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Title: A New Male Sterile Mutant in Cucumber (Cucumis sativus L.): Inheritance, Genetic Relationship to Other Mutants and Sex Types, and Response to Chemical and Environmental Factors.

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Dr. A. C. Gabert and

[redacted]

Dr. J. R. Baggett

A new male sterile (MS) mutant was found in 1988 in an inbred line of cucumber (Cucumis sativus L.) in the greenhouse of Sunseeds Company. The mutant is characterized by normal corolla in both male and female flowers, normal fertility in the female, and absence of pollen in otherwise normal-appearing male flowers. The inheritance of this pollen sterile (PS) mutant was studied in F_2 segregates derived from crosses between PS and male fertile (MF) plants, and by mating PS plants with MF sib plants of the heterozygous genotype (PS ps). Chi-square tests indicated that the PS character controlled by a single recessive gene, assigned the tentative

designation ps.

Six reciprocal crosses made between heterozygous PS and heterozygous apetalous (Ap) type produced a total 370 fertile : 0 PS F_1 plants, indicating that there is no allelic relationship between PS and Ap.

F_1 generations from each of gynoecious PS, and monoecious PS crossed with monoecious, gynoecious (silver ion treated) and hermaphroditic pollen parents had no PS plants. By variance analysis and contrasting the individual effects of three sex types, Gy, M and H, on PS, it was found that sex types do not significantly influence PS levels in F_2 families even though PS occurs in male flowers (a sexual character).

It was not possible to effect changes in the expression of PS by application of cytokinin, indole acetic acid (IAA) or gibberellin (GA_3), and there were likewise no changes in response to temperature and fertilizer treatment. Unlike gynoecy, which is responsive to some external factors, PS is a stable characteristic.

MS-1, a previously reported male sterile, was studied in different environments. It was found that MS-1 is heritable but significantly affected by environmental factors. The study indicated that MS-1 is a strong female sterile and semi-male sterile type. The male flower buds of MS-1 plants are able to open normally after the 10th to 15th node under greenhouse conditions. All staminate flower buds aborted in the field. The result of an allelism test indicated that MS-1 is not allelic to Ap.

A NEW MALE STERILE MUTANT IN CUCUMBER (CUCUMIS SATIVUS L.): INHERITANCE, GENETIC RELATIONSHIP TO OTHER MUTANTS AND SEX TYPES, AND RESPONSE TO CHEMICAL AND ENVIRONMENTAL FACTORS

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Qi Zhang

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APPROVED:

Professor of Horticulture in Charge of Major

Professor of Horticulture in Charge of Major

Head of Department of Horticulture

Dean of Graduate School

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Typed by Qi Zhang

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A NEW MALE STERILE MUTANT IN CUCUMBER (*CUCUMIS SATIVUS* L.): INHERITANCE, GENETIC RELATIONSHIP TO OTHER MUTANTS AND SEX TYPES, AND RESPONSE TO CHEMICAL AND ENVIRONMENTAL FACTORS

INTRODUCTION

Male sterility has been of practical importance in cucumber (*Cucumis sativus* L.) breeding because it can facilitate F_1 hybrid seed production without hand pollination. The several forms of male sterility that have been available are a.) gynoecious flowering (Gy); b.) an apetalous sterile mutant (Ap); c.) male sterile-1 (MS-1); d.) male sterile-2 (MS-2); e.) a closed flower type (cl); and f.) gingko leafed mutation (gi). However, only gynoecious flowering (a form of male sterility, but also a sex type) has been used in cucumber breeding because it can be easily maintained by selfing with chemically induced pollen. In the Ap mutant, the corolla is lacking in both male and female flowers. Because insects are not attracted, it is impossible to economically produce large amounts of commercial hybrid seed with this mutant. Due to semi-sterility of both male and female flowers, the MS-1 mutant has not been used in hybrid seed production. However, from observations made by the author it appears that MS-1 may have potential value for research and utilization because male flowers can open normally in certain environments. It

is anticipated that the fertility of the MS-1 female flowers may also be improved by some environments. MS-2 can be used as a form of "AB" line in seed production. However, the utilization of MS-2 is uneconomical and therefore its development has been impeded. The closed flower type and gingko leafed mutant have little breeding value because the former has closed male and female flowers, and the latter has abnormal plants.

In the Summer of 1988, a pollen aborted MS plant was found in an inbred line of cucumber. In this mutant, the corolla of both male and female flowers is normal and female flowers are fertile. The male flowers are generally normal in appearance but produce no pollen. The new mutant type was designated as "pollen sterile" (PS) and a prelimanary designation ps was assigned to the controlling gene. After the PS plant was found, five phases of study were undertaken. Study I addressed the inheritance of the PS mutant. Its mode of inheritance was determined by testing segregation ratios from progenies of selfed heterozygous plants (PS ps), and from sib-mating PS (ps ps) with fertile heterozygous plants (PS ps). In Study II the allelic relationship of PS to Ap and MS-1 mutants was determined in F_1 segregates from crosses of Ap plants (apap) and PS heterozygous plants (Psps) or from crosses of PS heterozygous plants (Psps) and MS-1 (ms-1ms-1) with partially fertile pollen). The MS-2, closed flower type and

gingko leafed mutant were not available and therefore their genetic relationship to PS could not be investigated in Study II. Due to the female sterility in MS-1, very few seeds were derived from crosses between MS-1 plants and heterozygous PS plants. Therefore, no information about the genetic relationship of MS-1 and PS could be obtained. In the search for suitable maintainer lines for PS, it was assumed that PS 1.) may be allelic to certain sex types, or other MS or semi-MS types; 2.) may be maintained by a certain sex type. To test these hypotheses, monoecious and gynoecious PS plants (treated with $Ag(S_2O_3)_2$) were selected and mated with monoecious, gynoecious and hermaphroditic fertile plants. Data from the F_2 progenies of these crosses were obtained in Study III. In view of the fact that male sterility based on sex expression in cucumber is very sensitive to chemical, hormonal and environmental factors, it was hypothesized that PS could be changed from sterile to fertile so that it can be maintained by selfing. Therefore, in Studies IV and V, the response of PS to some chemical, hormonal and environmental factors was investigated. Based on the Author's observation on MS-1, the male flower buds of MS-1 could open normally under greenhouse condition and the opened male flowers were partially fertile. However the male flower buds of MS-1 aborted in the field. Study VI was added to elucidate the behavior of the MS-1 mutant and determine the allelic relationship of MS-1 to the Ap mutant.

In summary, the objectives of the studies reported in this thesis were: to determine the inheritance of the PS mutant; to determine whether PS is allelic with other available MS types; and to determine if the PS mutant could be modified to produce pollen by combination with sex types, or by chemical treatment and environment modification.

