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Role of Male Sterility in Vegetable Crops

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Abstract:

Male sterility serves as a key method for efficiently producing hybrid seeds in various vegetable crops, contributing to increased yield, early maturity and pest-disease resistance. Progress in understanding its mechanisms has enabled the development of cost-effective hybrid seeds. Globally, cytoplasmic male sterility (CMS) and cytoplasmic genetic male sterility (CGMS) are widely employed for vegetable hybridization. CGMS finds commercial use in chilli, onion, and carrot, while CMS is utilized in cabbage, cauliflower, and onion. Additionally, genetic male sterility (GMS) is applied in chilli and cucurbit crops. This article underscores the importance of different male sterility systems: GMS, CMS and CGMS for plant breeders aiming to produce more efficient and economically viable hybrid seeds. By harnessing male sterility, breeders can enhance agricultural productivity and sustainability, meeting the growing demand for high-quality vegetables while optimizing resources. Understanding and leveraging these mechanisms are pivotal steps toward advancing vegetable crop production globally.

Keywords: CGMS, CMS, GMS, Male sterility, Vegetables.

Introduction:

Vegetables are essential for nutrition, but with a growing population, demand rises. Hybrid varieties are increasingly popular, replacing open-pollinated ones over the past two decades. Adoption of hybrids boosts vegetable production significantly, especially considering limited land availability. Hybrid technology, utilizing male sterility lines, offers efficient seed production, enhancing productivity and economic output. This advancement in agriculture promises to meet future challenges, ensuring food security and sustainability in our country.

Male sterility is a condition observed in flowering plants where the pollen is either absent or non-functional, while the female gametes function normally. This phenomenon can manifest in various forms, including:

1. Absence or malformation of male reproductive organs in bisexual plants
2. Failure to develop normal microsporogenous tissue, leading to impaired pollen production
3. Anomalies in microsporogenesis, resulting in the production of viable, deformed, or aborted pollen
4. Viable pollen development, but the anthers remain indehiscent, hindering the release of pollen

History of Male Sterility

Male sterility in flowering plants was first documented by Koelreuter in 1763. Subsequent reports in onions (Jones and Clarke, 1943), carrots (Welch and Grimball, 1947), peppers (Martin and Grawford, 1951), and cabbage and cauliflower (Nieuwhof, 1961) marked significant milestones in understanding male sterility.

These discoveries spurred further research into its genetic mechanisms. Today, male sterility is crucial in hybrid seed production and seedless fruit development, revolutionizing modern vegetable breeding and agricultural practices.

Classification of Male Sterility

Male sterility can be classified into five basic types, each with distinct characteristics and underlying mechanisms.

- Genetic Male Sterility,
- Cytoplasmic Male Sterility
- Cytoplasmic Genetic/Genic Male Sterility.

There are two other types, *viz.*, Chemical Induced Male Sterility and Transgenic Male Sterility.

A. Genetic Male Sterility (GMS)

Pollen sterility caused by nuclear genes is termed genic or genetic male sterility. It has been reported in crop plants such as tomato, pepper, brinjal, cucurbits, and cole crops. This type of sterility enables efficient hybrid seed production and ensures genetic purity in breeding programs, contributing to improved crop yields and high-quality varieties.

Hybrid Seed Production Procedure for GMS Based Hybrids

Hybrid seed production takes place in an isolated field known as the "hybrid seed production block." In this system (block), male and female lines are strategically interplanted in a 1:2 ratio (Fig. 1). The female line produces both male fertile and male sterile plants in a 1:1 ratio. To initiate cross-pollination for hybrid seed production, the male fertile plants are removed from the female line. In most cases, male sterile plants are indistinguishable from their fertile counterparts, except in a few instances where the male sterile flowers may be smaller, such as in chili plants.

