ms-2, ms-3, ms-4 and	Two commercial cultivars released in 1978. Due to the instability of this ms-1 gene in sub-tropical field condi-	Nandpuri *et al*. (1982) Lal, *et al*. (2007)

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		ms-5 (recessive and non-allelic genes)	-tions, the seed production of these hybrids has posed numerous problems consistently	Dhatt and Gill, (2000).
Water melon	X2 generation of Sugar Baby	Gms	Male sterile mutant in the X2 generation of Sugar Baby irradiated with gamma rays. Due to disruption in the reproduction system caused by the gms male sterile alleles found to be of less commercial applicability.	Watts, (1967)
Cole crops	*B. nigra*	*B.oleraceae* var *italic*	Flowers of pettaloid male sterile plants were less attractive to the pollinating insects, since pistils were enlarged, malformed and were lacking in nectarines	Pearson, (1972)
	Amphidiploids	*B.oleraceae* var *capitata* cv. Green Globe	flowers smaller, normal and with functional nectarines, however homozygous plants could not be recovered even after six generations of backcrossing in broccoli	Dickson, (1975)
	Ogura CMS Japanese radish cultivar and *Brassica oleraceae*	*Brassica oleraceae* var italiaca	Ogura based CMS lines developed in snowball cauliflower *viz*., Ogu1A, Ogu2A and Ogu3A for hybrid development in cauliflower. Cabbage hybrids H-64 & KCH-4 using cytoplasmic male sterility at IARI RS Katrain	Ogura (1968) Bannerot *et al*. (1974), Kalia, (2008)
	Anand cytoplasm B. tournefortii B. rapa to *B. oleraceae*	-	The presence of “Anand” chloroplasts with a *B. oleracea* nucleus did not result in cold temperature chlorosis, as seen in “Ogura” CMS plants.	Cardi and Earle, (1997)
	Tour cytoplasm Brassica tournefortii	-	Floral abnormalities and causes chlorosis in *Brassica* spp.	Arumugam *et al*.(1996)

to avoid the ambiguity. The male gametocide is advantageous as it is the rapid method of developing male sterile line as compared to conventional backcross breeding utilizing upto 6-8 seasons. Also as in CMS/ CGMS there is no need to maintain the A, B and R lines. Potential of certain chemicals (maleic hydrazide) to induce selective male sterility was first demonstrated during 1950 in maize (Moore, 1950; Naylor, 1950). CHAs has been widely used and reported by various coworkers (Prayaga *et al*. (2002) in safflower plants treated with 100 to 300 ppm GA, Sreedhar (2003) in Niger using GA3 (100 and 150 ppm) and detergent solution (1 and 2 per cent), Razzak *et al* (2015) in sunflower using 1.5% of detergent).However there are certain disadvantages with male gametocide such as incomplete pollen abortion, deformation of leaf tissue and wilting of leaf and the need for repeated treatment and need of specific growth stage (Sneep and Hendriksen, 1979, Parodi and Gaju, 2009). It was recognized that despite of certain disadvantages there might be some advantages, especially in terms of time required to economically viable hybrids. This is because chemical methods for inducing male sterility can obviate the lengthy time frame required to obtain male sterile and restorer lines.

Properties of an ideal CHA

- Must be highly male or female selective
- Should be easily applicable and economic in use
- Time of application should be flexible
- Must not be mutagenic
- Must consistently produce >95% male sterility
- Must cause minimum reduction in seed set
- Should not affect out crossing
- Should not be hazardous to the environment

Site and mode of action of CHA

Literature regarding compounds developed earlier suggest that FW-450, ethephon, RH-531, PPX 3778) produces specific effects depending upon treatment time and dosage interaction. The various phenomenon observed are disruption of meiosis and anther development. Pollen exine formation is disrupted and microspores are thin walled, distorted in shape and non-viable. Microspores vacuole abnormalities, decreased starch deposition and persistent tapetum. Pollen is non-viable but anthers are normal. Pollen is present and viable but anthers either do not dehisce or show delayed dehiscence. (McRae 1985).

Mechanisms of male sterility

Cytological changes: Breakdown in the process of microsporogenesis can occur at a pre or post meiotic stage. The abnormalities can involve aberration during the process of meiosis in the formation of tetrads, during the release of tetrad (the dissolution of callase), at the vacuolate microspore stage or at mature or near-mature pollen stage.

Biochemical changes: Besides cytological changes, male sterility also causes various biochemical changes *viz*., changes in magnitude of various amino acids, protein and amino acids. Kaul (1988) reported reduced levels of proline, leucine, isoleucine, phenylalanine and valine and increased levels of glycine, arginine and aspartic acids. Research on sterile anthers showed one-eighth amount of proline, lower soluble protein and fewer polypeptide bands in comparison to the fertile anthers (Kakihara *et al.,* 1988). Male sterile anthers also have reduced activity of enzyme callase which is responsible for breakdown of callase that surrounds PMCs and the tetrad is decreased. Also activity of isozyme esterase is decreased which plays an important role in hydrolysis of sporopollenin polymer that is required for pollen formation. Similar observations have been observed by Bhadula and Sawhney (1987) in tomato. Reduced activity of amylase leading to high starch content and reduced levels of soluble sugars has been noted in sterile anthers as compared to fertile anthers.

In spite of the detailed studies and understanding about the male sterility systems, still the phenomenon is not being widely used for hybrid seed production in many vegetable crops. The main reason for such under exploitation of this mechanism is non availability of stable male sterile lines, whereas in case of genetic male sterility seed multiplication of male sterile lines remains a problem. The use of biotechnology, molecular markers and transgenic can provide as an aid for overcoming such obstacles. After examining the cost benefit ratio, it has been found that efficiency of GMS system can be further increased by the utilisation of molecular markers like RAPD (Kalloo *et al.,* 1998). Development of practically feasible molecular markers may provide appropriate cost effective selection strategy to discard 50% male fertile sister plants at seedling stage, which may open the way to exploit monogenic recessive male sterile lines in several vegetables (Kumar and Singh, 2004). Identification of functional male sterile and EGMS lines also have great potential for being utilized in commercial hybrid seed production due to presence of functional pollen grains unlike genetic male sterility in which pollen grains are non-functional.

CONCLUSION

- Male sterility could reduce the cost of hybrid seed production by limiting the labour making it efficient and economical. .
- Identification of male sterile lines in commercial crops which offer stability under varied environmental conditions.
- Encompassing desired horticultural traits in male sterile systems.
- Incorporation of biotechnological tools in conventional plant breeding techniques could aid the breeders in limiting the drawbacks surrounding exploitation of male sterility for development of new hybrids.

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