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Exploitation of Male Sterility for Heterosis Breeding in Vegetable Crops

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ABSTRACT

The phenomenon of heterosis is extensively utilized in vegetable crops for the exploitation of hybrid vigor. Presently, hybrids are popular in majority of the vegetable crops due to their uniform shape, size, color and quality, providing better returns to the growers. The main worry regarding production of hybrid seeds is the scarcity and high cost of specialized labor, which increasingly raises the cost of hybrid seed production, especially for solanaceous crops, where manual emasculation and pollination mechanisms still play an essential role. Thus, there is need to exploit different mechanisms which results in decreasing the cost of hybrids. Among these mechanisms, male sterility is very efficient and economic method for the large-scale F_1 seed production. Many male sterile mutants have also been discovered which eliminates the need of tedious emasculation process. Undesirable type of plants at the early stage of growth can be identified by using certain marker genes and thus facilitates the production of hybrid seeds. Exploiting heterosis in vegetable crops is more profitable as it increases the uniformity, resistance against various insect-pests and yield.

Keywords Emasculation, Heterosis breeding, Hybrid vigor, Male sterility, Marker genes.

INTRODUCTION

Earlier, hybrids were generally produced by allowing natural crossing among the plants which generally results in the poor quality of hybrid seeds as there is no mechanism to control on self-fertilization (Goulet *et al.* 2017). Thus, it encourages the scientists to develop new methods of producing pure hybrid seeds at commercial scale. Male sterility is a significant phenomenon in the generation of high-quality hybrid seeds such as tomato, cauliflower, cabbage, and okra. It occurs spontaneously in nature or can be induced artificially by using different mutagenesis (Xu *et al.* 2022).

Phenotypically, the genetic male sterility (GMS) can be classified into three types viz., sporogenous male sterility, functional male sterility and structural male sterility and the non-genetic type is classified as

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physiological, ecological and chemical male sterility while genotypically, it has been classified into three types: Genic male sterility (GMS), cytoplasmic male sterility (CMS), and cytoplasmic-genic male sterility (CGMS) (Mishra 2013). Many genetic engineering techniques and the protoplast fusion are also utilized for producing male sterility in vegetables (Dhall 2010). Presently, the focus is to exploit different methods of male sterility in different vegetables like cabbage, cauliflower, radish, okra, onion for the production of large quantity and quality of hybrid seeds.

Different types of male sterility

GMS (Genetic Male Sterility)
CMS (Cytoplasmic Male Sterility)
CGMS (Cytoplasmic Genetic Male Sterility)
CHAs (Chemically Induced Male Sterility

Nuclear male sterility or genetic male sterility (GMS)

Mackenzie (2012) reported nuclear male sterility in large number of crops. It is governed by single recessive gene (ms) but in vegetable like tomato, cabbage and broccoli dominant genes govern male sterility. To keep the male sterile line viable, the heterozygous male fertile line "Msms" is employed. The mutation in genes which control : Microsporogenesis, stamen development or micro-gametogenesis can cause genetic male sterility in vegetable crops.

Types of genetic male sterility (GMS)

Environmental sensitive GMS (EGMS)

Temperature sensitive GMS (TGMS)
Photoperiod sensitive GMS (PGMS)

Transgenic GMS (TGMS)

Environmental sensitive GMS (EGMS)

The behavior of these type of mutants changes in different environment and thus these are known as conditional mutants. In one environment, these mutants may show male sterility but in other environment the same mutant results into male fertile. Both temperature sensitive and photoperiod sensitive male sterility is observed (Table 1) but TGMS is more common in vegetables. In tomato the mutant "7B-1" male sterility has been found and it is observed that it produces 100% male sterile plants in long day conditions. Thus it contains a very good potential for utilization in hybrid breeding in tomato (Sawhney 2004).

Table 1. EGMS in different vegetable crops.