LITERATURE REVIEW

Male Sterility in Cucumber

Six forms of male sterility in cucumber have been reported: 1) gynoecious flowering (Gy) (Robinson and Munger, 1976; Lower and Edwards, 1986), 2) an apetalous sterile mutant (Ap) (Grimbly,1980; Pierce, 1990), 3) an aborted male flower bud type or male sterile-1 (MS-1) (Shifriss, 1950), 4) a pleiotropic pollen aborted mutant or male sterile-2 (MS-2) (Whelan, 1973, 1974), 5) the closed flower type (GF) (Groff and Odland, 1963), 6) gingko leafed mutation (GI) (John and Wilson, 1952).

Gynoecious Flowering Gynoecious plants bear only pistillate flowers that appear, mostly solitary, in the leaf axillas of the main axis and the side branches (Kubicki,1965, 1969a, b,c,d,1972, 1974,1980; Robinson and Munger, 1976; Galun, 1961; Nandgaonker and Baker, 1981; Rosa, 1928). The gynoecious character, with other sexual types such as monoecious, androecious, and hermaphroditic, is under the control of at least three loci (m^+-m, F^+-F, a^+-a) (Robinson, 1969; Robinson and Munger, 1976; Shifriss and Galun, 1946; Shifriss, 1961; Lower and Edwards, 1986). The genotype $\underline{m^+m^+}$ $\underline{FF}$ $\underline{--}$ is typically gynoecious. Usually, this

type is considered to be a form of sex expression rather than male sterility because it always occurs with sex segregation (Lower and Edwards, 1986). The details about gynoecious expression have been previously described in the Author's Master thesis (1989) and will not be repeated here.

Apetalous Mutant. An unusual Ap mutant was first obtained by Hutchins (1936, 1940), and successively reported by Shifriss (1950) and Grimbly (1980). The most conspicuous features of this mutant are leafy flowers and leafy tendrils and a radical change in the position of the ovary. The flowers are hypogynous and the ovary is superior, as opposed to the typical Cucurbitaceae characteristics of epigynous flowers and inferior ovaries (Shifriss, 1950). The stems and leaves are normal, but the flowers and fruits are abnormal. The corolla of both staminate and pistillate flowers are reduced to a whorl of five green, reflexed appendages identical to sepals. The staminate flowers never mature and usually fall off while they are still small. The pistillate flowers develop to their usual size and, if left unpollinated, produce parthenocarpic fruit. Since all the sepals and petals are reflexed from a very young stage, the immature stigma is exposed and it is difficult to judge when it is receptive to pollen. Therefore, pollinations can be repeated on several successive days when the ovary and stigma appear to be receptive. When the mutant was crossed

with the wild type, all of the F_1 plants were normal and the F_2 families segregated into 3 normal : 1 ap plant. It was shown that the character is controlled by a single recessive allele which was named ap by Grimbly (1980).

Male Sterile-1 The most distinguishing external characteristic of this mutation is the failure of anthesis in the staminate flowers. In contrast, the pistillate flowers appear entirely normal in regard to anthesis. Pollen sterility varies from 30 to 90 per cent. Crosses between sterile and fertile plants result in fruits which contain zero to several good seeds per fruit. It seems that sterility involve both sexes but the failure of anthesis is confined to the staminate flowers. The frequency of occurrence of the MS mutants may be as high as 1:10000 and the same locus was mutated in the two cultivars, Black Diamond and A&C (Shifriss, 1950). Repeated backcrosses for eight generations failed to break the complete linkage between male and female sterility in Shifriss's studies. He concluded that the occurrence of this character is due to a recessive pleiotropic gene, ms-1.

Male Sterile-2 This MS mutant has been reported by Barnes (1960), Whelan (1972, 1974), Dax-Fuchs et al. (1978), Miller and Quisenberry (1978), and Robinson (1978). It is characterized by abortion of the staminate flowers. In rare

instances when the flowers matured, no pollen was present (Whelan, 1972). Barnes (1960) reported that staminate flower buds on the MS plants usually aborted when they were about 3/4 grown and pistillate flowers usually appeared 2 to 3 days later than those on fertile plants in the same line. Early in the flowering period, male sterile plants produced approximately the same number of pistillate flowers as fertile plants, but later there was usually an increase in the number of pistillate flowers on male sterile plants.

The preliminary studies of MS-2 indicated that male sterility was due to both cytoplasmic and nuclear factors. Complete male sterility appears to involve one dominant and one recessive gene (Barnes, 1960). Whelan (1972) reported that his MS mutant from induction by a cobalt-60 gamma source was due to a single recessive gene which was designated ms-2. Cytological examination revealed that pollen abortion did not occur until after the mitotic division of the pollen grain nucleus. At times, following this stage of development, the pollen grains and tissues of the anther degenerated and the staminate flowers aborted. Since male sterility is not expressed until late in development, a linked seedling marker gene would be useful in selection. Linkage studies of genes controlling MS-2 and other genes were carried out and reported by Whelan (1974). The results from Whelan's study showed that repulsion linkage occurs between ms-2 and the traits glabrate seedling

(glb) and determinate (de).

Closed Flower Mutant This variant (CF) is unique in that the flowers, both staminate and pistillate, fail to open at maturity. The calyx and corolla attain full size and in pistillate flowers the ovary develops to normal size. Pigmentation of the corolla approaches the intensity of the bright yellow color characteristic of normal cucumber flowers and, in the bud stage, the flowers do not appear abnormal. The closed-flower variant is genetically male sterile. Pollen grains are very scarce and are not viable when observed microscopically or germinated on nutrient agar media (Groff and Odland, 1963). The mutant is conditioned by the double recessive, clcl. Pollen sterility is considered to be is a pleiotropic effect of gene cl (Groff and Odland, 1963; Robinson and Munger, 1976).

Gingko Leafed Mutant John and Wilson (1952) reported that all of the parts of the true leaves in this (GI) mutant are much reduced except the veins which exceed the blades by an appreciable length. The leaves are scabrous, usually bipartite, rather brittle, and resemble gingko leaves to a certain extent. Growth is slower than that of normal plants during all stages. Flowers of both sexes appear very late and are completely sterile through the season. Number of flowers of both sexes is approximately normal. The ovary is

shorter and thicker than normal. The stigma is about normal size and the petals and sepals of the flowers of both sexes are nearly always reduced to the vascular tissue. The veins of the petals and sepals appear like tufts of very thick hairs which are covered with a downy pubescence. The tendrils are more conspicuous than in normal plants. John and Wilson's study indicated that this mutant is controlled by a single Mendelian recessive factor.

