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AN OVERVIEW ON EXPLOITATION OF MALE STERILITY FOR DEVELOPMENT OF HYBRIDS AND SEED PRODUCTION IN HOT PEPPER

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ABSTRACT : Male-sterility in chili was documented for the first time in the 1950's. Since then, considerable knowledge has been accumulated on the nature of the trait, the means of its identification, induction, inheritance of both genetic and cytoplasmic genetic male-sterility, maintenance of inbreds, and their potential for breeding hybrid cultivars. Heterosis for various economic traits like; maturity, fruit weight, size, number and total yield have been utilized. Today, several internationally known seed companies and research institute use the genetic mechanism [msms] on a large scale for producing hybrids of sweet pepper, whereas the cytoplasmic genetic sterility [(S) Rf rf] is used mainly for breeding pungent hybrids. The possibilities of exploitation of male sterility for crop improvement as well as production of F_1 hybrids have been reviewed and discussed in the present paper. Biochemical as well as biotechnological aspects of this phenomenon has also been vividly reviewed.

Keywords: *Hot pepper, male sterility, hybridization, heterosis, hybrid seed production.*

Chilli (*Capsicum annuum* L.) is an essentially old world genus native to tropical America (Thompson and Kelly, 76). The fruit, consumed either as green or red powdered form is a culinary item in all parts of globe. The fruits are quite rich in nutrients, phytochemicals and antioxidants. The global acreage for both chilies and bell pepper was 1.7 million hectares with a production of 26.06 million tones, whereas the dry chilies and pepper occupy an area of 1.56 million hectares and production of 2.35 million tonnes. India tops the list of chilies and dry pepper production, while China tops the list for green chilies and green pepper production. In last few years per capita consumption of all peppers has increased. The consumption of bell peppers grew from 9.2 pounds to 9.8 pounds, while chilli pepper consumption grew from 6.1 pounds to 6.6 pounds (Anon., 2). But, most of the area is covered by open pollinated varieties, which mainly account for lower production and productivity. Exploitation of heterosis through hybrid development is essential to augment production and ensure stability of yield. Deshpande (16) was pioneer in reporting heterosis in chilies from India. Heterosis for various economic traits like maturity, fruit weight, size, number and total yield have been reported by many other authors (Shankarnag *et al.,* 66; Satish and Lad, 64; Patel *et al.*, 54). Reddy *et al.* (60) reported 88.27% better parent heterosis and 60.51% economic heterosis for fruit yield per plant in the hybrid SKAU-SC-1003 × Arka Lohit. Male sterility is a handy tool for the exploitation of heterosis in many crops, including chili. The high labour cost, tedious process of manual emasculation and sensitivity of flowers to physical injury limits the commercial exploitation of heterosis. Breuits and Pochard (5) developed the first hybrid Lamuyo by using male sterile line *ms*-509. Singh and Kaur (73) recommended use of male sterility for production of F_1 hybrids. Hundal and Khurana (29) reported 235.71% and 138.0% standard heterosis for green fruit yield and red ripe fruit yield, respectively using male sterility system. Heterosis for yield and different yield related traits utilizing male sterility system has also been reported by Meshram and Ghongade (47), Shankarnag and Madalageri (65) and Patel *et al.* (54).

FLORAL BIOLOGY OF CHILIES

Chilli is a hermaphrodite plant. The flowers are borne solitary or in clusters in the axil of the leaves. Sepals and petals are usually five in number, stamens varies from five to 14, stigma is club shaped, ovary superior and consists of 2 to 4 or more locules. The anther dehiscence and stigma receptivity are influenced by weather conditions. The flowers have been reported to open by 5.00-6.00 hr and anther dehiscence occurs between 8.00-11.00 hr. The pollen fertility and stigma receptivity is highest on the day of anthesis, therefore self pollination is favoured. However, differential position of stigma (e.g. heterostyly with longer stigma), prevailing protogyny (reported in some lines) and preference of honeybees and other pollinating insects determine the extent of cross pollination. Most cultivated peppers are

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autogamous and cross pollination as high as 68% has been reported (Greenleaf, 22). In open field, out-crossing commonly ranges from 7% to 90%, which shows that *Capsicum* should be considered facultative cross-pollinating species (Franceschetti, 20).

