ACQUISITION OF MALE STERILITY FOR TOMATO HYBRID SEED PRODUCTION

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ABSTRACT

Tomato (Lycopersicon esculentum Mill. 2n=2x=24) belonging to family solanaceae is highly self-pollinated with perfect flower, therefore, use of male sterile lines is essential for developing commercial tomato hybrid. The acquisition of male sterile line with good combining ability, sufficient style length to expose the stigmatic surface for cross pollination and simple maintenance with low cost of production has been a paramount interest of tomato breeders. Male sterility either genetic or non-genetic makes the pollen unavailable or such tomato flowers are incapable of setting seed through selfing. Thus, a male sterile line can be used as female parent and can produce a bulk quantity of seed due to cross-pollination. The seed set on the male sterile plant is hybrid, which is used for growing commercial hybrid. On an average, under field conditions, a normal tomato variety yields 16-25 tons/ha while hybrids often produce 60-80 tons/ha. However, to exploit the benefits, farmers have to buy fresh seed in every cropping season. In this review article a brief survey on nature of tomato male sterility and its possible utilization in the development of cost effective hybrid development program has been discussed from breeding point of view.

Key-words: Genetics, tomato, seed production.

INTRODUCTION

The concept that heterosis manifests itself most strongly in F₁ hybrid and decreases progressively in the next segregating generations has been contemplated as a positive biological phenomenon caused by heterozygosity, as opposite to the phenomenon depression caused by inbreeding provides the basis of hybrid seed breeding programs in almost all autogamous and allogamous crop species. Crane (1915) described male sterility in tomato for the first time. Barnos and Lucas (1942) first suggested that male sterile plants must be used as female parents thereby reducing the time and cost associated with hand emasculation. However, a male sterile line must exhibit complete male sterility, normal fertility without defects in morphology and lastly that male sterility trait should be essentially recessive i.e., its segregation ratio must be 1:1 in test cross. One of the main advantages of tomato hybrid seed is its high yield compared to the commercial cultivars, for example, the average yield of a cultivar ranges 16-25 tons/ha whereas hybrid yields 60-80 tons/ha (Tiwari and Chaudhary, 1986). The acquisition of male sterile line with good combining ability, sufficient style length to expose the stigmatic surface for cross pollination and simple maintenance with low cost of production has been a paramount interest of tomato breeders. In the beginning, F₁ hybrid had to compete only with usual cultivars however this problem became more complicated later on when the new hybrids started to compete other hybrids thus need to develop male sterile lines with valuable attributes was strongly considered regarding fertility, early ripeness, uniformity in plant and fruit shape, complex resistance to diseases and strong adaptability to different environmental conditions. As a result, several approaches were undertaken for the characterization / selection of wild male sterile lines, their introgression into cultivated species and creation of new male sterile mutants with economic trait(s) of interest. A brief survey of such male sterility systems and their comparative use in tomato hybrid breeding programs has been reviewed.

Male sterility

The production of hybrid seeds comes at an economic cost, since self or sib-pollination must be prevented by emasculation of the female lines. The use of male sterile forms as female components excludes labor-intensive process of emasculation, isolation and marking of flowers. Male sterility research has been directed to identify genetically stable lines that can be used in hybrid seed breeding programs. Male sterility was used for the first time in tomato hybrid seed production by Rick (1945a) and up to the present this phenomena is still recognized as a useful trait in breeding programmes (Sawhney, 1994; Gorman and McGormick, 1997). There are several types of male sterility in tomato:

1. Cytoplasmic male sterility
2. Chemical induced male sterility
3. Genetic male sterility
   a. Radiation induced male sterility
   b. Genetically engineered male sterility
1. Cytoplasmic male sterility (CMS)