Response of the Male Sterility in Cucumber to Hormonal, Chemical and Environmental Factors

Response of the Gynoecious Type to Hormonal and Chemical factors

Endogenous and exogenous auxins as well as some other chemicals are associated with female flowering tendency of cucumber plants (Rudich et al. 1972; Gunlan et al. 1959, 1965; Ito and Saito, 1956; Tolla and Peterson, 1979). Ito and Saito (1957) reported that female flower formation of cucumber is hastened by indoleacetic acid (IAA), naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D). NAA was reported to be the most effective (Ito and Saito, 1956; Trebitsh et al, 1987). Ethylene in plant tissue enhances gynoecious flowering and reduces male flowering (Beyer, 1972, 1979; Iwahori et al. 1970; Xu, 1983 Iezzoni and Peterson, 1981; MaKus et al, 1975). Takahashi and Jaffe

(1984) concluded that ethylene is a major factor regulating feminization and that exogenous auxin promotes pistillate flower formation through its stimulation of ethylene production. Conversely, gibberellin (GA), aminoethoxyvinylglycine (AVG) and silver ions (Ag^+) can promote staminate flower development on gynoecious plants (Peterson, 1960; Fuchs et al, 1977; Staub and Crubaugh, 1987; More and Munger, 1986; Den Nijs and Visser, 1980; Hunsperger et al, 1983; Hayashi et al, 1971; Lower, 1978; Owens et al, 1980; Pike and Peterson, 1969).

Environmental Factors Change of female flowering of gynoecious plants with seasonal change was reported by Edmond (1931). Summer grown gynoecious plants have more staminate flowers and winter conditions result in more pistillate flowers. The GA-induced staminate buds on gynoecious plants do not abort in the summer plantings, while there is a high rate of abortion among the natural pistillate buds (Dax-Fuchs et al. 1978). The effect of seasonal change on the gynoecious character in cucumber could be attributed to the complex influence of temperature, light and other factors (Cantliffe, 1981; Ito and saito, 1953, 1957, 1958; Atsmon, 1968; Takahashi et al. 1983; Nienhuis et al. 1980; Della Vecchia and Peterson, 1982). Tiedjens (1928) found that pistillate flowering is favored by low light intensity and short photoperiod. An interaction of short days and cool nights favors formation of more

pistillate flowers (Ito and Saito, 1957, 1958; Ito and Kato, 1953; Della et al, 1982; Odland and Groff, 1962). However, Lower et al. (1975) found that photoperiod and temperature had no influence on flowering of a completely gynoecious cultivar. In regard to cultural factors, it was reported that pistillate flower formation is promoted by conditions which reduce the plant growth rate. More staminate flowers form under conditions that favor rapid growth (Takahashi and Suge, 1980; Atsmon, 1968; Ito and Saito, 1958). Ito and Saito observed that the first female flower differentiates on a lower node after stem pinching and lower nitrogen application (Ito et al. 1954, 1956, 1958). Inadequate fertilization does not affect the ratio of staminate to pistillate flowers, but sand culture can produce changes in it (Staub and Crubaugh, 1987).

Response of Other Male Steriles to Environmental Factors

It was reported that MS-2 plants in muskmelon are slightly retarded in growth rate and appear to be more sensitive to adverse conditions such as insufficient light during winter (Bohn and Principe, 1964). The plima spontaneous MS mutant in Brassica napus L. is sensitive to temperature. This mutant can be divided into three groups according to its sensitivity to temperature 1) high temperature forms (62.9%), 2) low temperature forms (28.6%), and 3) stable forms (8.5%) (Fu et al. 1990). Similarly, male floral bud

abortion in the MS-1 and MS-2 mutants of cucumber increases in winter planting. There are seasonal effects on the extent of spontaneous floral bud abortion and on the response to chemical treatments. Flower bud abortion could have various genetic and nongenetic interpretations. It was proposed that both sex expression and floral bud abortion are inherited identically, and are influenced by modifying and environmental factors (Miller and Quisenberry, 1978). There have been no reports about the response of the apetalous type, closed flower mutant, or gingko mutant to environmental factors.

Maintenance of Male Sterile Mutants in Cucumber

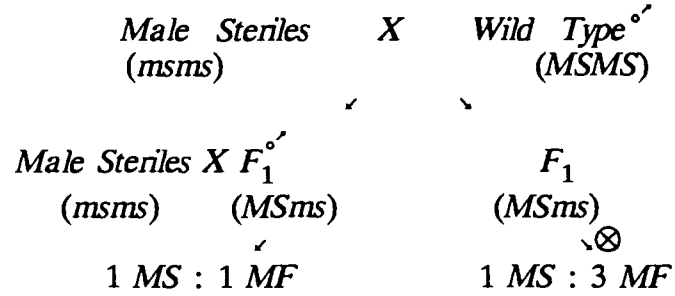
Maintenance of Gynoeciousness By Selfing or Sib-mating After gynoecious plants are obtained from a natural cultivar population or progenies of hybrids, they can be treated with GA, $AgNO_3$ or $Ag(S_2O_3)_2$ and selfed with pollen from the induced staminate flowers (Beyer, 1979; Staub and Crubaugh, 1987; Lower and Edwards, 1986; Briggs and Knowles, 1977; Veen, 1983). Gynoecious plants can also be maintained by sib-mating with predominantly gynoecious plants, but the recovery of gynoecious plants is lower than by selfing (Author's masters thesis, 1989). Roguing of monoecious segregates during the increase of gynoecious lines is important both for the development of stable inbreds and the

maintenance of gynoecious purity. Artificially stressing gynoecious lines has also been suggested as a means of more stringent selection for stability. To retain the stability of gynoecious lines, pollination must be done under controlled conditions. Pistillate and induced staminate flower buds must be tied prior to opening in fields. The most convenient way to produce stable gynoecious lines is to plant gynoecious materials under screen or in a greenhouse where the plants are treated with chemicals for induction of male flowers and pollinated by bees or by hand (Lower and Edwards, 1986; Tan and Chen, 1980).