Crop	Sterility type	References
Cabbage	Temperature sensitive and photoperiod sensitive GMS	Rundfeldt (1960)
Carrot	Temperature sensitive GMS	Kaul (1988)
Broccoli	Temperature sensitive GMS	Dickson (1970)
Brussels sprout	Temperature sensitive GMS	Nieuwhof (1968)
Pepper	Temperature sensitive GMS	Daskalov (1972)
Tomato	Temperature sensitive GMS	Sawhney (1983)

Transgenic male sterility

Different techniques of genetic engineering may be used to induce this type of male sterility in vegetable crops. Various pollen development processes like anther wall, filament are effected by the transgenes. The *Bacillus amyloliquefaciens* having barnase gene and results in the coding of extracellular RNase. It also contains an inhibitor gene barstar which helps to restore fertility has a corresponding inhibitor protein called barstar. The barstar gene forms a complex with the barnase gene and thus provides protection against the lethal effect of the barnase gene (Roque *et al.* 2019). Various molecular and morphological markers linked to "*ms*" gene are indicated in Tables 2 - 3.

Utilization of GMS

Chilli

PAU introgressed *ms10* gene into 3 chilli genotypes. MS-12 line is monogenic, recessive nuclear male

Table 2. Molecular markers linked to gene "*ms*".

Vegetable	Markers	References
Chilli	SCAR marker (*ms*-1 gene)	Lee *et al.* (2010)
	SSR markers (*ms* 10 gene)	Aulakh *et al.* (2016)
Muskmelon	SCAR and RAPD markers (*ms*-3 gene)	Park *et al.* (2004)
Cabbage	SSR marker (MS-*cd1* gene)	Zhang *et al.* (2011)

Table 3. Morphological markers linked to *ms* gene.

Crop	Markers	References
Broccoli	Bright green hypocotyles	Sampson (1966)
Tomato	Green stem and potato leaf shape	Kaul (1988)
	Parthenocarpy	Soressi and Salamini (1975)
Watermelon	Delay in green seedling	Zhang *et al.* (1996)

sterile line, possessing mutant gene ms 10 which is used for hybrid seed production.

PAU Ludhiana has developed three GMS based chilli hybrid:

a) CH-1: MS 12 × Local Ludhiana selection (LLS)
b) CH-3: MS 12 × S-2530
c) CH-27: MS 12 × S-343

Muskmelon

In 1978, *ms-1* gene (male-sterile gene) was introduced in India and different hybrids are developed by using MS-1 line

1 Punjab Anmol: MS-1 × Punjab Sunehri
2 Punjab Hybrid: MS-1 × Hara Madhu
3 MH 27: MS-1 × MM Sel.103

Okra

MS-1, Geneic Male Sterile (GMS) line is utilized for commercial F1 hybrid development. A pair of homozygous recessive genes control male sterility in okra (ms1ms1) and may be used to produce hybrid seeds. Gamma radiation may cause male sterility in okra (Asare *et al.* 2017). Environmental influences had no effect on the gene, although anther dehiscence was partial. Up to the tetrad stage, microsporogenesis was normal. As a result, there is a lot of room in a heterosis breeding program for hybrid seed production in the future (Pitchaimuthu *et al.* 2012).

Limitations of GMS

More tedious maintenance process.
GMS lines require more area as the plants will segregate into male sterile and fertile plants (1:1).
Identification error during screening.
Suitable marker genes are not available.

Cytoplasmic male sterility (CMS) system

It is governed by cytoplasmic genes (mitochondrial genes). Progeny of CMS line will always be 100% sterile. This system consists of A line and B line where A line is male sterile and B line male fertile. Ogura cytoplasm is an important source of sterile cytoplasm (Ogura 1968), which has been utilized in several cole vegetables to develop CMS system. A number of CMS genes have been discovered through molecular genetic research, with Ogura CMS from Japanese radish being the most thoroughly investigated and often utilized (Yamagishi and Bhat 2014).

