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Male sterility in vegetable crops

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Abstract
Male sterility in vegetables is a never-ending process due to rapid advancement in molecular techniques and their implementation. Substantial progress has been made in understanding the mechanism of male sterility in selected vegetable crops. On a global level, cytoplasmic male sterility (CMS) and cytoplasmic genetic male sterility (CGMS) are the most widely utilized in the majority of vegetables. In India vegetable hybrids based on CMS and CGMS system have been limited. In India, genetic male sterility (GMS) has been exploited commercially only in the cases of chilli and muskmelon to develop F_1 hybrid seed commercially. Sher-e- Kashmir University of agricultural science and technology (SKUAST), Kashmir, India has released two tomato hybrids (Shalimar tomato hybrid-1 and Shalimar tomato hybrid-2) based on the male sterility system. Similarly in tomato, work on GMS lines is in progress at PAU. The CGMS system has been commercially exploited in chilli, onion and carrot. In the recent past, chilli CGMS lines were introduced at the Indian Institute of Vegetable Research (IIVR) from AVRDC, which are utilized directly or indirectly to produce CMS-based hybrids, i.e Kashi Surkh (CCH-2) and Kashi Early (CCH-3). The Indian Institute of Horticultural Research (IIHR), Bangalore, India has also released chilli hybrids based on the CGMS system, i.e. Arka Meghna (MSH-172), MSH-149 and MSH-96. In carrot, the Indian Agricultural Research Institute (IARI) regional station, Katrain (HP), India has developed one hybrid, 'Pusa Nayanjyoti', which is based on petaloid CGMS. In the tropical group of carrot, IARI, New Delhi India has also reported CGMS system in different genetic back- grounds and evaluation of different hybrid combinations is in progress. In onion, IIHR, Bangalore has released two hybrids based on the CGMS system, i.e. Arka Kirtiman and Arka Lalima, and IARI, New Delhi has developed two hybrids in onion (Hybrid-63 and Hybrid-35) which are based on the same system. The CMS system has been commercially exploited in cabbage, cauliflower and onion.

Keywords: vegetables, genetic male sterility, cytoplasmic male sterility, cytoplasmic genetic male sterility

Introduction
Male sterility is defined as the deviant condition in normally bisexual plants with the failure of the plants to produce functional pollens, anthers or male gametes. It refers to a condition in which the plants are unable to produce or release the functional pollen grain as a result of failure of formation or development of pollen, stamens or gametes although the female gametes function normally. J.K. Koelreuter [11] observed anther abortion within species & species hybrids and was first to report male sterility in flowering plants. The male sterility attains diverse forms such as:

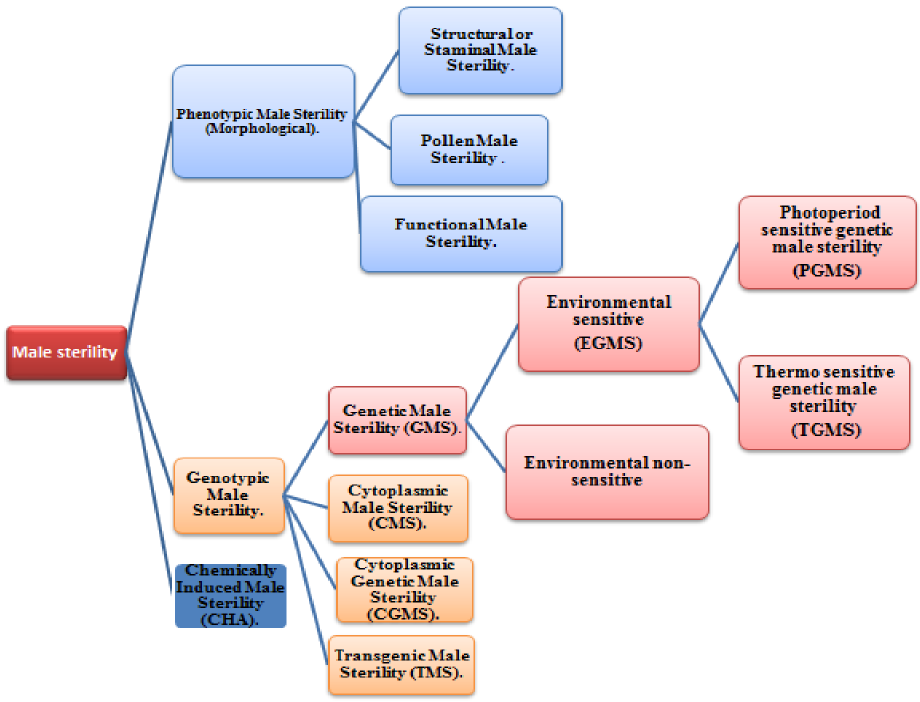
- Absence, malformation of male organs in bisexual plants.
- Fail to develop normal microsporogenous tissue.
- Anomalies in microsporogenesis yielding in viable, deformed or aborted pollen.
- Viable pollen development but anthers indehiscent.

