Male sterility in pepper (*Capsicum annuum* L.) [*]

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Summary

Male-sterility in peppers (*Capsicum annuum* L.) was first documented in the 1950's. Since then considerable knowledge has been accumulated on the nature of the trait, the means of its identification and induction, inheritance of both genic and cytoplasmic genic male-sterility, its line maintenance, and the potential for breeding hybrid cultivars. Today, several internationally known seed companies use the genic mechanism *msms* on a large scale for producing hybrids, whereas the cytoplasmic genic source is used mainly for breeding pungent (S) *Rf rf* hybrids.

Introduction

Male sterility in pepper, *Capsicum annuum* L., was first documented by Martin and Grawford (1951) and somewhat later by Peterson (1958). Since then, many reports dealing with the trait have appeared, including its isolation, mutagenic induction, inheritance, cytology and, particularly potential for hybrid seed production.

Interspecific hybridization

Sterility in pepper, as in other plants, can occur following interspecific hybridization due to chromosomal or plasmon-genome incompatibility. Egava and Tanaka (1986) suggested – based on chromosomal pairing in interspecific hybrids – the genetic distance between species. Hence, *Capsicum chinense* Jacq. and *C. frutescens* L. are considered closely related species (12 bivalents in the hybrids), whereas in *C. annuum* × *C. chinense* and *C. annuum* × *C. baccatum* L. combinations both quadrivalents and hexavalents due to reciprocal translocations were observed.

In the last cases F$_1$ hybrids are practically male-sterile, their anthers do not dehisce, and they contain few or no stainable pollen grains. Pollen fertility in such material can be restored by backcrossing to one or both parental lines. Many attempts were made (Andrasfalvy and Csillery, 1983; Csillery, 1983; Dumas de Vaulx and Pitrat, 1977; Egava and Tanaka, 1986; Hirose, 1965; Ledo et al., 1992; Ohta, 1962; Pickersgill, 1980; Rusenova-Kondareva, 1968; Saccardo and Sree Rumulu, 1977) to improve *C. annuum* via interspecific hybridization particularly with *C. chinense, C. baccatum, C. frutescens* and *C. chacoense* Hunz.

In the *C. baccatum* × *C. annuum* combination, a developmental process of male sterilization followed repeated backcrossing to *C. annuum*, resulting in antherless (frequently combined with reduced female fertility) progeny. Such plasmon-genome interaction leads to the cytoplasmic male-sterility (CMS) phenomenon (Andrasfalvy and Csillery, 1983; Shifriss, unpublished). Attempts of several workers to identify fertility restorer genes for this specific case were unsuccessful, as will be clarified later.

F$_1$ hybrids among *C. annuum* and the above mentioned species demonstrated different levels of male-sterility, depending on the accessions used in each interspecific cross.

Spontaneous and induced male-sterile ms mutants

Over a dozen single ms gene mutants have been found or induced following mutagenesis (X-rays, gamma rays and EMS) (Daskaloff, 1971; Deshpande et al., 1983; Hirose and Fugime, 1980; Meshram et al., 1992; Meshram and Narkhede, 1982; Milkova and Daskalov, 1984; Moor, 1986; Murty and Lakshmi, 1979; Pathak et al., 1983; Pochard, 1970; Prakash et al., 1987; Shifriss, 1973; Shifriss and Frankel, 1969; Shifriss and Rylski, 1972). Some of these *ms* genes appeared to be identical.

The *ms-509* gene that was induced by mutagenesis in France (Pochard, 1970) was found to be allelic to the *msk* allele isolated spontaneously in Korea (Woong Yu, 1985). In looking for spontaneous mutants, it is advisable to select among 'old' cultivars that contain more recessive mutants, than among lines (cultivars) that pass strict selection for uniformity.

Generally, the genic *ms* mutants are similar in function, and a few of them are linked with marker traits that can help in early identification of the *ms* individuals (Meshram and Narkhede, 1982; Murty and Lakshmi, 1979; Pathak et al., 1983).

Cytoplasmic genic male-sterility in C. annuum

Peterson (1958) isolated a male-sterile individual within accession USDA P.I. 164835. He classified several degrees of sterility, from 1 or 2 to 10 or 15 viable pollen grains per anther. The trait was found to be controlled by a major recessive *ms* gene interacting with a specific S plasmatype. A dominant *Ms* allele is necessary to restore pollen fertility. Hence, from the cross (S)*msms* $\times$ (N)*MsMs* one should obtain fertile (S)*Msms* progeny only. However, in several test crosses (Peterson, 1958) a dihybrid ratio was obtained (3 fertile: 1 sterile) suggesting an additional duplicate Ms locus, Ms_2ms_2.

