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Male Sterility in Tomato (*Lycopersicon esculentum* Mill.) and Brinjal (*Solanum melongena*)

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ABSTRACT

Normal development of the male reproductive organ (stamen) and male gametophyte (pollen grain) is essential for the successful completion of sexual reproduction in the angiosperms. Abnormalities at any stage of stamen and pollen development can result in male sterility and it may result from nuclear encoded gene action (GMS, or genic male sterility), cytoplasmic influence (CMS, or cytoplasmic male sterility), nuclear gene action and cytoplasmic influence (G-CMS, or genic-cytoplasmic male sterility), chromosomal aberrations and interspecific or intergeneric hybridization. This review article highlights the fundamentals of genic or nuclear male sterility in tomato and brinjal and their utilization in hybrid breeding programmes.

Keywords: distant hybridization, functional, genetic control, mutant, sporogenous

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INTRODUCTION

Male sterility in flowering plants indicates the absence or non-functioning of male gametophyte or pollen grains. The term male sterility implies a number of situations based on anther development and phenotype: the absence (due to abortion) or extreme paucity of pollen grains in anthers of bisexual or unisexual flowers (sporogenous sterility); non-functional, malformed or complete lack of stamens in bisexual flowers (staminal sterility) and non-dehiscence of an-

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thers with functional pollen grains. Abnormalities at any stage of stamen and pollen development can result in male sterility. Male sterility can result from mutations in either the nuclear or the cytoplasmic genes and accordingly can be classified in two major groups, viz., genetic (spontaneous or induced) and non-genetic (induced) male sterility (Kaul 1988). Based on the location of gene(s) controlling genetic male sterility, spontaneously isolated, artificially induced through mutagenesis, artificially incorporated through protoplast fusion or genetically engineered male sterility systems can be classified as genic male sterility or more appropriately nuclear male sterility (GMS or NMS), cytoplasmic male sterility (CMS) and genic-cytoplasmic male sterility (G-CMS) (reviewed in Roitsch and Engelke 2006).

Male sterility in general terms or sporogenous sterility in particular refers to the condition of the plants when they do not produce viable or functional pollen grains and if such sterility is exclusively maternally inherited, it is described as cytoplasmic male sterility. It is well established that the genetic determinants of CMS reside in the mitochondria (mt-genome) while the nuclear genes control the expression of CMS (Newton 1988) and the mitochondria are usually excluded from the pollen during fertilization ensuring maternal inheritance of the character. Once dominant restorer allele in nuclear genome for pollen fertility of a cytoplasmic male sterile line is identified, it is commonly known as genic-cytoplasmic male sterility (G-CMS). Hence, male sterility in CMS system is expressed under the presence of sterile mt-genome located in cytoplasm (S-cytoplasm) and recessive allele of the restorer (maintainer allele; *r*). It appears that CMS results because of a multitude of effects, all more or less related to insufficient or mistimed supply of necessary resources for developing microspores. Nuclear encoded, genic male sterility (GMS), a common occurrence in angiosperms can result from mutations in any one of a number of genes controlling pollen development, stamen development and pollen release. Accordingly, the phenotypes of GMS mutants vary and a number of cytological, physiological and biochemical processes are affected in GMS mutants (Sawhney 1997).

OVERVIEW OF MALE STERILITY IN TOMATO AND BRINJAL

Male sterility in tomato and brinjal which mostly occur due to natural mutation is generally controlled by the gene(s) from the nuclear compartment, referred to as genic male sterility (GMS). However, induced cytoplasmic male sterile system through interspecific hybridization has been reported in brinjal only (Rangaswamy and Kadambavamasundaram 1974; Fang *et al.* 1985; Isshiki and Yoshida 2002; Isshiki and Kawajiri 2002). Male sterility in tomato has been extensively studied (Rick 1948; Karbinskaya *et al.* 1985; Kaul 1988; Rastogi and Sawhney 1990; Georgiev 1991; Pekarkova 1993; Levin *et al.* 1994; Polowick and Sawhney 1995; Gorman *et al.* 1996; Ivanova *et al.* 1996; Sawhney 1997; Lu *et al.* 1998; Masuda *et al.* 1998; Atanassova 1999; Masuda *et al.* 1999; Staniaszek *et al.* 2000) and the majority of tomato mutants belong to the sporogenous male sterile (*ms*) or *stamenless* (*sl*) series, while the frequency of the mutations controlling functional sterility is lower (Kaul 1988; Sawhney 1994; Gorman and McCormick 1997; Atanassova *et al*. 2001). More than 55 male sterile alleles causing sporogenous, structural, and functional sterility have been reported in tomato (Kaul 1988; Kumar *et al.* 2000). Chromosomal location of some of these genes is also known viz., *ms-6*, *ms-32* (chr.1), *ms-2*, *ms-5*, *ms-10*, *ms*-15, *ms-26*, *ms-35*, *ps* (chr.2), *ms-9* (chr.3), *sl, ps-2* (chr.4), *ms-16*, *ms-33*, *cl-2* (chr.6), *ms-8*, *ms-17*, *vms* (chr.8), *ms-31* (chr.10), *ms-3*, *ms-7*, *ms-12*, *ms-14*, *ms-42*, *ap* (chr.11). The list of artificially induced and spontaneously isolated male sterile mutants is increasing constantly in tomato and three induced male sterile mutants of tomato have also been described (Masuda *et al.* 1998). In brinjal, only two cases of sporogenous male sterility have so far been reported, one each in *Solanum integrifolium* (Habib and Swamy Rao 1973) and *Solanum melongena* (Chauhan 1984) however, limited number of male sterile alleles causing functional male sterility due to non-dehiscent anthers could be identified (Jasmin 1954; Nuttall 1963; Kaul 1988; Phatak and Jaworski 1989; Phatak *et al.* 1991).