Male sterility can arise in plants due to following reasons
- Barrier of tapetal layer: Delayed degeneration of tapetal cells that block the availability of nutrient to microspore [15].
- Improper timing of callase activity: Callase is an enzyme required for breakdown of the callose that surrounds the pollen mother cells, helps in release of pollen ; early or delayed callase activity lead to sterility [16].
- Role of Esterase: Esterase play role in the hydrolysis of Sporopollenin, the polymer required for pollen formation. Decreased activity of esterase in male sterile plant has been observed in tomato [17] and in radish [21].
- Absence or malformation of male organs (stamens) in bisexual flowers: Failure to develop normal microsporogenous tissue- anther
- Abnormal micro sporogenesis: Deformed or in viable pollen

The phenomenon of male sterility is of special interest for the plant breeders to produce more efficient and economic hybrid seed, in number of crops. Vegetables are the most extensively utilized plants for exploitation of heterosis for development of hybrids. As the importance of heterosis breeding increased, male sterility proved an asset particularly in crops like onion and carrot which produce many but small sized flowers making hand emasculation tedious. Large scale hybrid seed production sometimes remains handicapped because of high labour cost, unavailability of trained labour at crucial times i.e. at blooming, bad weather conditions like continuous rains etc. The phenomenon of male sterility has always been of long term interest for researchers of various disciplines of applied, strategic and basic sciences. It is of special interest for plant breeders to produce more efficient and economic hybrid seed. Discovery of certain male sterile mutants which eliminate more laborious operations of emasculation combined with various marker genes further facilitate identification of undesirable types even at seedling stage has widened the very basis of hybrid seed production. This technique has also reduced the total cost of hybrid seed production. Onion crop provides one of the rare examples of very early recognition of male sterility [4, 7], its inheritance and use in hybrid seed production [8]. Since then male sterility is reported in fairly large number of crops including vegetables. These male sterile plants were either isolated in natural populations or were artificially induced through mutagenesis [10]. In recent past, male sterility systems are also developed through engineering [20] and protoplast fusion. Presently crops like muskmelon and chilli are very successful examples of utilization of male sterility system in India [9].

Classification of male sterility

Kaul [10] classified male sterility into two major groups: genetic (spontaneous or induced) and non- genetic (induced) male sterility. On a phenotypic basis, genetic male sterility has been divided into three classes (i.e. sporogenous, structural and functional) and non-genetic male sterility as chemical, physiological and ecological male sterility. In addition, on a genotypic basis, genetic male sterility has been grouped as genic, cytoplasmic and gene cytoplasmic male sterility as shown in figure below.



Kaul, 1988

Phenotypic male sterility

- **Pollen sterility**: in which male sterile individuals differ from normal only in the absence or extreme scarcity of functional pollen grains (the most common and the only one that has played a major role in plant breeding).
- **Structural or staminal male sterility**: in which male flowers or stamen are malformed and non-functional or completely absent.
- **Functional male sterility:** in which perfectly good and viable pollen is trapped in indehiscent anther and thus prevented from functioning.

Genetic male sterility (GMS)

GMS is reported in about 175 plant species [10] including important vegetable crops. As the name suggests, this type of male sterility is controlled by the gene(s) from the nuclear compartment. Most of the naturally occurring or induced male sterile mutants are recessive in nature with a few exceptions in Cole vegetables (e.g. cabbage and broccoli) and genetically transformed male sterile lines [10, 20]. Occurrence of predominantly recessive male sterility clearly indicates that GMS is the result of mutation in any gene(s) either controlling microporogenesis (pollen development process), stamen development or micro-gametogenesis (male gamete development process) or accordingly the phenotype of GMS mutants may vary. All the transgenic male sterile lines developed till date are GMS, since they are 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 [3, 5, 6], brinjal and several other vegetables [10]. Genetic male sterility is subdivided in 2 broad groups: (i) Environment sensitive genetic male sterility (EGMS) (ii) Environment insensitive genetic male sterility (GMS). In addition, genetic engineering has produced a novel type of dominant genetic male sterility referred to as transgenic male sterility.

EGMS: In EGMS, *ms* gene expression occurs within specified range of temperature and photoperiod regimes. Certain GMS lines are conditional mutants, meaning thereby, in particular environment male sterile mutant plants turn into male fertile. After determination of critical environment (usually temperature or photoperiod) for sterility and fertility expression, such EGMS is further divided into two groups i.e. (i) Temperature sensitive genetic male sterility (TGMS) (ii) Photoperiod sensitive genetic male sterility (PGMS). EGMS lines are reported in several vegetable crops. A majority of these, however, were previously identified as normal genic male sterile lines. Initially EGMS lines were of very less practical value, as they were unstable. But now they represent the most efficient system for hybrid seed production. 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.