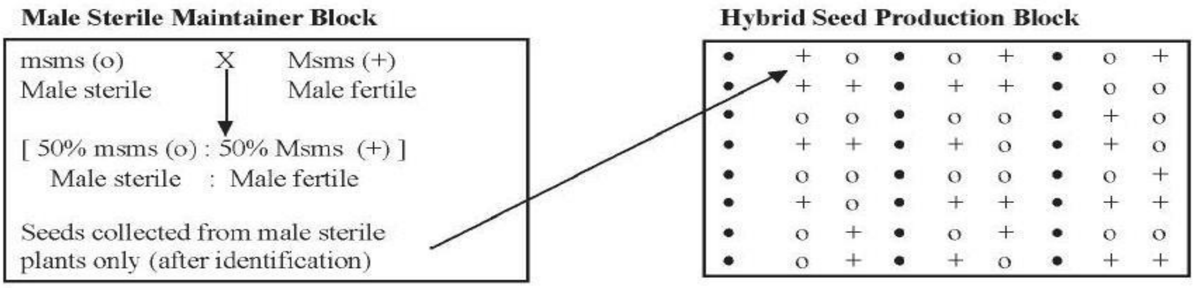

Fig.1: General Scheme of hybrid seed production utilizing GMS

Table 1: Utilization of GMS in vegetables

Crops	Gene number/condition	Gene	Variety developed
Tomato	Single recessive gene	*ps*-2	Shalimar Tomato Hybrid-1 Shalimar Tomato Hybrid-2
Chilli	Single recessive gene	*ms-12 & ms-3*	CH-1, CH-3
Muskmelon	Single recessive gene	*ms-1*	Punjab Hybrid-1

B. Cytoplasmic Male Sterility (CMS)

Cytoplasmic Male Sterility (CMS) is determined by the cytoplasm of the plant. As the cytoplasm comes mainly from the egg cell during zygote formation, progeny from male sterile plants will always be male sterile. CMS can be transferred to a specific strain by using it as a pollinator (recurrent parent) in successive generations of a backcross program. After 6-7 backcrosses, the nuclear genotype of the male sterile line becomes almost identical to the recurrent pollinator strain (Fig. 2).

Utilization of CMS For Hybrid Seed Production:

Cytoplasmic male sterility (CMS) can be maintained by crossing a male sterile line (A line) with the recurrent pollinator strain (maintainer line) in a backcross program, as their nuclear genotypes are identical. The male fertile line used for maintaining the male sterile line is called the maintainer line or B line. CMS is particularly valuable for hybrid seed production in vegetables where the economic value lies in the vegetative parts, such as onion, carrot, radish, cole crops and others.

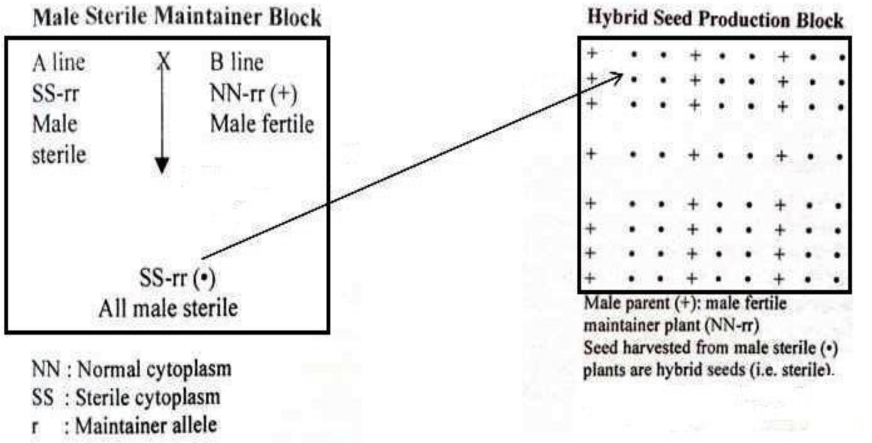

Fig.2 General Scheme of hybrid seed production utilizing CMS

C. Cytoplasmic Genetic Male Sterility (CGMS)

In this case of cytoplasmic male sterility, fertility restoration is possible due to a dominant nuclear gene known as the fertility restorer gene (RR). This gene can be found in certain strains of the species or transferred from a related species. The sterility factor results from the interaction of nuclear genes and cytoplasm, where neither of them alone can control sterility. This type of sterility has been reported in crops like carrot, onion, chili, capsicum, and *Brassica napus*.

To develop a new male sterile line, the same procedure as the cytoplasmic system is followed, but the pollinator strain's nuclear genotype must be *NN-rr* to ensure fertility restoration.