Cytoplasmic male sterility (CMS) is due to cytoplasmic factor ([S] rf1rf1, (S) rf1rf1rf2rf2 etc). The so-called S-factor in most cases is mutational event or rearrangement of mitochondrial DNA (mt-DNA) and is well-investigated source of sterility in different species, but does not naturally occur in Lycopersicon species. CMS based hybrid seed breeding programme involves three lines; the male sterile line (A) with S factor, the restorer line (R) which carries dominant nuclear genes that can suppress the male sterile effects of the cytoplasm and the maintainer line (B) with normal cytoplasm but is devoid of restorer genes. Melchers et al., (1992) obtained tomato CMS plants from protoplast fusion experiments conducted with L. esculentum and two different species of potato (Solanum acaule and S. tuberosum). The plants were similar to the cultivated tomato parent in chromosome number, morphology, physiology but depicted variations ranging from normal looking non-available pollen to a complex lack of stamens. Inheritance studies conducted over several generations proved that such CMS was stable; one of the most important feature of lines to be used in hybrid seed breeding programs (Gorman and McCormick, 1997). Fusions performed with S. lycopersicoides or with Nicotiana tabacum (Melchers et al., 1992) and similar experiments conducted with L. pennellii as the cytoplasmic donor (Mutschler, 1990) failed to produce CMS plants. It was therefore concluded that the types of mitochondrial rearrangements required can not be produced from species too closely related to L. esculentum (S. lycopersicoides and L. pennellii or from species too distant related such as N. tabacum.

This technique of CMS induction is very useful for breeding programmes because it allows CMS introduction in one step without any loss of desirable cultivar characteristics. However restorer alleles functional in the induced CMS plants would need to be identified and incorporated into the male parents. Melchers et al., (1992) reported weakly effective endogenous restorer alleles in some cultivars of L. esculentum. However, selection for strong alleles (e.g., from Solanum species) that will effectively suppress the de novo CMS and introduction of such alleles into L. esculentum has not been achieved so far (Gorman and McCormick, 1997).

2. Chemically induced male sterility

Plant hormones such as 2,4- dichlorophenoxyacetic acid (2,4-D), naphthaleneacetic acid (NAA) and 2,3-dichloroisobutyrate (DCB) have been extensively used to induce male sterility by various researchers (Rehm, 1952; Moore, 1959). Male sterility inducing effects of cytokinin and gibberellin treatments have been well studied by Sawheny and Shukla (1994). Although male sterile plants were produced yet lapses in achieving complete male sterility, necessity to apply continued hormone treatments to ensure sterility from all the flowers in an inflorescence and lower seed yield were the major drawbacks which do not permit their utilization in hybrid seed production.

3. Genetic male sterility

Genetic Male sterility in tomato is the effect of aberrations in nuclear genes and occurs relatively frequently in populations (Susan and McCormik, 1997). In the cultivated tomato (L. esculentum Mill.), genetic male sterility is most common (Stevens and Rick, 1986) and is classified into male sterile and functional male sterile mutants.

A large number of recessive male sterile mutants controlled by single genes are available (Mutschler et al., 1987). The majority of the male sterile mutants belong to male sterile (ms) or stamen less (sl) series. However, other male sterile mutants e.g., variable male sterile (vms), flordadel male sterile (fl) and pelican male sterile (pms), as well as those in which other floral parts are also affected e.g., vegetative (vg), pistillate (pi) and apetalous (ap) are also known (Nash et al., 1985). In addition, male sterile mutants with an effect on leaf characters e.g., blunt (bn) which affect leaf shape are also reported (Henderson and Brown, 1958). Mainly due to their accessible stigma that permits artificial pollination without stamen emasculation and complete male sterility, the male sterile (ms) and stamen less (sl) mutants seem to be the most attractive to be applied in breeding programs aiming at the facilitation of hybrid seed production (Gorman and McCormick, 1997).

Functional male sterile mutants produce viable pollen but are sterile for mechanical reasons. Relatively few mutants are included in this group: exerted stigma (ex), positional sterile (ps), positional sterile-2 (ps-2), and cleistogamous 2 (cl 2), dialytic (dl) however, cl 2 and dl have not been evaluated for breeding purposes (Atanasssova, 2000).

Exerted stigma sterility (ex)

This type of sterility is not associated with the alterations in the anthers as their dehiscence is normal and they produce normal pollen. It is due to excessive length of style that protrudes from the anther cone holding the stigma
out and away from the concentration of pollen in the central space that effectively produces a functional male sterile phenotype (Rick and Robinson, 1951). Such a position of stigma eliminates the necessity of stamen emasculation if producing hybrid seed.