Transgenic genetic male sterility
A gene introduced into the genome of an organism by recombinant DNA technology or genetic engineering is called transgene. Many transgenes produce genetic male sterility, which is dominant to fertility. Consequently, it is essential to develop effective fertility restoration system if these are to be utilized for hybrid seed production. An effective restoration system is available in at least one case called Barnase/Barstar system. The Barnase gene of Bacillus amyloliquefaciens encodes an RNAase. When Barnase gene is driven by TA29 promoter, it is expressed only in tapetum cells causing their degeneration. Transgenic tobacco and Brassica napus plants expressing Barnase were completely male sterile. Another gene, Barstar, from the same bacterium encodes a protein, which is a highly specific inhibitor of Barnase RNAase. Therefore, transgenic plants expressing both Barstar and Barnase are fully fertile. The Barnase gene is tagged with bar gene, which specifies resistance to be herbicide phosphinothricin. This male sterile line is maintained by crossing it with a male fertile line. The progeny so obtained contains 1 male sterile: 1 male fertile plant; the latter is easily eliminated at seedling stage by a phosphinothricin spray. The male sterile plants are crossed with the Barstar line to obtain male fertile hybrid progeny. This system of male sterility is yet to be commercially used.

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

Limitation of GMS
Due to a more tedious maintenance process and non-availability of suitable marker genes among vegetable crops, GMS has been utilized commercially only in chilli and muskmelon. Moreover, a number of crops (e.g. tomato, brinjal, pea etc.) which are highly self-pollinated, free out crossing is prohibitive, thus leading to poor seed and/or fruit set. This method could gain popularity in practice only if suitable insect pollinators or other means are found to raise the percentage of cross pollination.

Cytoplasmic male sterility
This type of male sterility is determined by cytoplasm. Since the cytoplasm of a zygote comes primarily from the egg cell, the progeny of such male sterile plants would always be male sterile. Cytoplasmic male sterility can be transferred easily to a given strain by using that strain as a pollinator (recurrent parent) in the successive generations of the backcross programme. After six to seven backcrosses, the nuclear genotype of the male sterile line would be almost identical to that of the recurrent pollinator strain.

Utilization of CMS
Cytoplasmic male sterility can be maintained by crossing a male sterile line (A line) with the pollinator strain (maintainer line or B line) used as the recurrent parent in the backcross programme since the nuclear genotype of the pollinator is identical to that of the new male sterile line. The basic method of utilization of a cytoplasmically male sterile parent for hybrid seed production is illustrated in figure 4. Cytoplasmic male sterility can be utilized for producing hybrid seeds in those vegetables where the vegetative part is of economic value (e.g. onion, carrot, radish, Cole crops etc.). But in those vegetables crops where seed is the economical part, like tomato, chilli, melon etc.it is of no use because the hybrid progeny will be male sterile.

Limitations of CMS
Cytoplasmic male sterility (CMS) is sensitive to environmental factors, e.g. a line may be completely male sterile in one environment and may have partial fertility in another. This phenomenon may lead to mixture of selfed seed in an otherwise hybrid seed. Certain diseases or disorders are associated with a particular type of cytoplasm which leads to genetic vulnerability, e.g. T-cytoplasm is associated with Southern Corn leaf blight in corn. Continuous incorporation of a small amount of male parent cytoplasm through each backcross during maintenance may lead to a partial or complete breakdown of male sterility.

Cytoplasmic-genetic male sterility (CGMS)
There is a case of cytoplasmic male sterility where a nuclear gene for restoring fertility in the male sterile line is known. The fertility restorer gene, Rf/Rf is dominant and is found in certain strains of the species or may be transferred from a related species. Thus the gene restores male fertility in the male sterile line, hence it is known as a restorer gene. The sterility factor is determined by the interaction of nuclear genes and cytoplasm but none of them singly can control sterility. This type of male sterility is reported in carrot, onion, sugar beet, chilli, capsicum and *Brassica napus*.

Utilization of CGMS in vegetables

Crops	Gene	Commercially utilized	Variety
Chilli	Single recessive gene	ms-2	Arka Meghna Arka Sweta Arka Harita Kashi Surkh
Onion	Single recessive gene		Arka Kirtiman Arka Lalima
Carrot	Single recessive gene		Pusa Nayanjyoti Pusa Vasuda

Limitation of CGMS

Although the CGMS system is the most commonly utilized male sterility, its use is restricted to specific species because of certain limitations, such as 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; breakdown of male sterility in particular environments; highly unstable sterile cytoplasm in several cases; poor cross pollination ability of flowers of plants with sterile cytoplasm due to altered morphology and technical complexity involved in seed production and maintenance of parental lines.

Chemically induced male sterility

CHA (Chemical Hybridizing Agents) is a chemical that induces artificial, non-genetic male sterility in plants so that they can be effectively used as female parent in hybrid seed production. Also called as Male gametocides, male sterilants, selective male sterilants, pollen suppressants, pollenocide, androcide etc.

The first report was given by Moore and Naylor (1950) [14], they induced male sterility in Maize using maleic hydrazide (MH).

Chemical hybridizing agents used in different crops.

S. No	Chemical	Crop
1	Gibberillic acid	Lettuce, maize, onion, rice
2	Malic hydrazide	Cucurbits, onion, tomato, wheat
3	Naphthalene acetic acid	Cucurbits
4	Ethereal	Rice, sugar beet, wheat
5	Sodium methyl arsenate	Rice
6	Zinc methyl arsenate	Rice

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