Utilization of CGMS For Hybrid Seed Production:

Cytoplasmic-Genetic Male Sterility (CGMS) can be maintained by crossing a cytoplasmic male sterile line (*SS-rr*) or A line with a pollinator strain (*NN-rr*) used as a recurrent parent in backcross programs. The nuclear genotype of the pollinator is identical to the new male sterile line (Fig. 3). The male fertile line used to maintain the male sterile line is called the maintainer line or B line.

For hybrid seed production, 2 to 3 rows of line A (*SS-rr*) are alternated with 1 row of line C, which is generally expected to have the genotype *NN-RR*. Commercial hybrid seed is harvested from line A. Line C may have genotypes *NN-rr*, *NN-Rr, SS-Rr,* or *SS-RR*. Hybrids developed using the first three genotypes will be sterile and suitable for situations where seed is not a commercial product. However, for cases where seed is important, the pollen parent should have the genetic constitution *NN-RR.*

The main advantage of CGMS over GMS is the ability to obtain 100% male sterile plants for direct use as female parents in hybrid seed production.

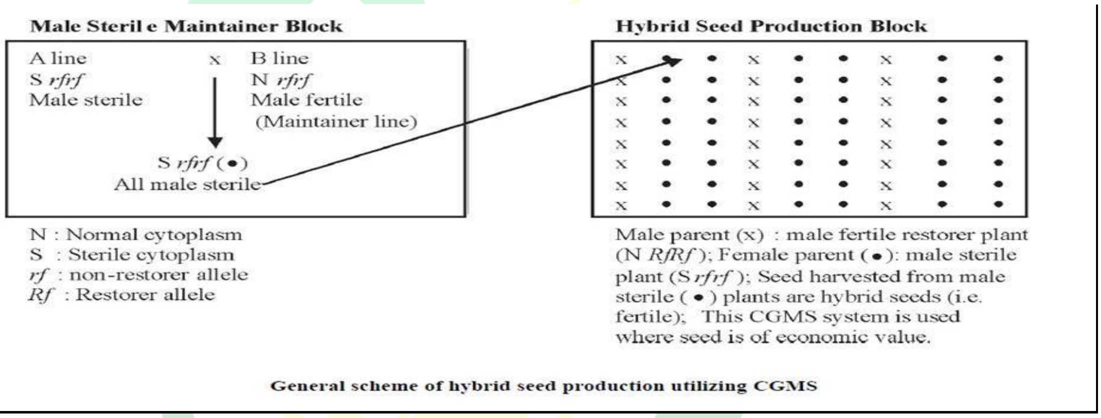

Fig.3: General Scheme of hybrid seed production utilizing CGMS

Table 2: Utilization of CGMS in vegetables

Sr. No	**Crops**	**Gene**	**Commercially utilized**	**Variety**
1.	Chilli	Single recessive gene	*ms-2*	Arka Meghna, Arka Sweta, Arka Harita, Kashi Surkh
2.	Onion	Single recessive gene		Arka Kirtiman, Arka Lalima
3.	Carrot	Single recessive gene		Pusa Nayanjyoti, Pusa Vasuda

D. Chemically Induced Male Sterility (CiMS)

CHA (Chemical Hybridizing Agents) are chemicals that artificially induce non-genetic male sterility in plants, making them suitable as female parents in hybrid seed production. They are also known as Male gametocides, male sterilants, selective male sterilants, pollen suppressants, pollenocide, androcide, and other names. The effectiveness of these compounds, such as FW-450, ethephon, RH-531, and PPX 3778, depends on treatment time and dosage.

The first report of using CHA for inducing male sterility was given by Moore and Naylor in 1950. They successfully induced male sterility in maize using maleic hydrazide (MH).

Table 3: Potential gametocides used for induction male sterility in vegetable crops

Gametocides	Concentration	Vegetable crops
GA3	2000-3000 ppm	Onion, lettuce
M.H	0.4-0.5%	Chilli, muskmelon
	2400-2600 ppm	Carrot
	100-500 ppm	Tomato
	400-500 ppm	Okra
FW -450 (Mendok)	0.2-0.4%	Tomato
	0.4%	Okra
	0.2%	Brinjal
	0.3%	Muskmelon
Phosphon –D	750-1000 ppm	Onion
2-4D	50 ppm	Tomato
	20 ppm	Brinjal
TIBA	50-100ppm	Watermelon, Tomato
GA3	1000ppm	Capsicum
Ethrel		Brinjal

E. Petaloid Male Sterility (PMS)

Petaloid sterility, a homeotic mutation, is widely used for commercial hybrid seed production worldwide. This mutation leads to the replacement of stamens with petals (white petaloidy) or both stamens and petals with green bract-like structures (green petaloidy) (Kitagawa *et al.*, 1994). Petaloid sterility remains stable across diverse environmental conditions during flowering and seed production.