Maintenance of Gynoeciousness by Crossing with Other Sex Types Although gynoecious lines can be maintaining by either selfing with induced male flowers or by sib-mating with male flowers from predominant gynoecious plants, chemical treatment is a cumbersome and expensive factor. Kubicki (1965), and Lower and Edwards (1986) suggested a possibility of producing female lines by utilizing hermaphrodite cucumber as pollinators. Hermaphrodite cucumbers, of an m/m F/F a^+/a^+ constitution, have been developed from the segregation of F which controls the degree of female tendency, m which governs the formation of bisexual flowers, and a which intensifies staminate tendency. Similar to gynoecious cucumbers, the hermaphrodites are homozygous in respect to allele F and can easily be reproduced through their development of bisexual

flowers. The F_1 generation ($\underline{m^+/m}$, $\underline{F/F}$, $\underline{-/-}$), derived from mating gynoecious lines ($\underline{m^+/m^+}$, $\underline{F/F}$, $\underline{-/-}$) with hermaphrodites ($\underline{m/m}$, $\underline{F/F}$, $\underline{-/-}$), is always completely gynoecious because of homozygosity for $\underline{F}$, and may be utilized as a seed parent in crosses with monoecious lines (Kubicki, 1965; Lower and Edwards, 1986). Since the F locus is only partially dominant in some backgrounds, this hybrid ($\underline{m^+/m}$, $\underline{F/F}$) is thought to result in a more stable gynoecious performance. Reproduction of gynoecious lines with andromonoecious plants (plants with male and bisexual flowers) as pollinators can produce the same result as above (Kubicki, 1974).

Maintenance of Other Male Steriles by Crossing Following Backcrossing or Sib-mating or Selfing Ap, MS-1, and CF mutants can be reproduced by crossing with the wild type after selfing heterozygous plants or backcrossing with heterozygous plants (Grimbly, 1980; Whelan, 1974; Shifriss, 1950; Groff and Odland, 1963). These male steriles are inherited as monogenic recessives. Therefore, only 50% MS plants can be expected when a male sterile is crossed to a heterozygous fertile plant and only 25% of the progeny from selfing a heterozygote are male sterile (Rao et al., 1990; Schwartz, 1951). A one-cycle scheme for maintaining these male steriles is shown as follows:



(Schwardz, 1951; Tan and Chen, 1980). If maintenance of the MS materials is continued by crossing MS segregates (msms or clcl) with fertile ones (Msms or Clcl) in the same lines, there should be a constant ratio of 1 MS plant (msms or clcl) : 1 MF plant (Msms or Clcl) in each successive generation. The maintenance procedure (for Ap and MS-1) is illustrated as follows:

Season	Procedure
1	Each of the MF parents (MsMs) are crossed by bees to MS plants in a parent serving as the source of male sterility. All of the F_1 plants are heterozygous (Msms) and 50% of their genes are from the MS parent.
2	The F_1 plants (Msms) are selfed pollinated.
3	The F_2 and F_1 populations are grown in the same season. Twenty-five percent of the F_2 plants will be male sterile. Pollen from the F_1 plants is used to

pollinate the appropriate MS plants. Seeds are only harvested in bulk from the MS plants to form the population which contains 50% MS (msms) and 50% MF (Msms).

4 The population is random pollinated by bees in isolation and seeds from the MS plants are harvested in bulk.

5 This generation, and additional maintenance generations, are done in the same manner as season 4. The segregation ratio in the population will be maintained as 1 Msms : 1 msms.

This system is usually called an AB system because it involves a MS line (A line) as well as maintainer line (B line) (Tan and Chen, 1980).

Maintenance of Male Steriles with Vegetative Propagation

The 50% MS plants from a segregating population can not be identified before flowering. It is uneconomical to discard half of the population at flowering and it takes a lot of time to determine the sterility or fertility of each plant in the population. One way to avoid this expense and time is to raise a clonal population of male steriles by vegetative propagation, which is relatively simple in cucumber (Rao et al. 1990). Two vegetative approaches have been used: 1)

regenerating plants by cell, tissue or organ culture in vitro (Gallun et al, 1963); and 2) rooting of cuttings taken from branches of cucumber plants (Rao et al. 1990; Nadolska-Orczyk and Malepszy, 1989; Whner and Lock, 1981). Hypocotyl, cotyledon, leaf, flower bud and axillary bud explants have been used for plant regeneration in vitro and have successfully produced plants (Hanhley and Chambliss, 1979). Cuttings have been taken from MS plants by the author since 1988 and it is observed that 100% success can be achieved if the age of the source plants, the treatment of cuttings with chemicals such as Rootone, and the environment for maintaining the cuttings is appropriate. Although tissue culture systems can maintain MS materials very well, there still are some concerns; 1) complicated regeneration procedures, 2) high cost, and 3) special environmental requirements. Vegetative propagation with branches of plants seems to be easier and more practical, but needs many mist chambers and requires a lot of time to manage the cuttings if large populations of male steriles are being reproduced.

Utilization of Genetic Male Steriles in Cucumber Breeding

Male sterility is of practical importance in cucumber breeding because it can facilitate (a) the production of

hybrid varieties and (b) inter- and intraspecific hybridization and back-crossing programs for the introduction of genetic variation into crop varieties without genetic emasculation and hand pollinations (Rao et al. 1990).

F_1 Hybrid Seed Production. Most commercial hybrids of cucumbers have a gynoecious line as the female parent. A major advantage of gynoecious cucumbers is the potential for an earlier harvest which is important in assuring a premium value for the crop. Even as the harvesting season progresses, uniformity in sex expression is important in maintaining a uniform production. A problem in commercial cucumber production as well as in commercial hybrid seed production, is that many of the gynoecious parent lines and their hybrids produce varying amounts of staminate flowers on the lower nodes. Therefore, the off-types (predominant female or monoecious plants) in female rows have to be rogued out at the beginning of anthesis during hybrid seed production. If the gynoecious lines used as female parents are very stable, the plants can be sprayed with ethephon to intensify female flowering tendency and reduce the possibility of other sex types occurring (Saito and Ito, 1963; Lower and Edwards, 1986; Rudich, et al. 1972; Hume and Lovell, 1983). Single-cross hybrids involving gynoecious (Gy) x hermaphrodite (H) parents have been developed to maximize pistillate expression in hybrids used for pickling

cucumber production (Pike and Mulkey, 1971; Mulkey and Pike, 1972; Staub and Grubaugh, 1978). Alternatively, an acceptable level of female flowering expression occurs in 3-way crosses utilizing (Gy x H) F_1 seed parents and monoecious (M) pollen parents (Pike, 1971). Unfortunately, hermaphrodite cucumbers are characterized by perigynous flowers, which overrides other fruit characteristics and gives rise to rounded rather than elongated fruit. In the heterozygous hybrid, this factor is recessive, allowing elongated fruit shape and the expression of underlying quality factors. Since the expression of fruit characteristics is obscured in a hermaphroditic background, evaluation of hermaphroditic inbreds for the characteristics they will confer in hybrid combinations is difficult (Lower and Edwards, 1986). However, the U.S.Department of Agriculture cucumber breeding program has made use of the hermaphroditic (H) characteristics in cucumber in an attempt to improve gynoecious sex stability. It was concluded that there are no differences in fruit quality or yield among Gy x Gy and Gy x H hybrids produced by genetically similar inbred lines (Staub et al. 1986). Staub and Crubaugh (1987) suggested that the hermaphrodite character may be potentially useful in stabilizing the gynoecious sex expression under environmental stress conditions.