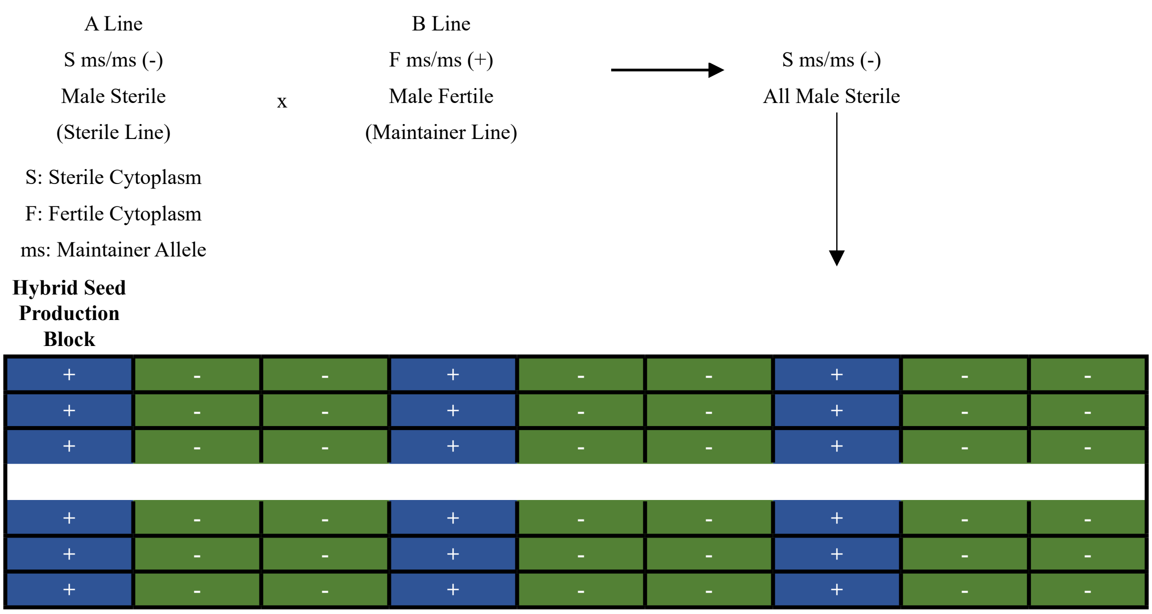
The resultant plants of *B. napus* show yellowing (chlorotic leaves) at temperature below 15^{o}C (Jeong *et al.* 2017) and is suggested as a consequence of incompatibility between *Brassica napus* nucleus and *Raphanus sativus* chloroplast. To overcome the problem of chlorosis, the cells of alloplasmic line of *B. napus* (male sterile) are fused with the normal *B. napus* (Kang *et al.* 2017). The regenerated plants were selected which show no chlorophyll deficiency and retain male sterility. The CMS line thus developed were used in hybrid breeding. This type of protoplast fusion was also used in *B. juncea* (Kumari *et al.* 2020).

Several new CMS systems

The wild allied species are the good source of new alleles and new genes including nuclear and organelle genomes which can be used for the production of new CMS systems in Brassicas. There are several different types of cytoplasms that have a high potential for use in this type of male sterility (Bang *et al.* 2011 and Kaur *et al.* 2014). Stable CMS lines (Fig. 1) in Brassicas may be created by introducing foreign cytoplasmic genes from wild related species through a hybridization process mediated by protoplast fusion (interspecific, intergeneric, or intertribal hybridization) (Prakash *et al.* 2009, Yamagishi and Bhat 2014).

Male-sterile *B. juncea* has been developed by using different male-sterile alloplasmic cytoplasm.

Fig. 1. Maintenance of CMS.



Male Parent (+): Male fertile maintainer plant (N ms/ms)

Female Parent (-): Male sterile plant (S ms/ms)

Seeds harvested from male sterile (-) plants are hybrid seeds (i.e. sterile).