Role of Male Sterility in Vegetables

1. Tomato:

Over 55 male sterile (*ms*) alleles causing sporogenous, structural, and functional sterility have been reported (Kaul, 1988). Some of these genes' chromosomal locations are known (Table 4). The list of artificially induced and spontaneously isolated male sterile mutants in tomato is continuously growing. There are four types of male sterility in tomato, each governed by a single recessive gene (Table 5). The stamenless type produces misshapen fruit in the F1 hybrid generation, and positional sterility is not stable. The pollen abortive type and functional sterilities are commonly used in F1 hybrid production.

Sher-E-Kashmir University of Agricultural Science and Technology (SKUAST) in Kashmir, India, has released two tomato hybrids (Shalimar tomato hybrid-1 and Shalimar tomato hybrid-2) based on the Genetic male sterility system. Similarly, work on GMS lines is underway at PAU for tomato.

Table 4: Chromosomal location of some *ms* genes in tomato

Chromosome	**ms genes**
1	*ms*-6, *ms*-32, *ms*
2	*ms*-2, *ms*-5, *ms*-10, *ms*-15, *ms*-26, *ms*-35, *ms*, *ps*
3	*ms*-9
4	*Ls*
6	*ms*-16, *ms*-32, *cl*-2
8	*ms*-8, *ms*-17, *vms*
10	*ms*-31
11	*ms*-3, *ms*-7, *ms*-12, *ms*-14, *ms*-42, *ms*, *ap*

Table 5: Description of different male sterile mutants in tomato

Mutant	**Description**	**Inheritance**	**Governing genes**
Pollen sterile	Pollen abortive	Monogenic recessive (except MS-48), monogenic dominant)	***ms*series (- 49 independent genes)**
Stamenless	Stamens absent	Monogenic recessive	*sl-1*, *sl-2*
Positional sterility	Stigma exerted	Monogenic recessive	*ps*

Functional sterility	Anthers do not dehisce	Monogenic recessive	*ps-2*

2. Chilli:

In chilli, Punjab Agricultural University (PAU) has developed the MS-12 line, which carries genetic male sterility (GMS) controlled by the recessive gene (*msms*). This male sterile line (MS-12) was created by transferring the sterility gene (ms-509, renamed as ms-10) from capsicum imported from France into the "Punjab Lal" cultivar through backcrossing (Singh and Kaur, 1986). Using this male sterile line, PAU has released two chilli hybrids, CH-1 and CH-3, showing 80-100% heterosis and outperforming all recommended chilli varieties. The popularity of these hybrids has led to a significant increase in chilli acreage in Punjab State and other states like Haryana and Rajasthan.

The World Vegetable Centre, Taiwan, identified two Cytoplasmic-Genetic Male Sterility (CGMS) lines in chilli, CCA-4759, and CCA-4757, which are reliably sterile under night temperatures below 15ºC (Liu and Gniffke, 2004). CGMS lines (CCA-4261) were recently introduced to IIVR in India and are being used to produce CGMS-based hybrids like Kashi Surkh. Several promising CGMS-based hybrid combinations have been identified at IIVR and IIHR, such as A2 x Pusa Jwala, A3 x Pusa Jwala, A2 x Pant C1, A3 x Japani Longi, and A7 x Pant C1. PAU Ludhiana is also actively working on utilizing CGMS in chilli hybrid breeding.

3. Onion:

The first CMS plant was reported in the progenies of the onion cultivar Italian Red (Jones and Emsweller, 1936). Male sterility in onion was controlled by a single recessive nuclear restorer locus (Jones and Clarke, 1943).

The first CMS source in onion was CMS-S type, which likely had an alien cytoplasm with differences in chloroplast and mitochondrial genomes compared to N cytoplasm. The CMS-S system has been widely used due to its stability in various environments. The CMS line (S *ms/ms*) and its near-isogenic maintainer line (N *ms/ms*) are crucial for breeding F_1 hybrids using the CMS system.