Although the CF character is of genetic interest, it does not appear to be promising as plant improvement

materials and is impossible to use in hybrid seed production due to the failure of the petals to unfold which inhibits insect pollinations (Groff and Odland, 1963). None of the Ap, MS-1 and GI mutants can be used in hybrid seed production; 1.) there are no normal corolla on female flowers of Ap sterile plants, which would attract bees or other insects to visit (Grimbly, 1980); and 2.) there not only is male sterility, but also a high level of female sterility in both MS-1 and GI mutants so that seed production per plant is very low and the seed production cost is very high (Shifriss,1950, and John and Wilson, 1952). The MS-2 type can possibly be used in the AB system to produce commercial quantities of hybrid seed, if male fertile segregates are eliminated in the female parent. However, the cost of hand roguing is too time-consuming and expensive for economical production of hybrid seed (Fehr, 1983). In contrast to cytoplasmic male sterility (CMS), genetic male sterility (GMS) such as in the MS-2 mutant has great advantages in breeding: 1) no special care is needed in selecting male lines and no special cytoplasms are needed to avoid association with negative agronomic performance or disease susceptibility which could be transferred to the hybrid; 2) no special requirement for an affective restorer gene in the male parent in order to achieve complete fertility in the commercial production field, and 3) GMS is more stable (Rao et al. 1990; Ruebenbauer, 1963).

In spite of the above-mentioned advantages of GMS, there are no practical methods available for obtaining pure stands of GMS plants in hybrid seed production fields. However, several approaches to produce hybrids with the MS-2 sterile can be considered: 1. Maintaining the MS plants by vegetative propagation (as mentioned above); and 2. using marker or pleiotropic characteristics to identify MS plants in seed parents.

Possible Applications in Population Improvement There have been extensive reports about the utilization of GMS in population improvement in many species. However, there has been no literature describing uses of the gynoecious type. Population improvement is an important long-term objective of most plant breeding programs for cross-pollinated species. Many breeding schemes have been outlined using recessive male steriles in recurrent selection programs (Sorrels and Fritz, 1982). Populations to be used for recurrent selection are developed with MF parents, that possess the desired characteristics, and an acceptable MS parent. One consideration in developing the population is a percentage of genes from the MS parent, other than the MS (ms) allele, that is acceptable. A percentage less than 50% requires backcrossing to the MF parents. A second consideration is the number of generations of recombination that are to be conducted before selection begins. Recombination is easily accomplished after the MS gene has

been incorporated into the population, but each generation of recombination takes additional time (Fehr, 1983). The basic population can be synthesized in several ways (e.g., from two-way crosses, backcrosses, and diallel crosses).

Several methods of recurrent selection can be used with GMS to improve a population. These methods depends on selection objectives, heritability of the characters under consideration, selection unit, and availability of greenhouse or winter nursery facilities. One principle applies to all methods: MS plants must be tagged at the time of pollination if they are indistinguishable from MF ones at maturity. The recurrent selection methods that have been used with GMS are: recurrent phenotypic selection (mass selection), recurrent selection among half-sib families, recurrent selection among and within half-sib families, recurrent selection among full-sib families, selection among selfed progeny, etc.. If two different populations in commercial production are to be improved simultaneously, reciprocal half-sib selection and reciprocal full-sib selection can be used (Brim and Stuber, 1973; Frhr, 1983).

Other Possible Uses Apart from providing a female parent for hybrid production and breeding superior populations, GMS can be employed in the incorporation of desirable genes such as those for disease resistance or better-quality proteins from other genera or species. Alternatively, wild types with some desirable traits can be converted to useful types using GMS (Rao *et al.* 1990).

MATERIALS AND METHODS

A large number of inbred lines were observed for sex expression and fertility at the SUNSEEDS Company in Brooks, Oregon. In 1988, a single new MS plant was found in one of these inbred lines. The corolla of both staminate and pistillate flowers of this mutant is normal and pistillate flowers are fertile. The plants produce a few aborted staminate flower buds only after they are loaded with fruit and/or they are physiologically mature. The staminate flowers are generally normal in appearance but have rudimentary anthers, and produce no pollen (Figure 1 and 2). The mutant under study has been designated as PS (for pollen sterility). To determine genetic mechanism of the new MS, its relation to other known MS mutants and sex types, its response to hormone and environment, and its potential use in cucumber hybrid production, five studies were conducted from 1988 through 1992.

Along with three other MS types; Gy, Ap, MS-1) and MS-2, PS was used as a seed parent. The three sex types, Gy, monoecious (M) and hermaphrodite (H) or perfect flower type, were used as maintainer parents in the above experiments (Table 1). All seed materials were provided by Dr. August C. Gabert.These experiments were carried out in greenhouses and

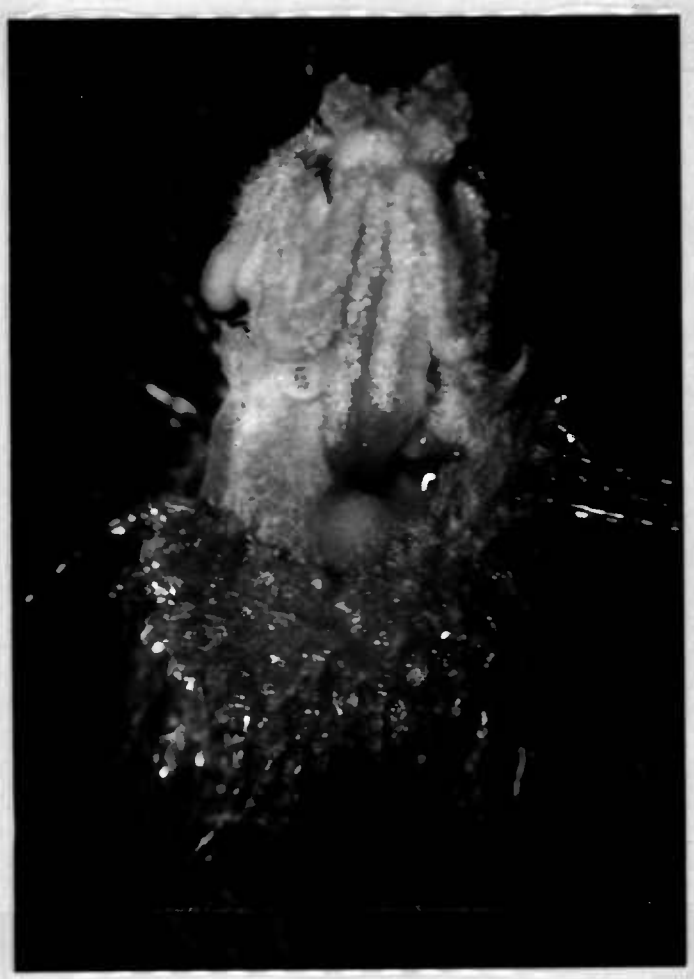


Figure 1. Anthers of a normal cucumber with pollen visible.