All the naturally occurring or induced male sterile mutants of both tomato and brinjal are recessive in nature. 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 microgametogenesis (male gamete development process) and the phenotype of GMS mutants may vary accordingly. Based on anther development and phenotype, the male sterile mutants are classified into structural or staminal, sporogenous and functional types. Nuclear or genic male sterility (NMS or GMS) in tomato and brinjal, as in other crops, is inherited as a Mendelian trait suggesting that male sterile genes act sporophytically, not gametophytically. Plants heterozygous for male sterility genes produce 50% each of normal and aborted pollen in the case of sporogenous sterility. The variance in the manifestation of different forms of male sterility may result due to an interaction between polygenes or environment-sensitive modifying genes and the major recessive gene conditioning male sterility character.

SPOROGENOUS MALE STERILE MUTANTS

Pollen contains the male gametes and is produced by the male organs of the flower, the stamens. Each stamen is initiated from the floral meristem and at maturity, is composed of a filament and an anther. The anther has a single cell layer of epidermis tissue that surrounds four microsporangia. Each microsporangium is composed of an endothecial layer, two parietal (middle) layers, a tapetal layer and two cell layers of sporogenous tissue that ultimately give rise to the pollen grains. The four sporangia of an anther are held together by sporophytic connective tissue that, at maturity, breaks down between pairs of microsporanga and leads to the appearance of two large chambers holding the pollen. Pollen development can be separated into two distinct phases: microsporogenesis and microgametogenesis. Microsporogenesis is the process of forming the microspore and encompasses the period from the formation of the sporogenous and tapetal initials through meiosis (which occurs in the pollen mother cells) and the appearance of free microspores. Microgametogenesis covers the period from the first mitosis of the free microspore through the second mitosis that produces the gametes. In tomato, this second mitotic division occurs after anthesis and germination of the pollen tube. Pollen development of tomato occurring within the anther has been divided into eight morphologically distinct stages based on observations from ultrastructural studies (Polowick and Sawhney 1992, 1993a, 1993b). Differentiation is a term used to describe developmental processes that cause structural or functional distinction between parts of an organism: the primary mechanism of differentiation is qualitative or quantitative changes in gene expression (Strickberger 1985). In tobacco comparison of RNA populations in floral and vegetative organs of revealed that more than 10,000 diverse anther mRNAs are undetectable in the nuclear RNA and mRNA populations of other organs and are anther-specific (Kamalay and Goldberg 1984) and are supposed to be involved directly or indirectly in the control of pollen development process. Several pollen-specific genes (expressed only in pollen) and their promoters have been isolated and characterized in different crops which can be utilized to design various strategies to develop transgenic male sterile systems (Williams *et al.* 1997). Like abnormal tapetum development several other cytological, biochemical and molecular changes have been reported to be associated with GMS. Hence, sporogenous male sterile mutants result from aberrations in sporophytic genes that affect pollen de-

velopment. The sporogenous mutants deviate significantly from the normal type in the absence or extreme scarcity of normal pollen. The only sporogenous male sterility so far reported in *Solanum melongena* is caused due to abnormalities in the development of tapetum and controlled by two recessive nuclear genes, *ms-1* and *ms-2* (Chauhan 1984). The sporogenous male sterile mutants (*ms*) of tomato can be categorized into 5 groups according to the stage at which pollen development aborts or breaks down (Gorman and McCormick 1997):

1) **Not determined**: Several male sterile mutants e.g., *ms-19*, *ms-20*, *ms-21*, *ms-22*, *ms-25*, *ms-38*, *ms-39*, *ms-44 and ms-48* in which the point of arrest in pollen development has not yet been determined.
2) **Premeiotic**: All the mutants in this group e.g., *ms-3*, *ms-15*, *ms-29* and *ms-32* display pollen mother cell collapse or abortion prior to meiotic prophase.
3) **Meiotic**: These mutants viz., *ms-1*, *ms-5*, *ms-7*, *ms-8*, *ms-10*, *ms-12*, *ms-16*, *ms-18*, *ms-30* and *ms-33* show a somewhat wider variety of phenotypes especially within the tapetal tissue and pollen mother cells do not abort until the meiotic prophase. Different abnormalities in the tapetal cells include slow maturity, less volume, small size, late collapse and incomplete degeneration. In some mutants, 3-5% of the microsporangia develop a normal tapetum and produce viable pollen.
4) **Tetrad**: In this group of mutants, *ms-2*, *ms-4*, *ms-6*, *ms-11*, *ms-17*, *ms-23*, *ms-34*, *ms-45* and *ms-46* normal meiosis is completed before pollens are aborted. Tapetal cells fail to expand to normal volumes producing small locules at anthesis. Roy (2006) recorded that 2 days before anthesis anthers of the male sterile plants of *ms-2*, ms-*45* and *ms-10*^{36} were empty due to abortion of microspores although such abortion of microspores was inconsistent.
5) **Microspore**: Twelve mutants viz., *ms-9*, *ms-13*, *ms-14*, *ms-24*, *ms-27*, *ms-28*, *ms-31*, *ms-37*, *ms-41*, *ms-43*, *vms* and a digenic mutant comprise the group that aborts pollens after formation of free microspores. The microspores do not enlarge but simply appear to abort after tetrad release. Tapetal development appears to be normal but is not synchronous in its degeneration.

STRUCTURAL OR STAMINAL MALE STERILE MUTANTS

These mutants result from aberrations in genes that affect the structure of the stamens or anther sacs. Often these flower parts are so deformed that no pollen are produced. The most studied structural mutant of tomato is the stamenless (*sl*) mutant. The stamens are shorter than normal, twisted, and laterally separate, thus preventing staminal cone formation. The stamens have pollen sacs containing aborted pollen, and there are clusters of external ovules on some stamens. The single gene, male sterile *stamenless-2* (*sl-2*) mutant at high temperature (28ºC day/15ºC night), produced normal stamens with viable pollen (Sawhney 1983), at intermediate temperature (23ºC day/18ºC night) produced flowers with half stamens and half carpels and at low temperature (5ºC change) or gibberellic acid treatment, stamen and pollen development is rescued and normal viable pollens are produced (Sawhney 1997).