Novak et al. (1971), working with Peterson's male-sterile material, suggested two contradictory digenic interpretations to his data. Based on the 1 : 3 and 9 : 7 (fertile : sterile) ratios in test crosses and F_2 populations, respectively, a complementary gene action was suggested. On the other hand, the 3 : 1 test cross data suggested independent gene action of the Ms_1 and Ms_2 loci. With the last concept, one would expect a 15 : 1 F_2 ratio, but such a F_2 segregation was never documented.

While *Ms* alleles were found in most wild hot pepper accessions, the *ms* alleles seem to be present in many sweet, large-fruited lines (Novak et al., 1971;

Ohta, 1971; Peterson, 1958; Verma et al., 1993; Woong Yu, 1985). Further studies revealed that late in the growing season, when the temperatures drop (under 25 °C and 17 °C day and night, respectively), pollen fertility is restored (Kubisova and Haslbachova, 1991; Ledo et al., 1992; Peterson, 1958; Shifriss and Guri, 1979; Verma et al., 1993; Woong Yu, 1985). According to Peterson (1958): 'Warm temperatures present the most critical environment for sterility expression in peppers'. The relative instability of the trait was attributed to an interaction between temperature and sterility modifier genes.

Woong Yu (1985), in a study of 270 pepper lines, found 152 to be 'stable B-lines', i.e., maintainer (N)*msms*, 66 to be restorer lines (N)*MsMs*, and the remaining 50 lines were defined as unstable, leading to segregating progeny following crossing with Peterson's male-sterile plants. In addition, the pattern of segregation demonstrated large deviations from year to year. These 'unstable lines' might be heterozygous to the sterility modifier genes and their progeny reacted to yearly or seasonal variation.

Eventually, selfing and selection in this material yielded both 'stable' maintainer lines and fertility-restorer ones.

In the studies of Woong Yu (1985, 1990), no maintainer lines were isolated among hot pepper pubescent accessions, suggesting a linkage between hairiness and the fertility restoring gene. In addition, among the sweet accessions only one, cv. DiQuneo was a restorer.

Shifriss and Frankel (1971) succeeded in isolating a S plasmatype following intraspecific crosses. Eventually, this source of male-sterility proved to be identical to that of Peterson.

Cytoplasmic male-sterility from interspecific crosses

Capsicum frutescens $\times$ *C. annuum* yielded cytoplasmic male sterile progenies following backcrosses with sterility-maintainer lines (Csillery, 1983; Woong Yu, 1990). The *C. frutescens* plasmatype was found to be identical to Peterson's type in its sterilization and fertility restoration potential when crossed with Peterson's maintainer and restorer lines, respectively. Hence, three independent sources of S plasmatypes, two in *C. annuum* and one in *C. frutescens*, were found to be identical.

C. baccatum $\times$ *C. annuum* hybrids backcrossed to *C. annuum* yielded an extreme type of male sterility, viz., antherless flowers (Andrasfalvy and Csillery, 1983; Csillery, 1983; Saccardo and Sree Rumu-

lu, 1977; Shifriss, unpublished; Woong Yu, 1990). Efforts to isolate fertility-restorer genes so far have been unsuccessful. A gradual feminization process which occurred in advanced backcrossing of *C. baccatum* × *C. annuum* suggests a polygenic plasmon-genome nature of inheritance. In other words, fertility restoration is recessive. Such an interspecific situation is not useful for producing male-fertile hybrids.

At this stage we should differentiate between the *ms* genes operating autonomously (*ms*$_1$, *ms*509, etc) and the male sterility genes interacting with N and S plasmatypes that were originally, also designated as *ms* genes (Peterson, 1958). The last category should be classified as *rf* genes. Hence, cytoplasmic gene male-sterile individuals will be marked (S)*rfrf* and the restorer allele as *Rf*. In several studies (Shifriss, unpublished; Woong Yu, 1990) it was demonstrated that *ms* mutants are non-allelic to the *rf* ones.

Cytological aspects of meiotic breakdown

Except for one case of meiotic breakdown at the pachytene stage (Murty and Lakshmi, 1979), all reported evidence on male sterility indicates that microspore breakdown occurs after the tetrad stage of meiosis. Microscopic observations (Hendrychova-Tomkova and Nguyen, 1982; Hirose and Fugime, 1981; Novak, 1971) and electron microscopy (Horner and Rogers, 1974) helped to clarify the functions of the tapetal layers and the callose during the process. In normal meiosis, the callose dissolves and normal microspores are released from the tetrads. In meiosis of cytoplasmic male-sterile plants, both the outer and inner layers of the tapetum degenerate and the microspores abort.