Figure 2. Anthers of PS mutant showing the absence of pollen.

Table 1. Pedigree, flowering type and source of male sterile mutants and related parents

Code Name[z]	Pedigree	Type[y]	Source
MS Mutants			
Ap	23B- x 705	Seg. Gy.	Sunseeds
MS-1	22676-6	Monoecious	Sunseeds
PS	23B- X 26	Seg. Gy.	Sunseeds
Pollen Parents			
Gy	23B-116	Gynoecious	Sunseeds
M	23B-113	Monoecious	Sunseeds
H	MSU669H	Hermaphrodite	Sunseeds

[z] Ap, MS-1, and PS = Apetalous, male sterile-1 and pollen sterile mutant, respectively. Gy, M and H = gynoecious, monoecious and Hermaphrodite sex type, respectively.

[y] Seg. Gy.: segregating gynoecious line.

fields of Sunseeds Company at Brooks, Oregon, and greenhouses at Oregon State University (OSU). General field cultural methods were similar to those in commercial use in the area. Culture and conditions in the Sunseeds and OSU greenhouses were similar.

Study I. Inheritance of PS Mutant

When the new PS plant was found, it was sib-mated (SB) with a male fertile (MF) plant of the same inbred line. Fourteen seeds (SB_1) from the PS plant were planted in the greenhouse in the spring of 1989. There were no PS plants in that generation. Therefore, each plant was selfed and seeds from the 14 plants were bulked. Thirty-five seeds from this bulk population produced seven PS plants, which resembled the original PS plant, and 23 MF plants. The PS segregates were sib-mated with several randomly selected MF segregates (MF_A, MF_B, MF_C ,....) and the MF segregates were selfed at the same time. However, only seven sib-mated PS plants and three MF plants MF_A, MF_B and MF_C) produced enough selfed seeds to test. Seeds were harvested separately from each fruit of each mating.

During the summer of 1989 to determine the genetic mechanism of PS, seven families from sib-mated PS plants and three from MF plants were grown in the greenhouse. Based on the fertility expression on the original PS plant and in F_2

families, MF and PS were considered to be qualitative variables so that no special experimental design was used. Forty seeds of each family were planted in seven pots as a plot, each pot (diameter 11 in. and height 9 in.) was filled with a special soil mix consisting of bark materials, soil, lime and fertilizers. Ten families were randomly arranged in a greenhouse with the same environmental conditions (temperature, light etc.). Plants were irrigated and fertilized with an automatic drip system. No supplemental lighting was used.

Plants were classified as PS or MF at anthesis according to the phenotype of anthers in the male flowers. The plants were classified as PS when they had open staminate flowers with aborted anthers on the first ten nodes. After the percentage of PS plants in F_2 populations and progenies of test crosses were obtained, the data were tested by Chi-square to determine whether PS character is controlled by a nuclear gene or cytoplasm-nuclear genes. Also, anthers were observed under a light microscope (20 and 40X) periodically for presence of pollen.

Study II. Genetic Relationship of PS to Ap and MS-1 Mutants

This study was designed to determine whether ps is allelic with apetalous mutant (ap) or male sterile-1 (ms-1). This experiment involved three steps: 1) getting F_1 plants heterozygous respectively for each male sterile mutant, ap and ps; 2) making reciprocal crosses by using male sterile plants, homozygous for ap or ps, as the female parent and male fertile plants that were heterozygous for ap or ps as the male parent; 3) determine if F_1 plants derived from step 2 were all fertile or segregated into 1 fertile:1 sterile plants. All F_1 progenies from these crosses would be fertile if the two genes in the cross were not allelic, but would segregate 1 fertile : 1 male sterile if they are allelic.

While making the crosses in step 1 during the fall of 1989, it was found that the sterility of the pistillate flowers of MS plants in the MS-1 mutant is much higher than that of staminate flowers. Only four F_1 seeds from 43 fruit from MS-1 (ms1ms1) x Fertile (MS1MS1) were obtained. Therefore, a procedure for testing the genetic relationship between PS and MS-1 was revised as c.) in Step 2 during the fall of 1990. PS, AP and MS-1 were carried through the complete test procedures, described as follows:

Step 1. a) *Ap(apap) X Fertile(ApAp)*

↓

F_1

(Apap)

b) *PS(psps) X Fertile(PsPs)*

↓

F_1

(Psps)

Step 2. a) *PS(psps) X F_1(Apap)*

Allelic F_1 (*Ap/ps*; *ap/ps*) 1 *MF* : 1 *MS*

Nonallelic F_1 (*Ap/Ap*; *ap/Ap*; *ps/Ps*) *All MF*

b) *AP(apap) X F_1(Psps)*

Allelic F_1 (*Ps/ap*; *ps/ap*) 1 *MF* : 1 *MS*

Nonallelic F_1 (*ap/Ap*; *Ps/Ps*; *Ps/ps*) *All MF*

c) *F_1(Psps) X MS-1(ms-1ms-1)*

Allelic F_1 (*Ps/ms-1*; *ps/ms-1*) 1 *MF* :1 *MS*

Nonallelic F_1 (*Ps/Ps*; *ps/Ps*; *ms-1/Ms-1*) *All MF*

Step 3. All F_1 progenies from Step 2 were planted in the OSU greenhouse during the spring of 1991. No special experimental design was used. When the two F_1 populations and their

reciprocals flowered, the type and number of male sterile plants in each population were recorded.