Structural male sterile mutants have not yet been reported in brinjal.

CAUSES OF SPOROGENOUS AND STAMINAL MALE STERILITY

In both GMS and CMS systems, male sterility is the consequence of breakdown of tightly regulated pollen development and fertilization processes at any of the pre or post-meiotic stages i.e., during meiotic process, during the formation of the tetrad, during the release of the tetrad, at the vacuolated microspore stage or at the pollen dehiscence stage (Kaul 1988; Horner and Palmer 1995). Expression of the male sterility trait is associated with a large number of morphological, physiological, histological, cytological, biochemical and molecular changes in male reproductive tissues at various stages of microsporogenesis and microgametogenesis. Involvement of different cells, enzymes and growth substances in the consequence of male sterility are discussed below.

Involvement of sporophytic tissue of the anther

In the sporogenous male sterile mutants, male sterile genes are probably expressed in the sporophytic tissues of the anther, particularly tapetal and stomial tissues. The number of mutants with defects in these tissues reflects the importance of the contribution of these tissues in anther and pollen development.

Anthers of the male sterile plants of the members of the family Solanaceae showed abnormalities in the endothecium, tapetum and connective region and histological investigations suggested that differentiation of the vascular strand was inhibited in the anthers which was confirmed by marked deficiency of nutrients in the anthers and this effect appeared to be common to the sterile anthers irrespective of the methods used to obtain sterility (Chauhan 1986). Tapetum cells are the innermost layer of the anther wall that surrounds the anther locule possessing sporogeneous cells (developing pollen). The tapetum is a transitory tissue surrounding and interacting with the pollen mother cells and whose function was shown to be critical to the development of pollen (Mariani *et al*. 1990). These cells are involved in the transmission of nutrients/energy to the sporogenous cells and are associated with synthesis of the enzyme callase which is required for the breakdown of callose (β-1,3 glucans) that surrounds pollen mother cells and after meiosis, the tetrads (Sawhney 1997). Microspores are released from the tetrads by the activity of callase and in some GMS and CMS systems, mistiming of callase activity results in premature or delayed release of microspores causing male sterility. Tapetal proteins are storage proteins that are secreted for use by the developing microspores (Aguirre and Smith 1993). The most striking histological feature associated with the majority of male sterile plants (GMS and CMS) is persistence or premature breakdown of the tapetum. Aberration in tapetum development leads to the failure of tapetum function, consequently failure of pollen development and, therefore, expression of male sterility. Ever since the first report on abnormal development of tapetum in male sterile plants (Monosmith 1926), there is growing experimental evidence in favour of a strong association between abnormal tapetum behaviour and nuclear or cytoplasmic male sterile plants (Nakashima and Hosokawa 1971; Horner and Rogers 1974; Pearson 1981; Polowick and Sawhney 1995; Su *et al.* 1995). In some mutant anthers, the inner tangential wall is excessively loosened allowing the passage of tapetal cell wall material and cytoplasmic contents into the anther locule. This presumably alters the osmoticum of the locule and results in plasmolysis of the microspores. Membranous fragments commonly observed in the normal tapetal cell wall, and presumed to have a role in transfer of materials from the tapetum to microspores, are absent from the *sl*-2 mutant. This is associated with reduced transfer of materials, such as lipids, to the developing pollen grains. In addition, a lining of sporopollenin-like deposits that coat the inner tangential wall of the normal tapetum is discontinuous in the mutant. In mutant anthers where the tapetal cell wall is not lost, the transfer of all materials is restricted and this result seen the collapse of sporogenous material (Polowick and Sawhney 1995).

Since the tapetum is a major source of a variety of substances into the locules, such as carbohydrates and proteins, that are either important for pollen growth and development or become components of the outer pollen cell wall (Pacini *et al*. 1985), it is evident that any deviation in its development pattern would inevitably lead to pollen sterility. In the

male sterile lines of different species, various abnormalities in tapetal development recorded so far (Overman and Warmke 1972; Stelly and Palmer 1982; Bino 1985; Graybosch and Palmer 1985; Grant *et al*. 1986; Sun and Ganders 1987; Sawhney and Bhadula 1988) have been summarized as i) a delay in tapetum degeneration along with a lack of exine deposition, ii) delayed degeneration and persistence of the tapetum, iii) considerable enlargement and/or vacuolation of tapetal cells, iv) smaller size of tapetal cells, v) premature degeneration of the tapetum and vi) more than one layered tapetum.

Involvement of enzymes

Callase

Callase is an enzyme involved in the breakdown of the callose that surrounds the pollen mother cells (PMCs), thus helps in the release of microspores (pollen) from the tetrad after meiosis. Early or delayed callase activity has been found to be associated with male sterility. Mistiming of callase activity led to the premature or delayed release of meiocytes and microspores resulting in male sterility (Izhar and Frankel 1971; Gottschalk and Kaul 1974).

Esterase

Esterase isozymes are believed to play role in the hydrolysis of sporopollenin, the polymer required for pollen formation. Decreased activity of esterase in male sterile plants has been observed in petunia (Marrewijk *et al.* 1986), tomato (Bhadula and Sawhney 1987) and in radish (Zhou and Zhang 1994). Hence, it has been proposed that decreased activity of esterase has an adverse effect on pollen development (Sawhney 1997). However, Marrewijk *et al.* (1986) emphasized that decreased esterase activity and composition in CMS petunia are the result rather than the cause of male sterility.

Adenine phosphoribosyl transferase

Moffatt and Somerville (1988) found that an *Arabidopsis* mutant that was deficient in Adenine phosphoribosyl transferase (APRT) activity was male sterile. The APRT enzyme is a component of the nucleic acid salvage pathway that scavenges the adenine by-product from methionine, polyamine or nucleotide metabolism and converts it to adenosine monophosphate. This male sterile mutant appeared to abort pollens development at some time after meiosis generally at the tetrad stage.

