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Review Article

EXPLOITATION OF HETEROSIS USING MALE STERILITY IN VEGETABLE CROPS

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ABSTRACT

Availability of cost effective techniques to produce large-scale F_1 seeds utilizing selected parental lines is an important factor, which ultimately determines the economics of the hybrid varieties. In vegetables, although experimental crosses (few seeds for research purpose) can be developed through manual emasculation (in case of hermaphrodite crops) followed by manual pollination of emasculated flowers or pistilate flowers (in case of monoecious crops with separate staminate and pistilate flowers) seed production of commercial hybrids (large quantity of seeds for cultivation) based on such methods is economically feasible only in tomato, eggplant, sweet pepper, cucurbits, cole crops and few other vegetables, in which a large number of F_1 seeds are obtained from one manually pollinated crossed fruits. Hybrid seeds harness heterosis therefore used for stimulated crop production. Since 1930s the discovery of male sterility in onion and self-incompatibility in cabbage, mechanisms and their proposed utilization in hybrid seed production, several mechanisms and methods have been evolved for the development of experimental and commercial hybrids. This review describes some genetic and non-genetic mechanisms utilized for hybrid development in selected vegetable crops with special reference to male sterility and chemical hybridizing agents.

KEYWORDS: Seed, pollination, commercial hybrids, genetic, non-genetic, chemical hybridizing,gms, cgms.

INTRODUCTION

Among the vegetables, first F_1 hybrid of eggplant was released during 1924 in Japan (Nishi, 1967). With the time hybrids of cucumber (1933), radish (1935), tomato (1940), watermelon (1930) and cabbage (1942) were developed (Liedle and Anderson, 1993). Seeds of most of these hybrids were produced through natural crossing between plants by exploiting competitive fertilization between self and cross pollen. Thus only it comprises of 40-80% of the seeds which were actual hybrids (Liedle and Anderson, 1993), and its level was far below than the current acceptable level for contamination in hybrid seeds. Therefore, there were need to search for methods to produce pure hybrid seeds at commercial scale. Pearson (1933) used self incompatibility mechanism in cole crops such as cabbage and cauliflowers. In onion Jones and Clarke (1943) utilized cytoplasmic male sterility mechanism and proposed methods of large scale pure hybrid seed production in onion. The achievement of complete male-sterility in the female-parent and the restored-fertility in F_1-hybrids are the major bottlenecks in the commercial hybrid seed production (Singh *et al*., 2015). At present F1 hybrid breeding method is commercially utilized to exploit heterosis in many vegetable crops like tomato, eggplant, hot and sweet peppers, onion, cabbage, cauliflower, other cole crops, radish, carrot, melons *etc*. Vegetable breeders prefer heterosis breeding because of its uniqueness to easy incorporation of resistant genes for biotic and abiotic stresses in F_1 hybrid. Moreover, despite the fact that cost of hybrid seeds is high, there has been increasing concern of the farmers on the cultivation of hybrids. This is because under optimum crop production and protection management, crop raised from the seeds of F_1 hybrid has several advantages like better yield, adaptability, uniformity and reactions to certain stresses in comparison to crop raised from the seeds of improved pure line or population.

Mechanism for hybrid development

Although experimental crosses (few seeds for research purpose) can be developed through manual emasculation (in case of hermaphrodite vegetables) followed by manual pollination of emasculated flowers or pistillate flowers (in case of monoecious vegetables), seed production of commercial hybrids (large quantity of seeds for cultivation) based on such mechanisms and methods is economically feasible only in vegetables like tomato, eggplant, many cucurbits, in which a large number of F_1 seeds are obtained from one manually pollinated crossed fruits. Nevertheless, in

these vegetables also, cost of F_1 seed production can be brought down, if practically applicable mechanism (s) to avoid selfing and maximize out crossing is resorted in the hybrid seed production field. For example, in tomato, sharp reduction in labour expenditure of hybrid seed production can be achieved by the elimination of manual emasculation process, as it represents about 40% of the total expenditure (Yordanov, 1983). Likewise, expenditure on manual pollination can be saved during pepper hybrid seed production, if considerable amount of natural cross pollination takes place on the plants of female parent (Kumar *et al*., 2002). Several mechanisms and methods have been reported for the development of hybrids in vegetable crops, however, only selected once are utilized to develop commercial hybrids of specific vegetable therefore in the table-1 some of the important most commonly utilized mechanisms for developing commercial hybrids in vegetables has been listed.