When testing ex lines for their usefulness in hybrid seed production it was established that it was really difficult to determine and to fix right rate of stigma exertion. On one hand, lines possessing 1.0-1.5 mm stigma exertion became occasionally normal ones that resulted in undesirable self pollination. On the other hand the F1 hybrids of the lines possessing steadily manifested exerted stigma more than 2.0 mm above anther cone, as performed sometime longuistylic, also resulting in lower percentage of fruit setting. Bearing in mind all these inconveniences as well as the fact that breeding ex-seed parent line would overcharge this process by manipulating two more polygenic characters (anther and style length), it was concluded that ex sterility would rather inapplicable in breeding programs (Atanassova, 2000).

**Positional sterility (ps)**

Petals showing coalescence of the corolla nearly to their extremity characterize this type of sterility. The greater lateral growth of the petals causes an overlapping and curling with the adjacent petals. The connate and pseudo-connate forms of the petals results in considerable construction of anthers and tends to hold them in exceedingly close contact with the pistil, particularly with the apex. Occurrence of selfing is a disadvantage that also limits application of ps sterility in practice (Atanassova, 2000).

**Positional sterility-2 (ps-2) or non-opening anthers**

Gene ps-2 resulted from a spontaneous mutation in the Czech cultivar Vrbicanske. It is closely linked to the fulgens, chromosome 4 (Atanassova, 1991) and characterized by the presence of fertile pollen and indehiscent anthers (Tronickova, 1962). The ps-2 sterility was found to be due to structural alteration in the zone of anther dehiscence (Oryol and Zhakova, 1977). Studies on the occurrence of anther dehiscence in tomato ps-2 lines have shown that anther dehiscence occurred usually not immediately at anthesis, but 2-3 days later and the percentage of flowers containing dehiscent anthers varied with the genotype. Probably for this reason, when producing hybrid seed using ps-2 sterile lines, it was noticed that the percentage of selfed seeds was significantly lower than the percentage of selfing of these same sterile lines at the end of period of hybrid seed production. Therefore the occurrence of some percentage of selfing (6-7 % maximum), in the ps-2 lines might not be considered as an insurmountable barrier for producing 100% of hybrid seed. Based on these facts, it was concluded that the usefulness of ps-2 lines had to be evaluated on the basis of both percentage of selfing and the hybridity of seeds (Atanassova, 1999).

Introgression of ps-2 gene into fertile genotypes showed that the expressivities of gene varied with the genotypes: in some F3 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. It made clear that strict control of percentage of self fertilization and the elimination of plants showing more than 5-7 % of selfing was necessary through out the entire breeding process (Atanassova, 1999). Emasculation during anthesis was found to be easier and almost two times rapid than emasculation applied on the fertile floral buds (Georgiev and Atanassova, 1981).

The easy maintenance of the sterile lines is the main advantage in using ps-2 sterility in hybrid seed production. The significantly higher hybrid seed yield obtained from the ps-2 lines might be considered as one more advantage in using this type of sterility in hybrid seed production (Georgiev, 1991). The only type of functional sterility applied up to date in practice is the ps-2 sterility. During last 10 years, the ps-2 sterility was introduced in the technology of producing hybrid seed in Bulgaria. About 90% of the Bulgarian hybrid cultivars possess ps-2 sterile seed parents (Atanassova, 1999). Tomato hybrids possessing ps-2 seed parents were developed also in Czech Republic and Moldova.

**a. Radiation induced genetic male sterility**

Powers (1945) suggested that tomato-seed should be X-rayed and a large population grown to obtain male sterile lines. Rick (1945b) in his survey of cytogenetics causes of unfruitfulness in tomato, suggested that genetic male sterile mutants, depending on simple recessive genes, might be most advantageous in hybrid tomato seed production. He stated that selection in large varietal plantings for the male sterile characteristics could be done more quickly than following the traditional method of back crossing. His observations indicated the possibility of obtaining one male sterile diploid in 5000 plants. Besides application of radiation as mutagenic agent for creation of male sterility (Lesley and Lesley, 1958; Yamakawa, 1968), Contant et al., (1971) concluded from exemplary large
scale experiments with tomato (L. esculentum) that irradiation of dry pollen or (premeiotic) pollen mother cells (present in flower buds of 3-4 mm) seems to hold little promise for mutation breeding process because of the “extremely low” frequencies of recessive visible mutations in M1 generations. Moreover deleterious effects caused by irradiation treatment are not efficiently eliminated. In these treatments irradiation of both gametes (24 h after pollination of emasculated flowers and at least 20 h before gametic fusion) resulted in much higher mutation frequencies in tomato plants than irradiation of pollen mother cells and pollen, but the mutation frequency of first group still remained lower than after irradiation of dry seeds. Irradiation of “both gametes” stage resulted in much higher load of sterility (indicating chromosomal aberrations) than treatment of pollen or pollen mother cells. Several male sterile mutants plants have been developed with gamma ray application (Driscoll and Barlow, 1976; Masuda et al., 1999) and with chemical mutagens treatments (Cross and Laddyman, 1991). However their utilization for industrial hybrid seed production of tomato is still underway.