Study III. Relationship of PS Mutant to Sex Types

The relationship of the pollen sterile mutant to sex expression was investigated from the fall of 1989 through the spring of 1991. Initially, an AB line was planted in 70 pots in the greenhouse of Sunseeds during the fall of 1989. When the flower buds could be identified, gynoecious plants were tagged and sprayed with ($Ag(S_2O_3)_2$). MS plants were classified into two types: 1) monoecious pollen sterile plants (M-PS) which are monoecious plants with anther-aborted male flowers; and 2) gynoecious pollen sterile plants (Gy-PS) which are gynoecious plants and with anther-aborted sterile flowers after treatment with $Ag(S_2O_3)_2$. After the MS plants were tagged, they were moved to the OSU greenhouse and crossed with gynoecious (GY), monoecious (M) and hermaphrodite (H) plants, to give the following combinations:

1. Gy-PS x Gy
2. Gy-PS x M
3. Gy-PS x H
4. M-PS x Gy
5. M-PS x M
6. M-PS x H

At the same time, Ap plants were crossed with normal Gy, M, and H parents. Seeds from each cross were bulked and planted in the OSU greenhouse in the winter of 1990. The gynoecious F_1 plants were treated with $Ag(S_2O_3)_2$ and the ratio of Gy : (Gy + M + H) plants in each cross was recorded. The plants were also checked every day to see if there were any MS plants. After the Gy plants were differentiated from M plants and tagged in three F_1 populations, Ap x Gy, Ap x M and Ap x H, the gynoecious and monoecious plants in each of the three crosses were selfed and seed from each type of plant was bulked.

This experiment was based on the hypothesis that PS character would be inherited with one of three sex characters and that this could be determined in the F_2 If this relationship exists,, a PS line could be maintained by a specific sex type or may be used in combination with a sex character in hybrid seed production.

During the spring of 1991, F_2 populations from each cross were planted in the Sunseeds and OSU greenhouses (as two blocks) to investigate fertility segregation in different F_2 populations, which derived from different sexual crosses. Because the numbers of F_2 seeds from crosses M-PS x Gy, Gy-PS x H, Ap X M and Ap X Gy were not adequate for replication, an augmented randomized complete block (ARCB) experiment design (Federer, 1956) was used for

9 F_2 populations:

1. *(M-PS X M)-M*$^{\otimes}$
2. *(Gy-PS X M)-M*$^{\otimes}$
3. *(M-PS X Gy)-M*$^{\otimes}$
4. *(Gy-PS X Gy)-Gy*$^{\otimes}$
5. *(Gy-PS X H)-Gy*$^{\otimes}$
6. *(M-PS X H)-M*$^{\otimes}$
7. *(Ap X M)-M*$^{\otimes}$
8. *(Ap X Gy)-Gy*$^{\otimes}$
9. *(Ap X Gy)-M*$^{\otimes}$

A replication for each treatment consisted of 5 pots and each pot contained 10 plants. The gynoecious plants were treated with $Ag(S_2O_3)_2$ for the production of male flowers and the male sterile plants were observed at anthesis.

The experiment used $n_r = 5$ replicated and $n_{uj} = 4$ unreplicated F_2 populations where n_{uj} is the number of unreplicated populations within the jth block. The total number of F_2 populations used is a function of n_r, the number of homogeneous experimental units (N) which can be found for a given block size, and the number of blocks (replications) (r).

Each block was made up of the 5 replicated (n_r) and 4 unreplicated F_2 populations; thus, the total number of experimental units (plots) in the jth block is $n_r + n_{uj} = N_j$. The total number of experimental units is

$$N = \sum_{j=1}^{r} N_j = rn_r + \sum_{j=1}^{r} n_{uj} = 2(5)+4=14$$

The 4 unreplicated populations were divided between blocks by putting $n_{u1} = 2$ and $n_{u2} = 2$. When the experiment was laid

out, F_2 populations were randomized within blocks. The unrandomized layout is shown in Table 2.

Table 2. The unrandomized experimental layout for the PS-sex relationship in cucumber ARCB experiment showing F_2 populations within blocks.

F_2 Population	Block I	Block II
Replicated	1, 2, 4, 6, 9	1, 2, 4, 6, 9
Unreplicated	3, 8	5, 7

The linear model for the ARCB is $y_{ij} = u + a_i + B_j + e_{ij}$ where y_{ij} is an observation of the ith replicated or unreplicated populations in the jth block, u is the population mean, a_i is the effect of the jth block, e_{ij} is the experimental error for the ijth experimental unit, j = 1, 2 and r is the number of complete blocks.

Study IV. Effect of Hormone Treatment on the Flowering Behavior of PS Mutant

It was assumed that the pollen sterility in cucumber is related to sex expression and affected by some hormonal factors, and that it might be possible to find an external

hormonal factor that could change PS plants to MF. That would enable cucumber breeders to maintain male sterile lines by treating them with specific chemical or hormonal products.

To facilitate the control of environmental factors, the research was carried out in the greenhouses of Sunseeds Company in the summer of 1990. The hormones studied, Kinetin, IAA and GA_3, were divided into three levels:

Cytokinin (ppm)	0	500	1500
IAA (ppm)	0	100	200
GA_3 (mg/L)	0	1	10

These were applied as a factorial set of 27 combinations arranged in a completely randomized block (CRB) design with two blocks. The bulked experimental seed, from sib-mating MS plants with fertile sibs (MF) was divided into 54 parts and sprouted in petri dishes. The sprouted seeds were immersed in the assigned hormone solution for 24 hours. After the sprouted seeds were sown in pots, they were put in the greenhouse. Repeat applications were made by spraying seedlings with the respective hormone treatment when their cotyledons were completely unfolded, and again when the fourth leaf was completely unfolded. At the same time, all of the plots were sprayed with silver thiosulphate $[Ag(S_2O_3)_2]$ for the promotion of male flowers on the gynoecious plants.

The characteristics to be observed and recorded were the same as those in study 1.

Study V. Effect of Temperature and Fertilizer Level on Performance of PS Mutant

To estimate the effects of temperature and fertilizer on the new male sterile, this study used several levels of temperature, nitrogen(N), phosphorus(P) and potassium(K) as treatment factors. Bulked seed, from sib-mated male sterile plants, was used as experimental materials. Two levels were used for each of the factors:

Temperature	Fertilizer		
	N	P	K
75-90°F	0	0	0
55-75°F	173ppm	39ppm	280ppm

A split-plot design was used in two greenhouses(GH-A and GH-C). The minimum temperatures were set at 55°F in GH-A and 75°F in GH-C. The maximum temperature was 75° F and 90°F respectively. Eight factorial combinations of the three fertilizers were repeated in each of the two main plots (GH-A and GH-C). There were two replications of each treatment in each greenhouse. For each treatment, 50 seeds were

planted in 5 pots filled with sand (10 seeds per pot) which were fertilized with the respective fertilizer combination in water solution. All plants were sprayed with $Ag(S_2O_3)_2$ three times to promote staminate flower production on the gynoecious plants. The flowers at the first few nodes of each plant were noted for the expression of male sterility.

An additional observation, without any special experimental design, was made on the effect of Boron (B) on male sterile plants during the autumn and winter of 1989. One plant in each of 10 pots was sprayed with H_3BO_4 (1000ppm) once a week for two months. The new staminate flowers were recorded continuously until the plants were senescent.