Extracellular invertase

Carbohydrates play a critical role in anther and pollen development. They are nutrients used to sustain growth as well as signal to influence development *in vivo* and *in vitro*. Accordingly, different male sterile lines were shown to be characterized by perturbed charbohydrate metabolism (Kaul 1988). Assimilates are produced in photosynthetically active source tissues and transported to photosynthetically less active or inactive sink tissue. An unloading pathway via the apoplasmic space is mandatory for symplastically isolated cells, such as developing pollen, and also can contribute prominently in other actively growing tissues. Sucrose is released from the sieve elements of the phloem into the apoplast via a sucrose transporter. An extracellular invertase ionically bound to the cell wall irreversibly hydrolyses the transport sugar to sucrose. The hexose monomers are taken up into the sink cell by high-affinity hexose transporters. These key reactions create a localized concentration gradient, thus promoting phloem unloading via an apoplastic pathway and increasing the sink strength of the corresponding sink tissue. Identification of extracellular invertase isoenzymes (Goetz *et al*. 2001) that are expressed in anther tissues supports a link between extracellular sucrose cleavage and anther and pollen development.

Involvement of plant growth substances

Endogenous plant growth substances (PGSs) play a very important role in stamen and pollen development (Kinet *et al.* 1985; Greyson 1994; Sawhney and Shukla 1994). All types of male sterility (functional, structural and sporogenous) have been reported to be associated with changes in a number of PGSs, rather than any specific substance and perhaps it is the altered balance of PGSs that affects the pollen development process. In vegetative and floral parts (except the pistil) of the *sl*-2 mutant, abscisic acid (ABA) content was observed to be more than the normal, especially in stamens. The increase in ABA content in stamens was found to coincide with the first sign of abnormality in anthers. At a low temperature, when fertility was restored, there was a drop in ABA level in leaves and stamens. Therefore, it is believed that male sterility in *sl*-2 is a manifestation of hormonal imbalance (high ABA) and low temperature regulation of male sterility is mediated through a reduction in ABA content (Singh and Sawhney 1998). Some such major changes in plant growth regulators documented in stamenless sterility tomato are:

- Reduced levels of GA and GA-like substances (Sawhney 1974; Singh *et al*. 1992).
- Affecting endogenous gibberellin levels, thus reducing sugar levels and impairing the development of pollen grains (Sawhney and Rastogi 1990).
- Affecting endogenous gibberellins in the *sl*-2 mutant which affects the activity of amylases, resulting in lower sugar levels leading ultimately to abnormal pollen development (Bhadula and Sawhney 1989).
- Increased level of IAA (Singh *et al.* 1992; Sawhney 1997).
- NAA inhibits the growth of all floral organs (Sawhney and Rastogi 1990).
- Reduced level of cytokinins and increased level of ABA (Singh and Sawhney 1998).

Involvement of amino acids and proteins

In several cases, developing anthers of male sterile plants have been found to be associated with qualitative and quantitative changes in amino acids, proteins and enzymes. In comparison to the anthers of fertile plants, variations in the levels of specific amino acids have been reported in different species. Generally, the level of proline, leucine, isoleucine, phenylalanine and valine is reduced and asparagines, glycine, arginine and aspartic acid are increased in the sterile anthers (Kaul 1988). Rastogi and Sawhney (1990) reported that elevated polyamine (putrescine, spermidine, spermine) levels contribute to abnormal stamen development in the *sl*-2 mutant. Differences in total protein content and polypeptide bands between sterile and fertile anthers have been determined. In general, lower protein content with fewer bands has been observed in the anthesis stage of sterile plants (Alam and Sandal 1969; Banga *et al.* 1984; Sawhney and Bhadula 1987). Bhadula and Sawhney (1991) observed that the mutant stamens contain low levels of soluble protein, which is related to a reduction in protein synthesis. The mutant stamens, however, possess many polypeptides similar to the normal stamens and synthesize a 53-kDa polypeptide at stages when there are abnormalities in tapetum development. The mutant stamens also possess a 23-kDa and some low molecular weight polypeptides that are considered as degradative proteins. The synthesis and the lack of specific polypeptides appear to be associated with pollen degeneration. Masuda *et al*. (1999) reported that in the pollen degradation mutant of tomato, the inhibition of starch degradation is associated with low pollen germination, but whether it causes pollen sterility is unclear. It was also found that there is a direct correlation between the reduced

expression of the LAT 52 protein and abnormal pollen function (Muschietti *et al.* 1994). It may be suggested that LAT 52 is involved in pollen hydration and/or pollen germination.

Environmental-hormonal interplay

The *sl-2* mutant in tomato is an excellent system for investigating the environmental-hormonal interaction in stamen and pollen development. Low temperatures or gibberellic acid treatment induce the development of normal and viable pollen and in contrast, high temperatures or indole acetic acid treatment result in the formation of carpels in place of stamens and in these organs megasporogenesis occurs instead of microsporogenesis (Sawhney 1983, 1997). A photoperiod-dependant single recessive gene male sterile mutant "7B-1" appears to be an ABA-overproducer, and photoperiod-regulated ABA levels may be responsible for the hypersensitivity of the mutant to exogenous ABA (Fellner *et al*. 2001).

Similarly, in *ms-15* and *ms-33* mutants, low temperature (<30ºC) is reported to be associated with fertility restoration (Sawhney 1997). The variable male sterile plants express male sterility at a temperature of 30ºC or above (Rick and Boynton 1967). Climatic conditions such as excessive temperature and high humidity have a detrimental effect on the expression of male sterility.