b. Genetically engineered male sterility

The most important avenue for eliminating hand emasculation in hybrid seed production is the use of transgenic plants that have been engineered for male sterility using biological molecular techniques. Gene transfer through protoplast fusion can regenerate novel male sterile plants, i.e., by cybrid formation. (Kofer et al., 1990). For example the promoter of a tapetal --specific gene might be used to drive expression of a cytotoxic protein. Self-destruction of tapetum would thus cause male sterility. Mariani et al. (1996) were the first to succeed in using such scheme. They used the tobacco TA29 gene promoter to direct the expression of either of two different Rnase genes (RnaseTI and barnase) in tobacco and oil seed rape. The resulting male sterile transgenic anthers lacked a detectable tapetum and contained collapsed pollen sacs without microspores or pollen grains. For easy selection of the male sterile plants in a back cross population, the transgene was linked to bar, a gene conferring tolerance to herbicide glufosinate-ammonium. Application of the herbicide to progeny of backcross then eliminates 50% male fertile plants, increasing the efficiency with which homozygote male sterile plants can be isolated. With out the ability to reserve sterility, such engineered male sterile plants are not better than spontaneous mutants. Mariani et al., (1992) solved this problem for the barnase male sterile plants by creating a second engineered gene that contained a ribonuclease inhibitor gene (barstar) under TA29 promoter control. The TA29-barstar plants acts as a restorer line and, when crossed with plants carrying TA2-barnase, produce male fertile plants. This type of engineered control of male sterility is still in its infancy but holds greater potential for hybrid seed production (Gorman and McCormick, 1997).

As an alternative, a number of dominant male sterile systems have been developed using genetic engineering, based on their ability to inhibit pollen function by interfering with the critical functions in the tapetum, a glandular cell layer surrounding the anther locule that is responsible for providing nutrients, cell-wall components and enzymes to developing microspores (Bedinger, 1992). These systems interfere with tapetal function by inhibiting flavonoid production or carbohydrate supply (Goetz et al., 2001) or by using sense or anti-sense suppression to inhibit essential genes (Luo et al., 2000; Xu et al., 1995), or they utilize tapetal-specific promoters to express cytotoxic genes such as barnase (Mariani et al., 1990), glucanase (Curtis et al., 1996) or genes encoding enzymes capable of converting relatively non-toxic compounds into toxic compounds (Dotson et al., 1996; Kreite et al., 1996). Except for the conditional male sterility systems, all of these systems suffer from the inability to produce pure stands of male sterile plants. One solution to this problem has been to link an herbicide gene and then cull out the 50% of the progeny that are male fertile by using herbicide spray (Reynaerts et al., 1993). However, this strategy requires either that seedlings be planted after spraying or results in uneven plantings. Burgees et al. (2002) developed a novel, two-component barnase system for cell lethality in contrast to one-component lethality system. This system allows pure stands of male sterile to be produced. In this system inbred A seeds are produced by crossing inbreeds A1 (pTap1: Bn5-2) and A2 (pTap2: Bn3-2), which are each homozygous for one of the two partial barnase genes. In the hybrid seed production field inbred A (pTap1: Bn5-2 x pTap2: Bn3-2) will be 100% male sterile because it has both partial barnase genes. When this A is crossed with inbred B to produce hybrid seed, the hybrid progeny will be 100% fertile if the two partial barnase genes are present at allelic position. Since both inbred parents A1 and A2 are homozygous for only one of the two components, therefore both are male fertile and can be maintained by selfing. pTap1 represents a tapetal specific promoter and pTap2 represents second tapetal-specific promoter. Using this system, Burgees et al., (2002) found that none of over 300 tomato seed plants resulted in viable progeny that inherited both transgenes and all were sterile. However, sufficient filed trials will show the practicality of this system in future.