MALE STERILITY SYSTEM IN CHILLI AND ITS EXPLOITATION IN HYBRID DEVELOPMENT

The flowers are small in structure, therefore, hand emasculation and pollination is a time taking process. Unlike tomato, the stamens are neither epipetalous, nor are the flowers bigger and stout as in brinjal. Besides, manual emasculation significantly increases the cost of hybrid seed production. To mitigate this problem, both genetic as well as cytoplasmic systems have been exploited for use in hybrid seed production. Male sterility is characterized by the absence of functional pollen in the hermaphrodite flower, which is used as an aid in the hybridization programme of chilies. In India, Punjab farmers are producing chilli hybrid seeds, using nuclear male sterile lines and natural cross pollination (Dash *et al.*, 11; Kumar and Singh, 39). CGMS system in chilies had also been reported from IIHR, Bangalore (Madhavi, 45). Male sterility system in chilies is governed by nuclear genes alone (genetic male sterility) or a combination of cytoplasmic and nuclear gene (cytoplasmic-genetic male sterility). Male sterility was first documented by Martin and Crawford (46) in *Capsicum* pubescence. A few years later, Peterson (57) isolated a male sterile line from a non-pungent collection of India (USDA PI 164835). The trait was reported to be controlled by a sterile cytoplasm with a recessive *ms* genes, while dominant *ms i.e., Ms* allele restores the pollen fertility in the hybrid. In many test crosses, a dihybrid ratio of 3 fertile: 1 sterile was observed and suggested presence of another restorer locus *ms-2*. Nevertheless, all other reports so far have concluded restoration to be monogenic dominant. Ms-509 line (Pochard, 58) was renamed *ms*-10 by Daskalov and Poulos (15), later brought to India and introgressed into Indian chilli lines and developed *ms*-12, *ms*-13 and *ms*-14 (Singh and Kaur, 73) lines. Later *Ms*-12 became a successful parental line and two chilli hybrids *viz.,* CH-1and CH-3 gained a lot of popularity in North India. Shifriiss and Pilowsky (72) reported a digenic male sterility in *C. annuum*, controlled by two loci viz., *Ms1 and Ms2*, the recessive forms of which were responsible for expression of male sterility. Yazawa *et al.* (80) developed a new line of capsicum by transferring Peterson's male sterile line into a Japanese accession and reported it to be stable under fluctuating temperatures. They also transferred this cytoplasm into the background of a Japanese variety Murasaki to obtain a non-pungent male sterile line. Kumar *et al.* (36) were successful in transferring genetic male sterility from *ms-12* to sweet peeper and obtaining non-pungent bell shape fruits by back crossing method.

MALE STERILITY THROUGH MUTATION: SPONTANEOUS AND INDUCED

Every open pollinated cultivar would be a potential candidate in which male sterile mutants could be found, particularly the exceptionally tall, poor bearing plants revealed sterility (Shifriiss, 67). While many times mutants appear in open pollinated populations of established varieties, it is observed mostly in segregating generations of intra and interspecific crosses. Peterson's male sterility system was a spontaneous one. Roussenova-Kondareva (61) obtained spontaneous mutants from the F_4 generation of two crosses viz., *C. pendulum* var. *bicoloratum* × *C. annuum* and *C. pendulum* var. *longisilicum* × *C. annuum*. The flowers were stamenless or sometimes with only rudimentary stamens. Dikii and Analkecko (18) succeeded in improving the morphological and biological characters by backcrossing with different varieties, but could not identify restorer lines for this. Shifriiss (67) suggested screening old cultivars for spontaneous male sterile mutants they might contain more of recessive mutants in comparison to lines, which were developed after strict selection for uniformity. Shifriiss (68) identified twenty four unfruitful plants in a population of ten thousand plants and one plant was found to produce small and shrunken anthers, which was unable to produce pollen grains.