TABLE 1. The most commonly utilized mechanisms/methods for developing commercial hybrids in vegetables.

Mechanism	Commercially exploited in crops
Hand emasculation + Hand Pollination	Tomato, eggplant, sweet pepper, okra, hot pepper
Pinching of staminate flowers + Hand Pollination	Cucurbits (bitter gourd, bottle gourd etc.
Male sterility + Hand Pollination	Tomato, hot pepper, sweet pepper
Male sterility + Natural Pollination	Onion, cabbage, cauliflower, carrot, radish, muskmelon, hot pepper
Self-incompatibility + Natural Pollination	Most of the cole vegetables like broccoli, cabbage etc.
Gynoecism + Natural Pollination	Cucumber, muskmelon
Pinching of staminate flowers* + Natural Pollination	Cucurbits including bitter gourd, summer squash *etc*.

Male sterility

Onion crop provides one of the rare examples of very early recognition of male sterility (Jones and Emsweller, 1936), its inheritance and use in hybrid seed production (Jones and Clarke, 1943). Since then male sterility has been reported in several vegetables. These male sterile plants were either result of natural population or were artificially induced through techniques such as mutagenesis, genetic engineering and protoplast fusion (Kaul, 1988, Williams *et al*., 1997; Kumar *et al*., 2000 and Pelletier *et al*., 1995).

Types of Male Sterility

Male sterility in crop species has been classified in to two major groups, *viz*., genetic (spontaneous or induced) and non-genetic (induced) male sterility (Kaul, 1988). On the basis of phenotype genetic male sterility has been categorized in three classes namely sporogenous, structural and functional. Similarly, chemical, physiological and ecological male sterility are the classification of non-genetic male sterility. Further, on genotypic basis genetic male sterility was grouped as genic, cytoplasmic and gene-cytoplasmic male sterility (Kaul, 1988). Based on the location of gene (s) controlling genetic male sterility male sterility systems can be classified as (i) genic male sterility (gms; more precisely nuclear male sterility) and (ii) cytoplasmic male sterility (cms; more precisely cytoplasmic-nuclear male sterility).

Nuclear or Genic Male Sterility (gms)

Nuclear male sterility which was earlier termed as gms 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 few exceptions in cole vegetables (*e.g*. cabbage and broccoli) and genetically transformed male sterile lines (Kaul, 1988, Williams *et al*., 1997). Certain mutants, which although produce functional pollen but pollen fail to self fertilize it may be due to non-dehiscence of pollen or their special flower morphology *e.g.* positional sterility in tomato (Atanassova, 1999) and functional male sterility in eggplant (Phatak and Jaworski, 1989). The occurrence of predominantly recessive male sterility clearly indicates that gms is the result of mutation in any gene (s) controlling microsporogenesis (pollen development process), stamen development or micro gametogenesis (male gamete development process).

EGMS: There are few gms lines where male sterile mutant plants turn into male fertile which is called as conditional mutants because of influence of particular environment. After determination of critical environment like temperature or photoperiod for sterility and fertility expression, such GMS mutants are classified under environmental sensitive genic male sterile (egms) lines. Sheng *et al*. (2015) have cloned a rice gene called *tms9* on chromosome 2, involved in thermo sensitive genic male sterility. In vegetable crops, mostly temperature sensitive Egms lines have been reported (Table 2). From practical utility viewpoint, it is required to identify critical temperature or photoperiod for the fertility/sterility expression in temperature and photoperiod sensitive genetic male sterility, respectively.