This two-component barnase system has a number of advantages over the one component barnase system currently being used for hybrid seed production (Mariani et al., 1992). First of all it allows pure stands of male sterile to be generated. Secondly it permits more effective targeting of lethality through the use of separate
promoters to enhance the specificity of expression and lastly restoration of fertility in the hybrids can easily be
achieved when inbred A male sterile is crossed to any non-engineered inbred B, since the two alleles will be forced
to apart by the laws of genetics. In contrast fertility restoration in one-component system requires engineering inbred
B with ribonuclease-inhibitor gene, barstar. (For more details, see technical advance: Burgess et al., 2002).

Modeling of F₁ hybrid

It is very difficult to rule out novel F₁ hybrid breeding model system as it is largely influenced by the objectives.
For the sake of simplicity, a model is discussed as described and illustrated by Georgiev (1991) with some
modifications. Like all technological processes, the effectiveness of F₁ hybrid breeding depends on the degree of
development of the corresponding sources, informational and methodical insurance, including the following
important steps:

a) Collection, Maintenance and Passportization of Parental lines

In the F₁ hybrid breeding, pure lines are used which preserves their phenotypic expression when reproduced by
the seeds, which need to be evaluated for the quantitative as well as qualitative characteristics including male
sterility. To assure a greater guarantee of purity, their evaluation and reproduction for the purpose of hybrid seed
production must be carried out with reliable isolation and space. A list of these characteristics is given as under:

Quantitative Characteristics

These characters are controlled by a greater number of genes strongly influenced by the environmental factors.
A strict individual plant selection on seed morphology and germination, cotyledon, leaf, stem, cluster, flower, fruit,
fruit yield and morphology are suggested to be made in order to avoid the harmful consequences for the combining
ability (CA).

Qualitative Characteristics

These characters are controlled by the small number of genes and are generally associated with the plant health
such as resistance to diseases and pest. The data obtained, fed into the computer will form the passport of each line
from the collection and would be available for any trait of interest aimed at the facilitation of hybrid seed
production. The assessment of the lines must be carried out by comparing them to the standard lines.

b) Selection of Parental lines

On the basis of information coded in computer memory regarding pure lines from the collection, such parental
lines should be chosen for crossing as may give the greatest chance for the realization of breeding model. First of all,
a selection of female lines should be made, which apart from other qualities, must have the appropriate
characteristics for hybrid seed production like male sterility, genetic markers, high yield of seed with good
germinibility and resistance to diseases, especially to those transferred by seeds. The male parents are chosen, which
will complement those characteristics that are not transferred through female parent. It is natural that not only one
but also several copies of the model hybrids would be expected.

c) Combining ability Inheritance, mode of gene action investigations

F₁ hybrid breeding is a game of “combiners”. Using a broad genotype as a tester, the general combining ability
of lines is tested in the top cross method. Line x Tester analysis is an extension of this method in which several
testers are used (Kempthorne, 1957). The design thus provides information about general and specific combining
ability of the parents and at the same time it is helpful in estimating various types of gene effects. Scaling test and
components of generations means have been found best for the assessment of transformations of trait(s) from
parents to offspring. However, for developing suitable hybrids, adequacy of scale must satisfy two conditions
namely, additive gene effects and independence of heritable components from non-heritable ones. The test of first
condition provides information regarding presence or absence of gene interactions. The test of adequacy of scales is
also essential because in most of cases, the estimation of additive and dominant components of variances are made
assuming the absence of gene interaction (Sing and Chaudhary, 1985). Mather (1949) and Hayman and Mather
(1955) gave four tests for scale effects, which can be applied depending upon the objectives, available labor and
time. The information derived will show the lineage of inheritance pattern with every increment of gene(s) in
hybrids between the two parents in a particular cross as a result of accumulation of heterozygous or homozygous
genes. Best combiner lines with additive gene effects and independent heritable components i.e., with out linkage
and pleiotropic effects are desirable for F₁ hybrid development (Georgiev, 1991).

REFERENCES


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