Several mutagens have been tried to create male sterile mutants in *Capsicum*. Daskalov (12) obtained 6 male sterile mutants after seed irradiation with γ or X-rays. Genetic studies revealed that five of the mutants were non-allelic monogenic mutants (*ms*-3, 4, 6, 7 and 8), whereas one of the mutants was found dominant (designated *Dms*). Pochard (58) induced male sterility through application of X-rays or EMS (ethyl methyl sulphonate) on monoploid material and obtained three different mutants viz., *ms*-9, *ms*-509 and *ms*-705. Thakur *et al.* (75) irradiated California Wonder seeds with different doses of X-rays; male sterile mutants were detected at 5 & 10 kR dose levels. Yu (82) found that a material isolated spontaneously (*msk* allele) was allelic to Pochard's material. Shifriiss and Frankel (70) isolated a sterile cytoplasm while studying intraspecific crosses, which was subsequently found identical to Peterson's male sterile. Many of the male sterility genes induced through mutagenesis were later reported to be identical among themselves or themselves or to some already existing system. An interspecific hybrid between *C. frutescens* and *C. annuum* following back crosses with sterility maintainer lines produced male sterile progenies (Csillary, 9). The cytoplasm of *C. frutescens* was found identical to Peterson's type in its sterility expression and response to the Peterson's maintainer and restorer lines. Cytoplasmic male sterile plants were isolated from segregating population of an interspecific cross involving *C. chinensis*, *C. baccatum* and *C. annuum* at Katrain centre of Indian Agricultural Research Institute (Anon., 3).

INDUCTION OF MALE STERILITY BY INTERSPECIFIC HYBRIDIZATION

The chromosomal or plasmon-genome incompatibility of interspecific hybrids may result in different degree of sterility. Shifriiss and Frankel (70) obtained male sterile forms after hybridization between *C. chinense* and *C. annuum,* followed by two backcrosses with *C. annuum*. The fertility restorer was identified in *C. chinense*. Dikii *et al.* (19) reported sterile plants as a result of hybridization between *C. peruvianum* and *C. annuum*. The fertility of progeny resulting from crosses between different species range between complete sterility (*C. chacoense* × *C. annuum*) to normal fertile hybrids (*C. annuum* × *C. baccatum*) (Kumar *et al.*, 35). Pollen sterility was also seen in three way hybrids (*C. annuum* × *C. chinense*) × *C. baccatum* (Yoon and Park, 81). The cross between *C. baccatum* and *C. annuum* were frequently found unsuccessful; in the few successful cases the F_1 hybrid was completely sterile or selfing was possible scarcely. In an attempt to obtain male sterility Andrasfilvy and Csillary (1) backcrossed the F_1 progeny to *C. annuum* repeatedly, which resulted in an antherless progeny frequently combined with reduced female fertility. Cytoplasmic male sterile plants were isolated from segregating population of an interspecific cross involving *C. chinensis*, *C. baccatum* and *C. annuum* (Anon. 3).

INDUCTION OF STERILITY USING PLANT GROWTH REGULATORS

Use of androecides had been suggested by many workers; 2-3 dichloroisobutyrate (Hirose, 26), maleic hydrazide @ 0.4-0.5% (Chauhan and Singh, 7), 2-2 dichloropropionate or dalapon (Hirose, 27; Chauhan, 6; Salgare, 63) to be used as inducers of complete sterility, whereas 2, 4-D was found to cause incomplete (up to 83%) male sterility. Kohli *et al.* (32) found spraying of GA_3 @ 1000 mg/l at 10 days interval from the onset of flowering for 3 times caused complete male sterility which lasted throughout the crop season. However, there are no reports of commercial hybrid seed production using this technology.