This CMS system is used where vegetative part is of economic value.

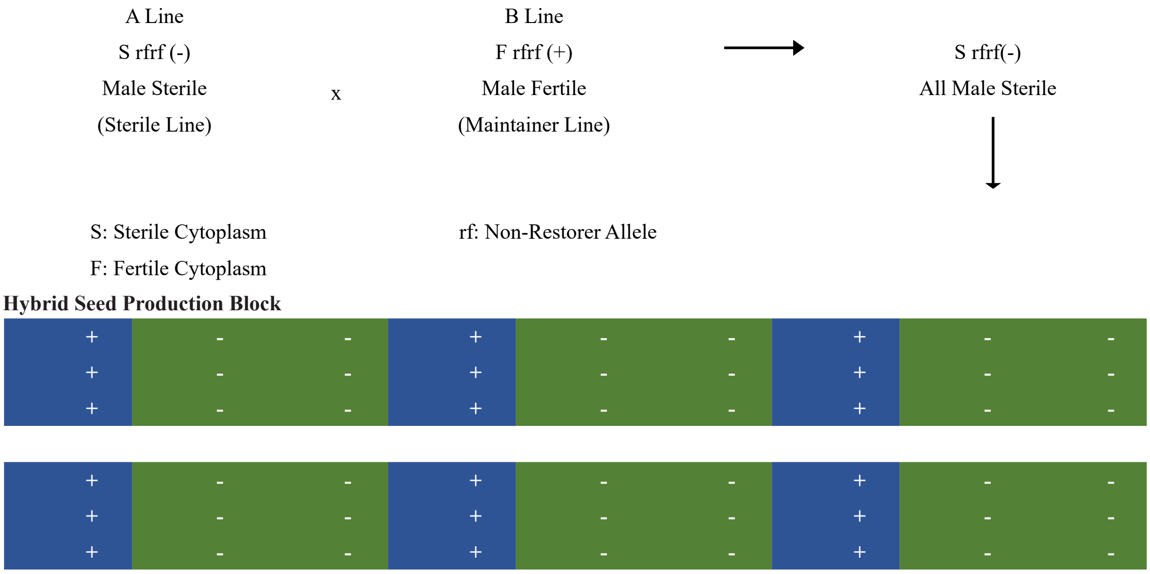
Through somatic hybridization sterile cytoplasm of *Moricandia arvensis* was transferred into *B. juncea* (Kaur *et al.* 2004). The CMS in *Brassica juncea* exchanged with the cytoplasms of *D. catholica, D. berthautii,* and *D. erucoides*, associated with *orf* 108, (Kumar *et al.* 2012) and thus it shows that CMS types differing in morphology may have same molecular basis. Recently, new male sterility system has also been developed in *Brassica juncea* which substitutes the nucleus of *Brassica juncea* into the cytoplasm of *Brassica fruticulose* (Atri *et al.* 2016).

Utilization of CMS

CMS has been reported in 150 species and extensively utilized to produce hybrids at large scale in vegetables (Kaul 1988). A line is back cross with selected B line for six to seven times. In the new genetic background, this generates the A and B lines. Cytoplasmic male sterility cannot be used in fruit vegetables (such as chillies and tomatoes), but it may be used in vegetables in which the vegetative part is more economically valuable (e.g. radish, carrot, cole crops). Different hybrids are produced by using CMS system in different vegetable crops like in cabbage (Quigan No. 1, KTCH-5, H-11), Cauliflower (Pusa SB Cauliflower Hybrid-1) and carrot (Jinhong No. 4, Jinhong No. 5), Pusa Vasuda (Tropical hybrid).