TABLE 2. Environmental sensitive male sterile mutants in vegetables

Vegetable	Mutant	Reference
Cabbage	TGMS, PGMS	Rundfeldt, 1961
Brussels sprout	TGMS	Nieuwhof, 1968
Broccoli	TGMS	Dickson, 1970
Pepper	TGMS, TCMS	Daskalov, 1972; Shifriss, 1997
Carrot	TGMS	Kaul, 1988
Tomato	TGMS	Rick, 1948; Sawhney, 1983

TGMS Temperature sensitive genic male sterlity
TCMS Temperature sensitive cytoplasmic male sterlity
PGMS Photo sensitive genic male sterility

Utilization of gms

The major bottle neck in utilization of gsm is the identification and removal of 50% male fertile segregants (*Msms*) before they shed pollen in hybrid seed production field and thereby it is maintained through backcrossing. In some gms lines, *ms* genes are tightly linked with the recessive phenotypic marker genes and such marker genes, especially which expresses at seedling stage, are good proposition for the identification of sterile/ fertile plants at seedling stage. The production of hybrid seed using 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. Due to its tedious maintenance process and non availability of suitable marker gene among the vegetable crops, utilization of gms is restricted only in few vegetables till date. The identification of fertilizing cytoplasm for specific nuclear male sterile gene (Horner and Palmer, 1995), is an interesting research area, which upon success, may provide opportunity for most efficient utilization of gms lines, like cms line.

Cytoplasmic (cms) Male Sterility

The expression of male sterility in cms plants is the result of incompatibility between recessive nuclear gene which is called as maintainer gene *rf* and male sterile cytoplasmic genome. Cytoplasmic male sterility is the result of nucleo-cytoplasmic conflicts. Such conflicts give rise to several rearrangements in organelle genome which leads to sterility. With the continuous effort it is well documented that specific open reading frame (ORFs) in mitochondrial genome (mt genome) are responsible for the expression of male sterile trait (Kumar *et al*., 2000). Once dominant restorer (*Rf*) gene which is located in nuclear genome is identified which is responsible for pollen fertility of a cytoplasmic male sterile line, it is commonly known as cytoplasmic- genic male sterility (CMS). Such lines can also be developed from the progeny of wild cross resulting into CMS line. Therefore, those cytoplasmic male sterile lines for which *Rf* gene (s) have been identified are commonly known as genic-cytoplasmic male sterility (g-cms) and treated as a separate class of male sterility system. However, both cms and cgms can be described under common head (*i.e*. cms) because of the fact that in both these systems, expression of male sterility is due to the defect in the cytoplasm (mt-genome). Based on mode of action of the pollen fertility restorer (*Rf*) and mainainer (*rf*) alleles, CMS are classified in to two types, *viz*., (i) gametophytic and (ii) sporophytic. In

gametophytic system, expression of restorer allele is pollen specific, therefore pollen grains decides the sterility or fertility (*e.g*. S-cytoplasm in corn, abortive cytoplasm in rice etc.). Therefore, a plant heterozygous for maintainer-restorer locus (*Rfrf*) produces 50% aborted (*rf*) and 50% normal (fertile) pollen (*Rf*). Pollen from such plant (*Rfrf*) crossed with a sterile plant (*rfrf*), will again produce plants with 50% each of aborted and normal pollen. In contrast, all pollens are either fertile or sterile in sporophytic system, which is most common (Pearson, 1981). A heterozygous restorer line (*Rfrf*) in this system produces all fertile pollen and when crossed on to a sterile plant (*rfrf*), produces 50% absolute male sterile and 50% absolute male fertile plants. Cytoplasmic male sterility may originate from inter-generic or inter-specific crosses and may be artificially induced through mutagenesis or antibiotic effects on cytoplasmic genes (Kaul, 1988). Cytoplasmic male sterile plants have also been developed in several vegeta3bles through protoplast fusion e.g. Cauliflower (Pelletier et al., 1995).