EFFECT OF ENVIRONMENT

Peterson (57) observed that instability in the male sterility could be attributed to interaction between temperature and sterility modifier genes. Later, it was reported that when the temperature drops (e.g. 25°C in day and 17°C in night) pollen fertility is restored (Kubisova and Haslbachova, 33; Ledo *et al.,* 42; Shifriiss and Guri, 71). The variation among cytoplasmic male sterile lines in the expression of sterility is possibly a result of differences in number and nature of sterility modifier genes. Yu (82) demonstrated that development of 'B'-lines with potential resistance to seasonal fluctuation was possible. Moreover, Shifriiss (67) suggested a system to take advantage of sensitivity of lines viz.; male sterile, maintainers and restorers to seasonal fluctuations for hybridization during summers when the temperatures are high (mean diurnal temperature 30°C). Multiplication of seeds of parental lines ('A'-line) during the cool season (diurnal mean temperature 17°C), when sterility remained stable in (S) *rf rf* could possible. Bashir (4) also recorded that male sterility was accentuated by higher temperatures. Hirose (24, 25) reported that high temperature a fortnight before anthesis causes pollen abortion and deterioration of pollination efficiency.

IDENTIFICATION OF MALE STERILE AND RESTORER LINE USING MOLECULAR MARKERS

Identification of restorer line and maintainer line is a tedious task, which requires screening of large number of genotypes. The fertility restoration in chili pepper is controlled by a major nuclear gene (Rf), along with several modifiers and some environmental factors. Kim *et al.* (31) identified molecular markers closely linked to fertility restorer locus (Rf) in chili pepper using bulk segregant analysis. The AFRF8 marker was successfully converted to CAPS marker for fast and reliable detection of restorer lines during F_1 hybrid seed production and breeding programmes in pepper. The distribution of RAPD markers linked with fertility restoration in chili was reported by Kumar *et al.* (37) and they found that Rf gene associated with two markers ($OPW19_{800}$ and $OPP131_{400}$) were not frequently distributed in the restorer inbred lines. Lee *et al.* (44) carried out bulk segregation analysis using 768 AFLP primer combinations and based on the sequence of the internal and flanking regions of the AFLP fragment closely linked to partial fertility restoration locus, the AFLP marker E-AGC/M-GCA_{122} was converted to CAPS marker, PR-CAPS. They further reported that this PR-CAPS marker could be useful in selecting fully fertile lines (Pr/Pr) and eliminating partially fertile (pr/pr) and heterozygous lines (Pr/pr) in segregant population during development of new inbred restorer lines. Mitochondrial DNA differences of CMS pepper line 21A and its maintainer line 21B were reported by Wang *et al.* (77). They utilized 100 random primers to differentiate mitochondrial DNA and found that specific RAPD marker CMSAG3 $_{430}$ was related to CMS gene.

MAINTENANCE AND RESTORATION

Yu (82) used Peterson's male sterile plants, which after crossing with fertile plants, were subjected to selfing and rigorous selection. Selection was eventually found effective and stable and efficient maintainers as well as restorers were isolated from this material. Yu (82) also found that hot pubescent pepper lines contained no maintainer lines and suggested a linkage between pubescence and fertility restorer genes. Among the sweet pepper lines, he was able to identify only one restorer viz., DiQuneo.

Male sterility (anther less flowers) was observed in a cross of *C. baccatum* × *C. annuum*, which was subsequently back crossed to *C. annuum* (Saccardo and SriRamulu, 62; Andrasfilvy and Csillary, 1; Csillary, 9) and gradual feminization was observed with advancement of backcrossing, thereby suggesting a polygenic plasmon-genome interaction and recessive fertility restoration. This condition was not conducive for male fertile hybrids. The *ms* genes interacting with sterile cytoplasm (*S*) and corresponding fertile cytoplasm (*N*) were initially designated as *ms* genes (Peterson, 57; Martin and Crawford, 46; Shifriiss and Frankel, 69). Instead, such genes should be ideally classified as *rf* genes. Only the genes operating independent of cytoplasmic interaction should be designated as *ms* genes.

Shifriiss and Frankel (70) indicated that hot pepper varieties contained only fertility restorer genes and bell type and other sweet varieties contained either maintainer or both maintainer and restorer genes. They also identified a new source of sterile cytoplasm collected from India, which behaved similar to Peterson's *S*-type.