In the onion cultivar (Italian Red), the first ms plant (13-53) was discovered (Jones and Emsweller 1936). It results from two recessive genes, ms1 and ms2. There are two forms of sterile cytoplasm documented, S and T, with S-cytoplasm being the most often used (Pelletier *et al.* 1995). Hybrids generated in onion using male sterility include Arka Kirtiman and Arka Lalima from IIHR (Bangalore) while Hybrid-63 and Hybrid-35 from IARI (New Delhi). The ms lines of onion brought from the United States, Japan and the Netherlands were very extremely unstable in India's high temperature and widespread photoperiod (Korla 1999, Hazra and Som 1999). Onion seeds lose

Fig. 2. Maintenance of CGMS.



Male Parent (+): Male fertile restorer plant (F RfRf)

Female Parent (-): Male sterile plant (S rfrf)

Seed harvested from male sterile (-) plants are hybrid seeds (i.e. fertile).

This CGMS system is used where seed is of economic value.

viability quickly, which makes it difficult for breeders to produce hybrids (Pathak and Gowda 1994, Kohli 1999). Furthermore, male sterility does not occur often in Indian onion varieties. Pusa Red and Nasik White Globe are two male sterile lines created in India (Pathak and Gowda 1994, Hazra and Som 1999).

Limitations of CMS

It depends on environmental factors i.e. may lead to partial fertility in another environment which result in mixture of seeds.

Genetic vulnerability is caused by some of the diseases or disorders linked with certain types of cytoplasm.

CMS is limited utilized as it produces sterile hybrids.

Cytoplasmic genetic male sterility (CGMS) system

It is governed by cytoplasmic and nuclear gene interactions (Fig. 2). Fertility restorer gene (RfRf) is dominant in carrot, onion, chilli, and Brassica napus.

In CGMS, three lines are involved:

A-line (Sterile cytoplasm and recessive nuclear alleles “rfrf”).

B-line (Fertile cytoplasm & recessive nuclear alleles “rfrf”).

C-line (Either sterile or fertile cytoplasm but a dominant fertility restorer gene).

Utilization of CGMS

Chilli

The CGMS was first observed in chilli by Peterson (1958) in an introduction (PI-164835) of *Capsicum annuum* from India (Kumar *et al.* 2007). The World Vegetable Center in Taiwan revealed two chilli CGMS lines that were sterile when night temperatures fell below 15 degrees Celsius, CCA-4759 and CCA-4757 (Liu and Gniffke 2004). AVRDC created chilli CCA-4261 (CGMS lines) that were utilized to create hybrid

(Kashi Surkh, "CCH-2"). Three CGMS-based hybrids have been developed at IIHR, Bangalore:

Arka Meghna (MSH-172)
Arka Harita (MSH-149)
Arka Sweta (MSH-96)

Whereas, CGMS-based onion hybrids includes Hybrid-63, and Hybrid-35 from IARI, New Delhi while Arka Kirtimaan and Arka Lalima from IIHR, Bangalore.

Carrot

Carrot has been reported to have three forms of male sterility:

Brown anther "ba" (Welch and Grimbal 1947)
Petalloid "pt" (Thompson 1962)
Gummifer "gugu" (Nothnagel 1992).

Brown anther type male sterility

Welch and Grimbal identified and published brown anther (ba) type of male sterility in the cultivar Tendersweet in 1947. This type of sterility in brown anthers is caused due to the interaction of "sacytoplasm" with two distinct recessive nuclear genes, resulting in malformed brown anthers with no functional pollen.

Petaloid male sterility

For hybrid seed development, petaloid sterility is more often employed. Morelock (1974) attributes male sterility to an interaction with "Sp cytoplasm" and two separate (dominant) genes "M1 and M2". It causes stamens to be replaced by petals or by both petals and stamens to be replaced by green bract-like structures (Kitagawa *et al.* 1994). It is more stable in a variety of settings. In 2009, at IARI regional station in India, Katrain (HP), petaloid CGMS was transferred into nantes kinds and then crossed with the indigenous variety Pusa Yamdagini to create Pusa Nayanjyoti (hybrid).