Utilization of cms

The cms system is the most commonly utilized male sterility to produce commercial hybrid seeds of several vegetables like cauliflower, chili. The cms based hybrid development is often termed as three line method of hybrid breeding involving A line which is male sterile (S-*rfrf*), B line called as maintainer line (N-*rfrf)* and C or R line known as restorer line (S-or N-*RfRf*). As mentioned, cms line without restorer male parent cannot be utilized in fruit producing vegetables (*e.g*. chilli), but it can be utilized in vegetables where vegetative part is of economic value (*e.g.* onion, cole vegetables, carrot, radish, leafy vegetables etc.). The cms system though is the most commonly utilized; its utilization is restricted in specific species because of the following limitations:

- Non-availability of cms 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.
- Highly unstable sterile cytoplasm in several cases.
- Poor cross pollination ability of flowers of plants with sterile cytoplasm due to altered morphology.
- Technical complexity involved in seed production and maintenance of parental lines.

Vulnerability of sterile cytoplasm to specific diseases is a major risk in utilization of cms due to monopolistic cultivation of hybrids derived from single source of sterile cytoplasm. Devastation of corn hybrids derived from T-cytoplasm by *Helminthosporium* blight in USA during 1970's may be taken as example (Levings, 1990).

Transgenic male sterility systems

Genetic transformation new genetic approaches have been proposed and implemented to develop male sterility systems from the beginning of 1990's (Mariani *et al*., 1992). It became possible because development of techniques of the isolation, cloning and characterization of anther or pollen specific genes and promoter sequences. These genes either expressed in pollen themselves (gametophytic expression) or in the cells and tissues (sporophytic expression) support pollen development directly or indirectly, such as tapetum, filament, anther wall. These developmental pathways are genetically controlled and are least affected by the external environment but are highly influenced by internal environment. Williams *et al.* (1997) reviewed the reports on genetically engineered male sterility systems under dominant male sterility, recessive male sterility, targeted gametocide and dual method. However, based on mechanism of male sterility induction and fertility restoration, all transgenic male sterility can be classified under five classes, *viz.*, (i) abolition-restoration system, (ii) abolition reversible system, (iii) constitutive reversible system, (iv) complementary gene system and (v) gametocide targeted system. Although in transgenic (s) developed within one system, mode of action of trans gene (s) remains the same, there can be variations in trans gene constructs including promoter, targeted site (depending upon the promoter used) and methodology adopted within one system. 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.

Present arena of development and use of male sterility

With the advent of molecular biology and genomics any trait can be exploited as per requirement. Male sterility has been exploited by several researcher worldwide via recombinant DNA technology (Mariani *et al*., 1992; Goldberg *et al*., 1993 *etc*.), transfer of foreign mitochondrial gene (Hernould *et al*., 1993) and silencing that with antisense RNA technology (Zabaleta *et al*., 1996), modifying promoter or other regulatory regions to over express or repress the gene (Singh *et al*., 2015), chloroplast transformation (Ruiz and Daniel, 2005) *etc*. Genomic tools will be helpful to restore male fertility in transgenic by site specific recombination as done in tobacco (Bayer and Hess, 2005) and egg-plant (Cao *et al*., 2010). Many researchers have reported about the use of artificial micro RNA based transgenic male sterility (Toppino *et al*., 2011). Systems can be made such that non transgenic hybrid seeds can be produced from male sterile transgenic parents, as it has been developed in maize (SPT system: an USA patent) by DuPont Pioneer (Wu *et al*., 2016). Studies have found that small RNA may also have role in the photo thermosensitive male sterility system as reported in rice by Zhang *et al.* (2016). Epigenetic changes may also have very significant contribution in development of novel environmentally sensitive male sterility system. Hu *et al.*, (2015) reported flavone and flavonol biosynthesis, circadian rhythm, photosynthesis and oxidative phosphorylation pathways were involved in sterility-fertility transition of PA64S rice variety, regulation of such gene was found to be controlled by differentially methylated regions in genome.