Meshram and Mukewar (49) and Gill and Gill (21) found that manual pollination brings much better effects and obtained 68-100 seeds through manual pollination as compared to 10-12 seeds when pollination was carried out by insects. Nowaczyk and Nowaczyk (52) observed distinct differences based on location of plants in a protection structure. The seed setting was found to be better in the plants situated in the central part of the polyhouse (75.3% setting) as compared to plants near the walls (52.6% setting). There was more stable temperature in the central part of the polyhouse. They found microclimatic conditions in the close neighbourhood of the tent wall less conducive for hybrid seed setting.

CYTOLOGICAL ASPECTS OF MALE STERILITY

Microscopic observations and electron microscopy revealed that after the tetrad stage of meiosis of CMS plants, where both the outer and inner layers of tapetum degenerated and the microspores aborted. Horner and Rogers, 28). Kumar *et al.* (41) by crossed a set of eight maintainer and restorer inbreds on four CMS lines possessing two independently isolated and commercially utilized S-cytoplasms (Peterson's and Reddy's). Based on fertility restoration/maintenance reaction of 32 resulted F1s and on the presence of two SCARs (atp6607 and coxII708) in both the S-cytoplasms, concluded that although two S-cytoplasms were isolated and commercially utilized independently,

LINKAGE WITH MARKER TRAITS

Generally the genetic male sterile mutants are similar in function and in certain cases they have been found to be linked with marker traits, which may be helpful in the identification of male sterile individuals at early stages (Meshram and Narkhede, 48; Murty and Lakshmi, 50; Pathak *et al.*, 56). Gulyas *et al.* (23) attempted to estimate the physical distance between restorer marker and the *rf* gene based on a closely linked molecular marker identified by Yanagawa *et al.* (79). Based on the pollen analysis and the use of *rf* marker in F_2 generation, the physical distance between the restorer gene and the marker gene was found to be 20 cM while it was 5.3 cM and 4.8 cM in F_3 and F_4 generations, respectively. Through, the environment did not alter the expression of a molecular marker (Tanksley, 74). Therefore, the sterility expression was considerably influenced by environmental factors a more precise estimate of the physical distance between the restorer and marker genes could be possible by eliminating the environmental effects. Pakozdi *et al.* (53) identified a molecular marker for the restorer of CMS system and the genetic distance was 20 cM. A CAPS marker linked to a genic male-sterile gene in the colored sweet pepper, 'Paprika' (*Capsicum* *annuum* L.) was identified by Lee *et al*. (43). Woong *et al.* (78) were also able to develop new molecular markers for the identification of male sterile cytoplasm in peppers (*Capsicum annuum* L.).

POLLINATION REQUIREMENT

Capsicum is generally considered a self-pollinated genus, but a high level of cross-pollination has also been reported. Cochran (8) observed that cross pollination takes place more frequently than it is generally believed. Although honeybees are considered the most common pollinators for capsicum, ants and bumble bees have also been observed to effect cross pollination in this crop. Moreover, the expenditure incurred on labour deployed in emasculation and hand pollination was found to be as high as 78 % of the total expenditure on labour employed in hybrid seed production (Kumar and Thakur, 34). Availability of male sterility has ruled out the necessity of selfing and done away with the need of manual emasculation. Several attempts had been reported to make hybrid seed production more economical by modifying the mode of manual pollination (Kumar *et al.*, 40), use of honeybees to transfer pollen from male to male sterile female parent proved an effective method of pollination for hybrid seed production on commercial scale. Several workers had explored the possibility of use of natural cross pollination for hybrid seed production and the commercial exploitation of male sterility system largely depends on natural cross pollination of the male sterile lines. Murty and Murty (51) have recorded as high as 68% natural cross pollination in chilies. Patel *et al.* (55) observed considerable amount of natural cross pollination in male sterile plants surrounded with fertile plants. Kumar *et al.* (38) reported that considerable amount of natural cross pollination takes place on male sterile plants of chilli. They further suggested that during CMS (CCA-4261) based chilli hybrid seed production, expenditure on manual pollination could be saved, without compromising the yield of hybrid seeds. Farmers in Punjab state of India produce chili hybrid on a large scale by using female: male ratio of 2:1 and honeybee for natural cross pollination.