Stability of male-sterility

All *ms* mutants are highly stable and hence promising sources for hybrid seeds production. In exceptional cases (Daskaloff, 1971), a few aborted pollen grains were found in anthers of male-sterile plants. In cytoplasmic-genic male-sterility (*Rf, rf* genes and N and S cytoplasms), the trait is highly sensitive to temperatures, as found by Peterson (1958) and others (Kubisova and Haslbachova, 1991; Ledo et al., 1992; Shifriss and Guri, 1979).

The variation among CMS lines in the expression of sterility presumably is caused by differences in number and nature of male-sterility modifying genes. Hence, one can select B-lines with potential resistance to seasonal (temperature) fluctuations (Shifriss, unpublished; Woong Yu, 1985).

During the hottest August–September period in Israel (when mean temperatures reach 30 °C) male-sterility in (S)*rfrf* plants is stable. However, when the temperature drop below the optimal for pepper production (24 °C, 17 °C day and night), meiotic breakdown is either stopped or delayed, resulting in pollen fertility. Hence, one can take advantage of the different seasons for both hybridization during late summer and seed increase of the (S)*rfrf* parental lines during the cool season.

Maintenance of male-sterile lines

Genetic male-sterility. Single gene *ms* mutants can be propagated vegetatively but the resulting explants have low juvenility and produce flowers at an early stage. A more common procedure is sibbing *msms* × *Msms*, resulting in a 1 : 1 segregation of male-fertile (*Msms*) and male-sterile (*msms*) plants. At seed production the *Msms* individuals have to be rogued. A seedling marker linked with the *ms* gene might be helpful for early identification and planting of only the desirable *msms* individuals. Csillery (1989) and Shifriss and Pilowsky (1993b) succeeded in developing a digenic system, *ms*$_1$*ms*$_1$*ms*$_2$*ms*$_2$ × *Ms*$_1$*ms*$_1$*Ms*$_2$*ms*$_2$ which, due to complementary gene action, yielded 3 male-sterile vs 1 fertile progenies.

The digenic procedure, however, requires vegetative maintenance of the two crossing components and close protection from viral contamination.

Shifriss (1983) tried to build an 'XYZ system' as proposed by Driscol (1972) by using *C. annuum msms* (ann) and *C. chinense MsMs* (chin). However, the desirable 12 II *msms* (ann) + I *Ms* (chin) Y – maintainer line was not achieved.

Cytoplasmic genic male sterility. The main advantage of the CMS system over the previous one, stems from the fact that one can obtain 100% male-sterile plants by intercrossing (S)*rfrf* × (N)*rfrf* for direct use of an A line in a hybrid seed production system. In addition, based on the relative instability of male-sterility we can multiply the (S)*rfrf* plants under off-season conditions that induce pollen restoration for selfing. This procedure was found applicable in the Arava region of Israel, during the winter season (Shifriss, unpublished).

How to look for male sterility?

A search for ms ms *mutant plants*
Every open-pollinated cultivar is a potential candidate in which one can find *ms* mutant plants. Exceptionally tall, poor-fruiting plants are usually suspected of being *ms ms*.

A search for novel (N*) plasmatype in C. annuum
This search is based on the suggestion of Frankel and Galun (1977) that a standard *msms* plant my actually be a (S*)*rfrf* type and the fertile plants are (S*)*RfRf*. Such a model should be tested through a search for an alternative (N*) type cytoplasm. How to isolate such novel (N*) cytoplasm? Crosses of tested accessions with the putative (S*)*Rfrf* male genotype, like (S*)*RfRf* × (S*)*Rfrf* should yield F_1 hybrids in which 50% will segregate for male sterility in their F_2 generation. However, rare exceptional crosses may show fertile progenies only in their F_2 generation. Such exceptional finding can be derived from the crosses between (N*)*Rf* or *rf* × (S*)*Rfrf*. Its F_2 progenies, (N*)*RfRf*, (N*)*Rfrf* and (N*)*rfrf*, are expected to be fertile and the new (N*)*rfrf* line will serve as a unique maintainer B-line to generate 100% male-sterile plants for hybrid seeds production. Such a system is ideal, because all pepper accessions may serve as restorer (S*)*RfRf* – C lines.

We tested this assumption with a few dozen accessions and two different *ms* mutant genes, but did not find the anticipated (Frankel and Galun, 1977) N*-type cytoplasm.

Male-sterility and hybrid seeds production

Different genetic and field procedures are being used on experimental and commercial scales in order to obtain pepper hybrids.