Over 50% of globally cultivated onion varieties are F1 hybrids derived from S-cytoplasm. In India, CMS research gained momentum in the 1980s at IIHR (Bangalore), IARI (New Delhi), and MPKV (Rahuri). IIHR has released two hybrids, Arka Kirtiman and Arka Lalima, after developing CMS lines and maintainers from a commercial variety Pusa Red.

CMS and CGMS are the most widely used male sterility systems globally, particularly in crops like onion and carrot with many small-sized flowers that make hand emasculation difficult. By utilizing male sterility, hybrid seed cost can be reduced by 40 to 70%. Genetically engineered male sterility offers breeders significant opportunities to enforce pollination control in hybrid seed production systems.

4. Cucurbitaceous vegetables:

Cucurbit vegetables, with their larger male and female flowers, allow for various pollination control strategies. Most genetic male sterile mutants in cucurbits are monogenic recessive. In muskmelon, male sterility has been successfully commercially exploited. Five recessive and non-allelic male sterile genes (*ms-1, ms-2, ms-3, ms-4, and ms-5*) have been identified in melon (*Cucumis melo* L.). However, greenhouse studies have shown that male-sterile plants in ms-1 and ms-2 progenies are challenging to identify, as aberrant flowers can also be observed on genetically fertile siblings, leading to unstable gene expression and potential genetic impurity in F1 hybrid seed (McCreight, 1984).

In India, the male-sterile gene ms-1 was introduced in 1978 and used to release two commercial cultivars, "Punjab Hybrid" and "Punjab Anmol."

The first male sterility in watermelon (*Citrullus lanatus*, Thunb.) was reported by Watts (1962), who discovered a male sterile mutant in the X2 generation of 'Sugar Baby' irradiated with gamma rays. The mutant, known as a glabrous male sterile (gms), exhibited a lack of hairs on the plant foliage alongside male sterility. Both glabrousness and male sterility were inherited together as a single recessive nuclear gene, indicating close linkage or a pleiotropic effect of the involved locus (Watts, 1967). However, the gms gene not only affected male reproductive function but also reduced female reproduction, resulting in limited commercial application (Zhang *et al*., 1994).

5. Cole crops:

In cole crops, F_1 hybrids offer significant advantages, including uniform maturity, high early and total yield, and improved curd/head quality in terms of compactness, color, and resistance to insect pests, diseases, and heat.

In *Brassica oleracea* L., the first CMS system was developed by Pearson in 1972 through interspecific hybridization between *B. nigra* and *B. oleracea* var. italica. Backcrosses were made between the resulting amphidiploids and the cabbage cultivar Green Globe, leading to the establishment of two CMS systems: petaloid and vestigial anther male sterility. The flowers of petaloid male sterile plants were less attractive to pollinating insects due to enlarged, malformed pistils lacking in nectarines (Pearson, 1972). In vestigial anther types, although the flowers were smaller and normal with functional nectarines, homozygous plants could not be obtained even after six generations of backcrossing in broccoli (Dickson, 1975).

6. Carrot:

Brown anther (ba) male sterility was first observed in the cultivar Tendersweet and reported by Welch and Grimball in 1947. Studies by Hanshe and Gabelman (1963) and Banga *et al.* (1964) indicated that the expression of brown anther sterility was caused by a homozygous recessive locus *Ms5* or a dominant allele for *Ms4*. However, the fertility could be restored by a dominant allele of either of the two complementary loci.

In India, the CMS system was established for the first time in asiatic carrot germplasm at IARI (Indian Agricultural Research Institute). IARI, New Delhi, developed the first public sector tropical carrot hybrid, Pusa Vasudha, using this male sterility. Additionally, the first temperate carrot hybrid, Pusa Nayanjyothi, was developed at IARI regional station, Katrainutilizing CMS. Introducing CMS into carrot breeding materials has proven to be an efficient tool for mass-scale pollination control, facilitating hybrid seed production in carrots.

Conclusion:

Vegetables are vital for nutrition, but with a rising population, demand surges. Hybrid vegetable varieties, utilizing male sterility for production, have become popular to meet the recommended 300g per capita daily consumption. Male sterility in flowering plants allows efficient hybrid production, essential for increasing vegetable output and addressing the growing need for nutritious food in our country.

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