FUNCTIONAL MALE STERILE MUTANTS

Since tomato and brinjal possess neither nectar nor nectaries, dehiscence cannot be related to sugar secretion, as has been demonstrated in some other plants. The anthers seem structurally adapted for water conservation and the only transpirational loss of the flower appears to be through the petals. Transpiration from the anthers themselves thus appears not to be involved in regulating dehiscence. Anther opening is preceded by dehydration of the locule, with water being exported through the filaments to the petals along an osmotic gradient generated by starch/sugar conversion. However, dehiscence cannot primarily be a desiccatory process. Rather, anther dehiscence is regarded as an orchestrated programme of physiological and structural events (protoplast degeneration and cell wall collapse) exhibiting environmentally linked hygroscopic absorption and leading to the desiccation of specific domains of the anthers (Bonner and Dickinson 1990). In tomato, pollen are released through vertical splitting of anthers inside and in brinjal, pollen are released through development of two pores at the tip of the anther (Roy 2006).

The functional male sterile mutants produce viable pollen but their sterility is caused due to some mechanical reasons. They either have non-dehiscent anthers or have dehiscent anthers with morphological abnormalities that prevent pollen from reaching the stigma.

FUNCTIONAL MALE STERILE MUTANTS IN TOMATO

Relatively few mutants of tomato are included in this group (Gorman and McCormick 1997; Attanassova 1999) and these are *positional sterile* (*ps*), *positional sterile 2* (*ps-2*), *cleistogamous* (*cl*), *cleistogamous-2* (*cl-2*), *dialytic* (*dl*) and *exserted stigma* (*ex*) mutants.

Cleistogamous mutant

In the *cleistogamous* mutants (*cl*, *cl-2*), although failure of the petals to open is the most obvious defect, stomial tissue is also affected in these mutants and the phenotype of the mutant must be due in part to a hormonal effect because application of 4-chlorophenoxyacetic acid could rescue fertility to a small extent (Rick and Robinson 1951).

Dialytic mutant

In this mutant (*dl*), growth of the epidermal hairs on the anther surface is prevented resulting in the failure of anthers to hold together around the pistil, and the pollen is not directed toward the style and rarely reaches the stigma (Rick 1947).

Exserted stigma mutant

This type of sterility (*ex*) is not associated with alterations in the anthers as their dehiscence is normal and they produce viable pollen. It is due to the excessive length of the style that protrudes from the anther cone holding the stigma out and away from the concentration of pollen in the central space and effectively produces a functional male sterile phenotype (Rick and Robinson 1951). Such a position of the stigma eliminates the necessity of stamen emasculation for the production of hybrid seeds. A number of *ex* genotypes were obtained by hybridization of *Lycopersicon esculentum* cultivars with *Lycopersicon pimpinellifolium* (Smith 1966), by treatment with GA_3 (Honma and Bucovac 1966), or by low temperature treatment of seeds (Dorossiev 1970). Studies on the genetic variability of *ex* sterility have shown that this character should not be evaluated based only on the length of the style part that protrudes from the anther cone. It depends on the lengths of both the style and anther, these lengths being polygenically controlled and varying signifycantly – in particular of the style – depending mainly on temperature (Thakur 1970; Georgiev and Atanassova 1977). When testing *ex* lines for their usefulness in hybrid seed production it was established that it was really difficult to determine and to correct the right rate of stigma exsertion. The lines possessing 1.0-1.5 mm stigma exsertion became occasionally normal ones that resulted in undesirable self-pollination (Atanassova 1999). Observations on stigma exsertion variability within a number of sporogenous sterile lines (*ms*) viz., *ms-10* showed strong variation depending on the environment and on the genotype (Levin *et al*. 1994).

Positional sterile mutants

This type of sterility (*ps*) is characterized by petals showing coalescence of the corolla nearly to their extremity. The greater lateral growth of the petals causes an overlapping and curling with the adjacent petals. The connate or pseudo-connate form of the petals results in considerable construction of anthers and tends to hold them in exceedingly close contact with the pistil, particularly at the apex. The main disadvantage of using this type of sterility in hybrid seed production was the necessity of stamen emasculation (Larson and Paur 1948). Dorossiev (1976) found a normal flowered *ps* functional sterile plant in the F_2 progeny between a *ps*-mutant and fertile line. It was characterized by a lack of coalescence between anthers and petals, the anthers however being in exceedingly close contact with the style, particularly at the apex. Based on this plant, a line No 159 that also carried gene *aw* (*anthocyanin without*) and *ex* was developed (Dorossiev 1976). Gene *aw* is closely linked to *ps* that enables not only rapid determination of the hybrid seed hybridity, but also easier breeding of sterile lines, as in the segregating progenies the *ps* plants could be assessed at the seedling stage. Occurrence of selfing is a disadvantage that also limits the application of *ps* sterility in practice. It was found that the percentage of selfing in a number of *ps* lines varied depending on temperature and humidity, the highest being under high temperature (Simonov 1967). The *ps* mutant was included in a number of breeding programs (Nickeson 1957; Singh *et al.* 1966; Simonov 1967; Dorossiev 1976) and according to Staniaszek *et al*. (2000) the idea of facilitating hybrid seed production based on this type of sterility resulted in practical utilization in Poland.

Positional sterile-2 mutant

Positional sterility of tomato is controlled by a single recessive gene *ps-2*. The *ps-2* male sterile mutant was identified by Tronickova (1962) in the Czech variety 'Vrbicanske nizke' and was found to be closely linked to *fulgens*, on chromosome 4 (Atanassova 1991). The mutant is characterized by the presence of fertile pollen and indehiscent anthers (Tronickova 1962). According to Oryol and Zhakova (1977) the *ps-2* sterility is due to structural alterations in the zone of anther dehiscence. It was confirmed further that the functional anther non-dehiscent character was controlled by a recessive allele and was not influenced by cytoplasmic genomes (Lu *et al.* 1998). The tests of *ps*-2 lines under a large scale of environmental conditions showed that occasionally the retention of the pollen was not totally consistent and undesirable selfing that depended on the environmental conditions was observed (Tronickova 1962; Philouze 1978; Atanassova and Georgiev 1986). Different morphological markers such as *potato leaf* (*c*), *anthocyaninless of Hoffmann* (*ah*), *anthocyanin without* (*aw*), etc. were introduced in *ps*-2 seed parents of a number of commercial hybrids (Atanassova *et al*. 2001).