Male Sterility and its Commercial Utilization in Vegetable Crops

In tomato (*Lycopersicon esculentum*; 2*n* = 24), approximately 19 male sterile based hybrids have been released of which 17 are based on functional sterility system which is being controlled by gene positional sterility (Single recessive gene *ps-2*). The *ms-10*35 gene is linked with a recessive marker gene (*a*) responsible for absence of anthocyanin which helps in identification of male sterile line at early stage and fertile plant can be rouged out in the nursery itself (Georgiev, 1991). Atanassova and Georgiev (1986) based on their observation advocated that genes *ms*-1035, ms-1526 and *ps-2* combined with short styles are most promising for hybrid development.

In pepper (*Capsicum annuum*; 2*n* = 24), the induced male sterile gene in France (*mc*-509; Pochard, 1970; renamed *ms*-10 by Daskalov and Poulos, 1994) was found allelic to *msk*allele isolated spontaneously in Korea (Shifriss, 1997). The *ms-2* line identified by Shifriss and Rylski (1972), was foundnon-allelic to *ms-1* isolated by Shifriss and Frankel (1969). The *ms-509* line (bell pepper type) of Pochard was introduced in India at Punjab Agricultural University (PAU) and *ms-10* was introgressed in three chilli, genotypes, *viz*., MS-12, MS-13 and MS-41 (Singh and Kaur, 1986). The MS-12 (*ms 10ms-10*) line is being utilized to produce hybrid seeds of CH-1 and CH-3 hybrids of hotpepper in Punjab state. Male sterile lines possessing *ms-3* gene are being utilized in Hungry to produce hybrid seeds (Kumar *et al*., 2000). In the recent past, development of stable cms lines of hot pepper has led to its increased utilization in hybrid development. In South Korea (Shifriss, 1997), China (Boaxi *et al*., 2000) and India, cms lines are being utilized to develop hybrids of hot pepper. However, non-availability of *Rf* genes in most of the sweet pepper genotypes is still a handicap in developing cms based commercial sweet pepper hybrids.

Among cole vegetables (*Brassica* spp. 2*n* = 18), although gms based experimental crosses have been developed, it has not been commercially utilized mainly because of the

difficulty in multiplication of male sterile seeds and availability of self-incompatibility and cms systems. The cms cybrid plants developed by Pelletier and his associates were of normal flower morphology with good nectar production and found highly stable. These promising cybrids, contained genomes resembling more to the *B. oleracea* type than the Ogura and being utilized by seed companies in France to develop hybrids of cabbage and cauliflower (Pelletier *et al*., 1995). Private seed companies in India are also engaged in developing cms based hybrids of cabbage and cauliflower (Singh 2002).

On the basis of floral morphology, radish (*Raphanus sativus*; 2*n* = 18) cms lines are generally classified into three types: (i) degenerative corolla, (ii) shrivelled stamen and (iii) abortive pollen. Success was achieved in identifying maintainer of Ogura cytoplasm and transferring of sterile cytoplasm in the new genetic background in recent past (Nieuwhof, 1990; Hawaldar *et al*., 1997). Ogura cytoplasmhas been found widely distributed among the wild Japanese radish plants and most of the early European radish populations, in which availability of maintainer allele is more frequent. Whereas, most of the Asian radish cultivars including Japanese cultivars possess normal cytoplasm except few cultivars from Tibet and Taiwan (Nieuwhof, 1990; Yamagishi and Terachi, 1994 a; b; 1996). In a study by Yamagishi (1998), restorer allele was found to be widely distributed in wild radish, European and Chinese cultivars; while occurrence of maintainer allele was more frequent in the Japanese cultivars. The male sterility in radish is being utilized by seed companies in Taiwan, China, Korea and Japan. In India also there are many private seed companies are using cms system for hybrid development (Singh *et al*., 2001).