The *ms* mutants are widely used (Breuils and Pochard, 1975; Csillery et al., 1987; Daskaloff, 1972, 1976; Hirose, 1965; Hirose and Fugime, 1980; Miladinovic and Stevanovic, 1977; Rabinovitch et al., 1993; Shifriss and Pilowsky, 1993a; Shifriss and Sacks, 1980; Velazquez Feria, 1983; Woong Yu, 1990). Despite the need to identify them early, the 50% fertile (*Msms*) plants are nevertheless often identifiable at anthesis and are removed.

The CMS system is particularly advantageous (for hot pepper hybrids) because it supplies 100% male-sterile plants and, in addition, restorer *Rf* genes are widely distributed in hot pepper accessions (Novak et al., 1971; Ohta, 1971; Peterson, 1958; Shifriss and Frankel, 1971; Verma et al. 1993; Woong Yu, 1985). In sweet pepper, we can use the (S)*rf rf* × (N)*rf rf* scheme to obtain a 100% male-sterile seed line, but the Rf genes have to be introduced from hot pepper lines by repeated backcrossing.

The only sweet accession that was found to be *Rf Rf* is cv. DiQuneo (Woong Yu, 1985). In practice, the new backcrossed C-lines are of the (S)*Rf Rf* type. The breeding procedure required to build sweet C-lines is a burden when compared with the genic system in which each line is potentially a *Ms Ms* 'restorer line'.

When (S) F_1's were compared with their isogenic (N) F_1's, in both hot (Woong Yu, 1990) and sweet groups (Shifriss, unpublished), no agronomical or other differences were found.

In addition, for breeding hot pepper hybrids, Shifriss and Sacks (1980) suggested to use sweet-fruited CMS mother plants, which contain a larger number of seeds per fruit than do common hot varieties.

Woong Yu (1990) suggested to combine both genic and CMS components of sterility in order to obtain double-cross-hybrids. Such a program could exploit the yielding heterosis well known among hot pepper accessions. Daskaloff and Mihailov (1988) demonstrated an elegant system in which the C-line is a conditional-female-sterile (the gene symbol is *cfs*) which continues to flower and to supply pollen. In addition, a CMS line that contains a lethal gene (*l*) serves as the seed parent. True F_1 seeds will be obtained from the cross (natural or manual): (S)*rfrf ll Cfs Cfs* × (N)*RfRf LL cfs cfs*.

Although the system is attractive, its applicability and the involvement of the lethal (*l*) gene remain unclear.

Following research on natural pollination of male-sterile plants, maximal yield of F_1 seeds per unit area was obtained within the 2 : 1, and 3 : 1 ratios of male-sterile and male fertile rows (Breuils and Pochard, 1975; Csillery et al., 1987; Daskaloff, 1972, 1976; Hirose, 1975; Miladinovic and Stevanovic, 1977; Shifriss and Sacks, 1980). Highest yield of hybrid seeds per male-sterile plant was obtained from the 1 : 1 ratio, however the yield was less than half that obtained from normal male fertile plants. Studies of insect pollination demonstrated that both honey bees (*Apis mellifera*) and bumble bees (*Bombus terrestris*) prefer fertile plants when exposed to both fertile and male-sterile

ones (Rabinovitch et al., 1993; Shifriss and Pilowsky, 1993a).

Rabinovitch et al. (1993) found that male-fertile flowers produce more nectar and a higher sugar concentration than sterile ones. In addition, a 'significant correlation between sugar quantity and number of honey bee visits per flower was evident'. However, additional studies are needed, particularly on the effect of pollen on the selective attractiveness of bees to fertile vs male-sterile flowers.

Economic importance of male-sterility

Male-sterility, genic and CMS sources are widely used on both experimental and commercial bases in the hybrid seed industry. Both manual and open pollination procedures are used for large scale hybridization. The increasing importance of male-sterility stems from the growing involvement of seed companies in producing elite F_1 hybrids.

Although sweet F_1's are considered the most important in the seed industry, more hot hybrids are becoming popular. In Korea, for example, 197 pepper hybrids – mostly hot ones – were registered up to 1994, and all of them were multiplied with genic or cytoplasmic mechanisms of male-sterility. Today, the CMS system is the dominant one (Woong-Yu, personal communication). Besides the general importance in the seed industry of male-sterility, the latter has been suggested as an instrument for selecting genotypes with high potential of parthenocarpy (Shifriss and Eidelman, 1986). Parthenocarpy or pepper cultivars with low seed dependence for normal fruit development, might be of economic importance.

Acknowledgement

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