Introgression of the *ps*-2 gene into fertile genotypes showed that the expressivity of the gene varied with the genotypes. In some F_3 progenies 70-80% of the sterile plants had to be eliminated because of their high percentage of selfing, while in other progenies the percentage of the plants to be eliminated was rather low. This information was useful for establishing the methodology of breeding *ps*-2 lines and it made clear that strict control of the percentage of self-fertilization and elimination of the plants showing more than 5-7% of selfing was necessary throughout the entire breeding process (Atanassova 1999). The easy maintenance of the sterile lines by forced selfing and significantly higher hybrid seed yield are the main advantages in using *ps-2* sterility in hybrid seed production (Atanassova 1999). Though undesirable selfing and the necessity of stamen emasculation were recognized as the main two disadvantages that limited the use of *ps-2*-sterile seed parents in tomato hybrid seed production (Atanassova and Georgiev 1986; Stevens and Rick 1986), this functional male sterility proved to be a useful tool in hybrid tomato breeding in Bulgaria, the Czeck Republic, Poland and Moldavia (Pekarkova-Tronickova 1993; Atanassova 1999; Atanassova and Georgiev 2002; Staniaszek *et al.* 2000).

FUNCTIONAL MALE STERILE MUTANTS IN BRINJAL

In brinjal, only one type of mutant (non-dehiscent anther) is included in this group where viable pollens are trapped inside the anther locule because the anthers fail to dehisce through the development of pores at the tip of the anther.

Jasmin (1954) first found a male sterile character due to failure of anther dehiscence despite normal pollen development in a population of Blackie variety of eggplant growing in a greenhouse in Canada and complete anther dehiscence character in the F_1 populations of the male fertile and male sterile parents indicated that this character was governed by recessive genes. However, backcrossing the functionally male sterile line "ED1" originating from Canada, to the male fertile Bulgarian variety failed to yield male sterility forms of value for hybrid seed production (Popova and Daskalov 1971). Another functional male sterile mutant of brinjal UGA 1-MS was released from the Department of Horticulture, Coastal Plain Experiment Station, University of Georgia, Tifton, USA (Phatak and Jaworski 1989). This functional male sterility was governed by a single recessive allele, for which the symbol *fms* was proposed and the *fms* gene is linked to purple fruit colour at the X/x locus with no linkage between functional male sterility and fruit shape (Phatak *et al.* 1991). Detailed evaluation of this functional male sterile line under Indian conditions showed that only 63.5% plants of UGA 1-MS on average exhibited non-dehiscent anther character under open field conditions revealing three distinct categories of anther dehiscence and pollen release viz., non-dehiscence, partial dehiscence (development of one pore) and complete dehiscence (development of two pores) and from the study of F_1 and F_2 populations of six cross combinations it was proposed that male sterility of UGA 1-MS was controlled by one major recessive gene in combination with at least two modifier genes in recessive condition which may be symbolized as *fms/fms* ++ (Roy 2006).

One plant from over 60 collections of *S. insanum*, a weedy relative of brinjal was detected with indehiscent anthers which showed regular bivalent formation during microsporogenesis, high pollen viability, and normal fruit and seed set following artificial self-pollination but the anthers did not dehisce and the pollen grains degenerated within the anther locules (Karihaloo and Malik 1995).

Mechanism of functional male sterility due to anther non-dehiscence

Transcriptional processes control anther-specific gene expression programmes and anther dehiscence involves the programmed destruction of specific cell types (Goldberg *et al*. 1993). Anther dehiscence is regarded as an orchestrated programme of physiological and structural events (protoplast degeneration and cell wall collapse) exhibiting environmentally linked hygroscopic absorption and leading to the desiccation of specific domains of the anthers (Bonner and Dickinson 1990). Cell wall fortifications in the endothecial cell layer of the anther are required for the anther dehiscence process (Steiner *et al.* 2003). A model is presented (Ishiguro *et al*. 2001) in which jasmonic acid synthesized in the filaments regulates the water transport in stamens and petals and thus pollen maturation, flower opening and anther dehiscence are synchronized. In a functional male sterile mutant of tomato (*positional sterile*, *ps* and *ps-2*) defective stomium is also involved in non-dehiscence of the anthers (Gorman and McCormick 1997). A longitudinal section of the non-dehiscent anther of the functional male sterile line of brinjal UGA 1-MS showed persistence of the cell layer and some depositions in the zone of pore development (Roy 2006).

Several studies (Bonner and Dickinson 1990; Goldberg *et al.* 1993; Maekawa and Inukai 1994; Vijayaraghavan and Chaudhry 1994; Gorman and McCormick 1997; Lu *et al.* 1998; Dawson *et al*. 1999; Steiner *et al.* 2003) indicated the following reasons for non-dehiscence of anthers resulting functional male sterility in different crops:

- Obstruction in the loss of water of the radial-thickened fibre layer near the endothecia between one anther sac and another one on the same side by the close ranked cuticle cells of the anther.
- Non-desiccation of anthers because of obstruction of water being exported through the filaments to the petals along an osmotic gradient generated by starch/sugar conversion.
- Non-shrinkage of the close coronal and fibrous layer.
- Absence of lysis in the septum or pore in the anther.
- Absence of endothecial wall thickening showing lack in cell wall fortification.
- Toughness of the anther wall, which fail to rupture.
- The labiate cells at the breaking point of the fibrous layer do not open.
- Defect in stomial tissue.