Carrot (*Daucus carota*; 2*n* = 18) is one of the few vegetable crops in which male sterility was documented long ago *i.e.* in the year 1885 (Kaul, 1988). Several other genic male sterile mutants have been described (Kaul, 1988), however, none of them have been utilized for commercial seed production due to the availability of more efficient cms system in carrot. There are two important types of sterile cytoplasm have been reported in carrot namely, (i) petaloid and (ii) brown anther (Welch and Grimball, 1947; Morelock, 1974). A Japanese seed company (Taki Seed Company) developed the first F1 hybrid variety in 1982 using cms (Pelletier, *et al*., 1995). In USA, majority of hybrids are produced from one cytoplasm *i.e.,* Cornell cytoplasm. Considering the risk of disease vulnerability of hybrid variety due to the monopolistic use of Cornell sterile cytoplasm, USDA has released a petaloid type new cms line derived from sterile cytoplasm of Wisconsin (Morelock *et al*., 1996).

In onion (*Allium cepa*; 2*n* = 16), the first male sterile plant was reported within the progenies of an onion cultivar Italian Red by Jones and Emsweller in the year 1936 (Jones and Emsweller, 1936), which was found to be inherited cytoplasmically and male sterility was under the control of single recessive nuclear restorer locus (Jones and Clarke, 1943). Worldwide more than 50% onion varieties currently cultivated are F1 hybrids derived from S-cytoplasm (Pelletier *et al*., 1995).

In muskmelon (*Cucumis melo*; 2*n* = 22), first recessive *ms* gene was reported by Bohn and Whitaker (1949), since then at least four additional male sterile recessive alleles, *viz*., *ms-2* (Bohn and Principe, 1962), *ms-3* (McCreight and Elmstrom, 1984), *ms-4* (Pilrat, 1990) and *ms-5* (Lecouviour, 1990) have been identified. The *ms-1* line has been successfully utilized in India, to develop first commercial hybrid (Punjab Hybrid-1) in vegetable crops, through the exploitation of male sterility at PAU, Ludhiana (Kumar *et al*., 2000).

CONCLUSION

The research on male sterility in vegetables crop has to be continued for achieving goal in future breeding and it can be possible with the application of new molecular techniques and their implementation. Substantial progress has been made in understanding the mechanism of male sterility in many vegetable crops like onion, chilli, tomato, melon *etc.* at global as well as in India male sterility governed by cytoplasm and cytolasmic- genic are the most widely utilized in most of the vegetables. For example success has been achieved in case of chilli and muskmelon, where GMS is being utilized to develop F_1 hybrid seed commercially. In the recent past, chilli CMS lines were introduced at IIVR, Varanasi from AVRDC, which were utilized to produce CMS based hybrids namely Kashi Surkh and Kashi Anmol. Similarly in tomato (PAU, Punjab), cabbage, cauliflower (IARI, New Delhi), okra (IIHR, Bengaluru) work on GMS or CGMS based male sterility is in progress. At many other horticulture institutes and state agricultural universities, male sterility in vegetables is being also utilized to develop experimental hybrids. But the development of commercial hybrids on the pattern of chilli and muskmelon should be the immediate aim of the vegetable breeders. In fruit bearing vegetables like tomato, brinjal, chilli, muskmelon *etc*., identification and utilization of functional male sterility are more attractive. This is because unlike in sporogenous genic male sterility (50:50 ratio of male sterile and male fertile plants), 100% male sterile progenies can be obtained after forced selfing of male sterile plants, since functional pollen can be extracted from the non-dehiscing anther. The attempts should be made to collect GMS lines of important vegetables at one place, so that these can be evaluated against sensitivity to temperature or photoperiod. This helps in the identification of EGMS lines, which are like functional sterility, believed to be having more potential for the production of commercial hybrid seeds. In India, research on transgenic male sterility system was initiated in selected vegetables from different institution but remarkable success has not been achieved yet. Our priority should be given for the utilization of male sterility systems especially in less popular minor vegetable crops like sweet potato, pointed gourd, ash gourd etc. With the application of such novel sex determination techniques the cost of hybrid seed production can be minimized. This will not only promote adoption of hybrid vegetable technology by economizing the

cost of hybrid seeds but also provide basic material and scope for the development of more efficient male sterility system in respective vegetable crops.

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