Gummifer type male sterility

It is due to the interaction of gummifer cytoplasm with recessive allele (gugu) and results in complete reduction of petals and anthers.

Limitations of CGMS

Maintenance of A, B and C-lines is laborious.
Male sterility may breakdown partially under certain environmental conditions.
Non availability of fertility restorer line.

Chemical hybridizing agents (CHAs)

Chemically induced male sterility is non heritable and induced through chemicals. Maleic Hydrazide, Ethephon, Gibberellic acid, Mendok, Dalapon are used as male gametocides which causes pollen abortion (Kempe and Gils 2011).

Need of CHAs

Multiple male sterility systems, like GMS, CMS, and CGMS, are utilized to induce male sterility, eliminating the need for the time-consuming emasculation procedure. In chemically induced male sterility, there is no requirement to maintain and determine ms (male sterile) and restorer lines (Tinna 2019). As a result, it is a more time-saving strategy. Aside from them, there is no requirement to identify and route male viable lines (Saxena and Hingane 2015).

Characteristics of an ideal gametocide

An ideal gametocide induces full male sterility while having no effect on female functioning (Tinna 2019). It is neither affected by environmental factors nor genotypic variances, thus has a broad range of application rates and times. They should not be phytotoxic and should not be mutagenic in nature. Seed set should be normal and unaffected by its use. In hybrid seed manufacturing, it must be cost efficient (Adhikari 2012).

Effect of gametocides

The effects of CHAs range from the early stages of sporogeneses cells when viable pollens failed to dehisce (Gomez *et al.* 2015). It disturbs meiosis at an early stage, causing pollen mother cells (PMC) to degenerate, resulting in the development of thin-walled non-viable microspores and the emergence of aberrant vacuoles in the microspores (Wang and Dobritsa 2021). Abnormal tapetal layer growth, delayed dehiscence of regularly grown anthers and inhibition of germination of pollen on stigmatic surface or halt of pollen tube elongation, limits the fertilization process (Kumar and Singh 2005).

Factors affecting CHAs technology

In CHAs technology, male sterility (functional) in female parents is induced chemically rather than genetically to create hybrid seeds (Singh *et al*. 2021). The use of CHA is determined by a number of factors including source of viable pollen (which is a male parent that can outcross with the male sterile female parent), configuration of the female plant, an adequate pollinating agent that transfers pollen grains from the male fertile parent to the male sterile parent, and the synchronization of flowering in male and female parents (Tinna 2019, Kempe *et al.* 2014).

Method of application of gametocides

CHAs are applied in a variety of ways, including aqueous sprays before the commencement of flower buds (Yahaya *et al.* 2019). Meer and Bennekom (1973) employed three different techniques of CHAs application.

Dipping the bulb bases in GA3 solution
Sprays of GA3
Injection with a micro-syringe (into the inflorescence stalk)

CONCLUSION

Male sterility is one of the most efficient genetic tool in hybrid seed generation. The process of male sterility in vegetable crops such as tomato, onion, carrot, chilli, melon, and so on should be understood. In the instance of chilli and muskmelon, GMS is being used to commercially create F1 hybrid seed. Cytoplasmic and cytolasmic-genic male sterility are commonly used in most vegetables, while CMS-based hybrids such as Kashi Surkh and Kashi Anmol have been produced at IIVR, Varanasi from AVRDC utilizing CMS lines. Many vegetable crops, including tomato (PAU, Punjab), cauliflower (IARI, New Delhi), okra (IIHR, Bengaluru), and others, are undergoing research on GMS or CGMS-based male sterility. For commercial production of hybrid seeds in vegetable crops, greater emphasis should be placed on transgenic male sterility systems, which have yet to reach spectacular success. Morphological markers must be found, and the introduction of molecular markers may enhance selection strategies, which are also required in all crops. Development of resistant hybrids to various biotic-abiotic stresses, will encourage the use of hybrid vegetable technology for large-scale vegetable crop production.

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