Induction of male sterility

Tomato

Sporogenous male sterility of different kinds in tomato could be induced artificially by seed radiation with gamma-rays and a dose of 200 Gy was sufficient to induce mutations (Masuda *et al*. 1998). Although pollen fertility in M_1

plants decreased according to irradiation dose, complete pollen sterility was not detected while plants with male sterility controlled by a single pair of recessive genes could be identified in the M_2 generation and three types of induced male sterile mutants could be characterized (Masuda *et al.* 1998):

i) Pollen degradation process: This type is indistinguishable from the non-irradiated plants even at the flowering stage. Pollen degradation of this type becomes evident at microsporogenesis.
ii) Morphological differences in the floral organs: This type is morphologically distinguishable by an exerted style because of a degenerating anther, which becomes yellow-brown acropetally. Pollen degradation of this type becomes evident around meiosis stage.
iii) Anther colour: This group develops a normal pistil and stamen with yellow anthers. The pistil becomes longer than the stamen a few days before anthesis, and the top of the staminal column splits at anthesis so that the stigma is disclosed. Pollen degradation of this type becomes evident from the tetrad to early microspore stage.

Brinjal

Interspecific hybridization of brinjal, *Solanum melongena* with other *Solanum* species resulted in the development of mostly cytoplasmic male sterility in brinjal carrying the cytoplasm of the distantly related *Solanum* species.

Rangaswamy and Kadambavamasundaram (1974) studied the fertility of an F_1 hybrid between *Solanum violaceum* and *Solanum melongena*, and its backcross progenies. Pollen fertility in the F1 was only 41.5% and in the amphidiploid it was 35%. An examination of chiasma frequency at diplotene indicated that chromosome pairing in the F_1 was normal. The occurrences of petaloid anthers in some of the male sterile segregates in the F_1 and *S. melongena* backcross progenies revealed that hybrid sterility was due to diplontic sterility. The pollen fertility levels in the F_2 and backcross progenies ranged from 0 to 89% with a continuous variation, indicating that sterility is governed by major genes with modifiers. Isshiki and Kawajiri (2002) also produced alloplasmic lines of brinjal by crossing S. *violaceum* with *S. melongena* followed by repeated backcrossing with *S. melongena* until the BC_4 generation to develop a male sterile line in brinjal. Analyses of chloroplast and mitochondrial DNA confirmed that the cytoplasm of these BC_4 plants was that of S. *violaceum*. The pollen stainability remained low in all the backcross generations, although there were high frequencies of 12 bivalents at meiotic metaphase1 and the numbers of seeds per fruit increased in successive generations. The anthers of some BC_1 and BC_2 plants and all BC_3 and BC_4 plants did not open to release the pollens which are attributed to disharmony between the cytoplasm of S. *violaceum* and the nuclear genes of *S. melongena*. This cytoplasmic male sterility was a functional male sterility caused by anther non-dehiscence however, fruit set by cross pollination by hand, number of seeds per fruit and seed germination rate were almost equal to those of *S. melongena*, indicating that the S. *violaceum* cytoplasm had no significant effect to seed fertility of *S. melongena* (Isshiki and Yoshida 2002). Fang *et al*. (1985) developed two cytoplasmic male sterile lines in brinjal by 3 backcrosses to *S. melongena* after crossing *S. gilo* with *S. melongena*. Line 9334A has petaloid anthers and the line 2518A has vestigial, pollenless anthers. In test crosses with 22 S. melongena cultivars, no male fertile plants were identified suggesting that the male sterility is under cytoplasmic control.

LINKAGE OF *MS* GENE WITH THE MARKER GENE

More than 55 non-allelic recessive nuclear male sterility genes (*ms*) have been reported in tomato but only few are linked with the marker character (**Table 1**) to identify sterile plants at the seedling stage and fertile plants can be rogued out in the nursery itself. One such potential male sterile line is *ms-10*^{35} *aa* in which the *ms* gene is linked with a recessive marker gene (*aa*) responsible for the absence of anthocyanin (Philouze 1974).

Table 1 Different marker characters linked with male sterility.

Crop	Marker gene	Reference
	Potato leaf shape and green colour	Kaul 1988
	Parthenocarpic fruit	Soressi and Salamini 1975
	Enzyme marker	Tanksley *et al.* 1984
	Absence of anthocyanin	Philouze 1974
Tomato	RFLP and flanking markers	Gorman *et al.* 1996
	RAPD markers linked to the *ps* gene	Staniaszek *et al.* 2000
	Qualitative variation in the A protein locus	Stoilova *et al.* 2000
	Linkage of *ps*-2 gene with *fulgens* gene	Atanassova 1991
Brinjal	Purple fruit colour	Phatak *et al.* 1991

TRANSGENIC MALE STERILITY SYSTEMS

New genetic approaches have been proposed and implemented to develop male sterility systems in many crops by genetic transformation through 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 that directly or indirectly support pollen development such as tapetum, filament, anther wall, etc. (sporophytic expression).

The locus *ms-14* conferring male sterility of tomato under microspore category (Gorman and McCormick 1997) has been mapped on RFLP marker and with the help of identified flanking markers 610 kb of YAC clone possessing *ms-14* locus has been cloned through map based cloning technique (Gorman *et al*. 1996).

The monogenic recessive positional sterility gene *ps-2* confers non-dehiscent anthers and is the most suitable for practical uses in hybrid seed production of tomato. In order to have tools for molecular-assisted selection (MAS) Gorguet *et al*. (2006) fine mapped the *ps-2* locus which was done in an F_2 segregating population derived from the interspecific cross between a functionally male sterile line (*ps-2/ps-2*; *Lycopersicon esculentum*) and a functionally male fertile line (*L. pimpinellifolium*). The procedure has led to the high-resolution fine mapping of the *ps-2* locus in a 1.65 cM interval delimited by markers T0958 and T0635 on the short arm of Chromosome 4. The presence of many COS markers in the local high-resolution map allowed to study the synteny between tomato and *Arabidopsis* at the *ps-2* locus region however, no obvious candidate gene for *ps-2* was identified among the known functional male sterility genes in *Arabidopsis*.