Both manual and open pollinated methods are being practiced for pollen transfer for large scale hybridization. Capsicum flowers produce both pollen and nectar; hence, are attractive to honeybees. Kubiasova and Hslbachova (33) observed that honeybees visit both male sterile and male fertile plants and changed lines frequently. Rabinowitch *et al..* (59) reported that pepper genotypes varied in frequency of honeybee visits and there was significant correlation between sugar quantity and honeybee visit per flower. Increasing number of visits increased the number of seeds in the pepper fruits. Considerable variation in nectar characteristics could be exploited for facilitating bee pollination in commercial hybrid seed production. Some workers have suggested possibility of capsicum hybrids seed production under protected cultivation. Jarlan *et al.* (30) assessed the possibilities of using syrphid fly (*Eristalis tenax* L.) as a pollinator of sweet pepper under glass house conditions. Higher seed set was observed in insect pollinated flowers as compared to fruits from unvisited flowers and the duration of visits also increased the seed set significantly.

CONCLUSION

It took a long time from discovery of male sterility system to its utilization for hybrid seed production. The male sterile mutants have been commercially used in hybrid seed production of hot pepper despite the need to identify and rogue out the sterile plants from the female line. Because of the lack of tightly linked marker systems, this process is carried out after flowering which is the biggest drawback in the use of genetic male sterility. Csillary (10) proposed accumulation of several male sterility genes to produce a line that segregates into a large proportion of male sterile plants containing two sterility genes viz., *ms*-3 and *ms*-8. Shifriss and Pilowsky (72) proposed that the F_2 derived from two near isogenic lines (NILs) differing for male sterility genes (*ms*-1*ms*-1*Ms*-2*Ms*-2 × *Ms*-1 *Ms*-1 *ms*-2 *ms*-2) should segregate either in 3:1 or 9:7 (fertile: sterile) ratio. However, they did not clearly indicate as to how crossing would be effected between these two male sterile lines. Daskalov and Mihailov (13, 14) proposed a technique for hybrid seed production based on male sterility (genetic or cytoplasmic), combined with a lethal gene whose action may be inhibited by a specific chemical. The aim of using such gene was elimination of the selfed plants. The cytoplasmic male sterility system is free from this disadvantage and it supplies a population comprised completely of male sterile plants.

A cytoplasmic male sterility (CMS) system in pepper is characterized by a known restorer gene required to overcome the sterility in the F_1 progeny and ensure fruit setting in the commercial crop. The CMS is better known as CGMS (cytoplasmic genetic male sterility) system and consists of three lines, namely; male sterile (female) ‘A’-line, male fertile ‘B’-line and fertility restorer ‘R’-line. This is the predominant method of pollination control for hybrid seed production in hot pepper. There are two major limitations in use of CGMS system in hybrid seed production of sweet pepper.

Firstly, restorer genes are very rare among sweet pepper lines. Moreover, transfer of male sterility system from hot pepper to sweet pepper requires crossing with hot pepper which results in inadvertent movement of genes for pungency into sweet pepper which is not eliminated even after several backcrosses. Otherwise no agronomic differences were found in hot pepper when F_1 hybrids containing sterile cytoplasm were compared with their isogenic F_1 hybrids containing fertile cytoplasm. To harvest higher yield of hybrid seed, sweet pepper lines can be used for producing hybrid seed of hot pepper hybrids.

The chili hybrids, CH-1 and CH-3 released by Punjab Agricultural University, Ludhiana, India based on GMS system are very popular in North India. Dhungel *et al.* (17) recorded maximum hybrid seed yield of chilies by planting in a ratio of 2:1 (female: male) followed by spray of 1% aqueous solution of urea at 30 and 40 days after transplanting. Similarly, two hot pepper hybrids viz., Arka Harita and Arka Shweta based on CGMS system, released by Indian Horticultural Research Institute, Bangalore are gaining popularity in South India and among several hybrids of private seed companies.

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