According to Williams *et al.* (1997) all transgenic male sterility systems developed so far can be described under five classes *viz*., i) abortion-restoration system (pollen development process is disrupted by exogenous *trans*-gene constructs), ii) abolition-reversible system (male sterility is induced by exogenous male sterility gene), iii) constitutive-reversible system (monogenic recessive male sterile plants are induced to revert into male fertile plant), iv) complementary gene system (crossing between two versions of transgenic plants lead to 100% male sterile plants) and v) gametocide-targeted system (male sterility is induced through pollen specific disruptive gene under the control of certain chemical specific inducer).

UTILIZATION OF MALE STERILE LINE IN HYBRID SEED PRODUCTION

The benefit of incorporating male sterility into hybrid breeding programmes was recognized not long after the appreciation of the advantages of heterosis and the detection

of male sterile genotypes in tomato. For the first time male sterility was used in tomato hybrid seed production by Rick (1945) and up to the present this phenomenon is still discussed as the most promising way for facilitating the process of hybrid seed production (Sawhney 1994; Gorman and McCormick 1997; Sawhney 1997; Atanassova *et al*. 2001). Economizing the process of hybrid seed production includes not only facilitating the process *per se*, but also increasing its efficiency by improving the quality of the final product, i.e. of the hybrid seed. Besides the high germination ability that is an obligatory characteristic for each kind of commercial seed, the high percentage of hybridity is ranked of primary importance for hybrid seed. Using a male sterile seed parent would be a warranty for producing 100% of hybrid seed and would eliminate the necessity of testing the seed for hybridity.

The sporogenous male sterile (*ms*) and stamenless (*sl*) mutants seem to be the most attractive to be applied in breeding programs aiming at the facilitation of hybrid seed production mainly because of complete male sterility and their accessible stigma (Stevens and Rick 1986; Sawhney 1994; Gorman and McCormick 1997). Because of anther deformation, some *ms* mutants such as *ms-10*, *ms-15*, *ms-32* exhibit an exserted stigma, i.e., accessible for pollination without emasculation. By developing *ms-10*^{35}*aa* genotypes, the main disadvantage in using *ms*-sterility in hybrid seed production (assessment of sterile plants at anthesis) was eliminated (Philouze 1974). The anthocyaninless sterile plants are easy to be distinguished at early developmental stages. Moreover, no effect of *ms10*^{35}*aa* genes on plant and fruit characteristics were established (Gardner 2000). This technology might be applied also using *ms 15 anthocyanin without* (*aw*) genotypes as the two genes are closely linked (Clayberg 1965). The *sl* mutants, mainly due to their accessible stigma that permits artificial pollination without stamen emasculation and complete male sterility seemed to be the most attractive to be applied in hybrid breeding programmes of tomato (Stevens and Rick 1986; Sawhney 1994; Gorman and McCormick 1997); however, this sterility has not yet been utilized in commercial hybrid breeding programmes. According to Bar and Frankel (1993), some *ms* mutants (*ms-14*, *ms-17*, *ms-18*, *ms-31*, *ms-33*, *ms-47*) were found to exercise pleiotropic effects on a number of economically important traits such as percentage of early marketable yield, average fruit weight and total marketable yield. Pistil and anther cone length and the difference between them are controlled by the interaction of the *ms-10* gene and polygenes (Levin *et al.* 1994). These findings suggest that detailed studies on *ms* sterile lines are necessary before including them in breeding programs

Different functional male sterile mutants were also evaluated as they offer the advantage of reproduction by artificial selfing to produce 100% sterile progeny. The *cl-2* and *dl* mutants of tomato have not been evaluated as useful for practical breeding purposes so far. Despite this advantage they have not been used widely in commercial hybrid seed production of tomato because of two significant disadvantages: occasional lapses in their expressivity that could result in undesirable selfing and, except in *exserted stigma* sterility, necessity of stamen emasculation. To our knowledge two types of functional sterility, *ps* and *ps-2* were applied to date in commercial hybrid seed production of tomato. Although functional male sterility due to non-dehiscent anthers has not been used in commercial hybrid seed production of brinjal so far, it holds ample promise if stability in character expression coupled with protrusion of the stigma to obviate hand emasculation is induced.

In conclusion, it has to be underlined that the problem of improving the process of hybrid seed production in tomato and brinjal using male sterility is quite complex. It has to be discussed keeping in mind not only the requirements concerning the performance and the maintenance of the sterile genotype but also the rate of overcharging the breeding process when developing the genotype desired. For example, exserted stigma, including the one due to another malformations in some sporogenous mutants, is always mentioned as a necessary characteristic of the sterile flower morphology. A number of studies have provided evidence however that the presence or absence and the rate of stigma exsertion depends on the lengths of both, style and anther, these lengths being polygenically controlled and varying significantly, especially of the style, depending mainly on temperature. Therefore, when developing male sterile genotypes possessing flowers with an exserted stigma, breeders have to take into consideration two more polygenically controlled characters that would complicate the breeding process and consequently might diminish the profit and the interest of developing such a genotype.

The recent advances in molecular genetics, the rapidly increasing number of investigations aimed at identification and isolation of male sterile genes in tomato and brinjal, engineering of transgenic male sterile plants, synthesis of tight linkage between an appropriate marker gene and male sterile gene through genetic transformation are promising ways that would help geneticists and breeders not only create better systems of hybrid seed production but made them applicable in the breeding process, as well. The most promising avenue for eliminating hand emasculation in hybrid seed production is the development of transgenic male sterile plants with exerted stigma character and a tapetal-specific gene causing self destruction of the tapetum followed by microspore abortion because the role of abnormal tapetum development in causing male sterility in both GMS and CMS system has been established beyond any doubt.

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