Study VI. Elucidation of the Behavior of MS-1 and the Genetic Relationship of MS-1 to Ap

Because of the difference between Shifriss's (1950) description and my observation of the MS-1 mutant, progenies of 9 selfed individuals derived from a line, Sunseeds 22676-6, which carried the ms-1 gene, were planted in three environments for observation from the fall of 1990 through the spring of 1992. The three environments used were the OSU greenhouse (65°F-70°F), the Sunseeds Co. greenhouse (75°F-95°F), and the Sunseeds field plots.

During Study II, it was found that MS-1 plants have a

strong female sterile tendency and could not be used as seed parents to obtain heterozygous genotype. Because pollen of MS-1 is partially fertile, PS and Ap heterozygous plants were used as seed parents and were repeatly pollinated with the MS-1 pollen derived from opened male flowers in the greenhouses during the allelic test. The procedures were as follows:

Step 1. Same as in Study II.

Step 2. a)

$$F_1(Apap)\ X\ MS\text{-}1(ms\text{-}1ms\text{-}1)$$

Allelic F_1	Nonallelic F_1
$(Ap/ms\text{-}1;\ ap/ms\text{-}1)$	$(Ap/+;\ ap/+;\ +/ms\text{-}1)$
$1\ MF : 1\ MS$	$All\ MF$

b)

$$F_1(Psps)\ X\ MS\text{-}1(ms\text{-}1ms\text{-}1)$$

Allelic F_1	Nonallelic F_1
$(Ps/ms\text{-}1;\ ps/ms\text{-}1)$	$(Ps/+;\ ps/+;\ +/ms\text{-}1)$
$1\ MF : 1\ MS$	$All\ MF$

Step 3. Same as in Study II.

Statistical Analysis

Due to the small populations, the qualitative data from

Study I and II were analyzed with the Chi-square Test (X^2):

$$X^2=\sum_{K=0}^{\infty}(O-E)^2/E$$

where O and E = observed and expected percent male steriles, respectively.

In the other three studies (III, IV, V and VI), the data, which were in the form of percent of male sterile plants, were transformed by arcsine prior to analysis of variance (ANOVA) by using SAS PROC ANOVA and GLM procedures (Peterson, 1985; SAS Institute Inc., 1991).

Analysis of the ARCB data in study III was carried out by using SAS PROC GLM. The analysis of variance of replicated and unreplicated populations is shown in Table 3 and Table 4.

The objective of an ARCB experiment was to determine whether or not there were significant differences among populations and to characterize those differences using a mean separation method, e.g., LSD. Different standard errors were used to test for differences between means of replicated and unreplicated populations. The standard error

$$\hat{\sigma}_{r,r} = \sqrt{2\frac{M_e}{r}}$$

was used to test differences between means of replicated populations. The standard error

$$\hat{\sigma}_{u,\mu} = \sqrt{2M_e}$$

was used to test differences between means of unreplicated populations residing in the same block. The standard error

$$\hat{\sigma}_{u,\mu} = \sqrt{2M_e\left(1+\frac{1}{n_r}\right)}$$

was used to test differences between means of unreplicated populations residing in different blocks. The standard error

$$\hat{\sigma}_{r,\mu} = \sqrt{M_e\left(1+\frac{1}{r}+\frac{1}{n_r}+\frac{1}{rn_r}\right)}$$

was used to test differences between means of replicated and unreplicated F_2 populations.

These standard errors have been used to estimate the Least Significant Difference (LSD) statistics or other mean separation statistics. The LSD is

$$LSD_\alpha = t_{\alpha[df_e]}\hat{\sigma}_{i,j}$$

where $t_{a[dfe]}$ is critical value from the t-distribution with df_e degrees of freedom and a probability of a Type I error of a and $\hat{\sigma}_{i,j}$ is the appropriate standard error for the comparisons described above.

Mean separations were then tested by Fisher's Protected Least Significant Difference (FPLSD) with transformed means and differences indicated by letters on the data tables in

their natural form. When the F test in ANOVA for any set of factorial treatments was not significant, the mean separations were not further carried out. The main statistical programs used were SAS and Quattro Pro.

Table 3. Analysis of variance (ANOVA) (Type III SS) of the ARCB experiment design[z].

Source of Variation	DF	Sums of Squares	Mean Square
Block	$df_b = r-1$	$S_b = R(\beta/u,\alpha)$	M_b
F_2 Population	$df_f = n_r+\sum_{j=1}^{r} n_{uj}-1$	$S_f = R(\alpha/u,\beta)$	M_f
Between Replicated F_2 Populations	$df_{f_r} = n_r-1$	$S_{f_r} = R(\alpha_r/u,\beta)$	M_{f_r}
Between Unreplicated F_2 Populations	$df_{f_u} = \sum_{j=1}^{r} n_{uj}-1$	$S_{f_u} = R(\alpha_u/u,\beta)$	M_{f_u}
Replicated Versus Unreplicated F_2 populations	$df_{f_{ru}} = 1$	$S_{f_{ru}} = R(\alpha_{ru}/u,\beta)$	$M_{f_{ru}}$
Error	$df_e = (n_r-1)(r-1)$	S_e	M_e

[z] ARCB means Augmented Randomized Complete Block.

Table 4. Analysis of variance of the replicated families of the ARCB experiment design[z].

Source of Variation	DF	Sums of Squares	Mean Square
Family	$df_f = n_r-1$	S_{fr}	M_{fr}
Block	$df_b = r-1$	S_b	M_b
Error	$df_e = (n_r-1)(r-1)$	S_e	M_e

[z] ARCB means Augmented Randomized Complete Block.

EXPERIMENTAL RESULTS

Study I. Inheritance of PS Mutant

All offspring from sib-mating the original PS plant with a PF sister plant were PF (SB_1, Table 5). Segregation among the 30 F_2 plants produced from these SB_1 plants closely fit the expected 3 PF: 1 PS ratio.

When three PF F_2 plants were selfed, the F_3 progenies from plant PF_A and PF_C segregated 3 PF:1 PS, while the progeny from plant PF_B had only PF Progeny.

Of the F_3 families derived from sib-mating PS F_2 segregates, four families derived from mating PS plants with either PF plant PF_A or PF_C produced ratios of 1 PF:1 PS plants. The families involving PF plant PF_B as pollen parent produced only PF plants. These data indicate that PF plants PF_A and PF_C in the F_2 generation had PS ps genotypes, and that PF plant MF_B had an PS PS genotype. All of the PS plants had an ps ps genotype.

It can be concluded from the analysis of selfed and sib-mated progenies that expression of the PS character was controlled by a single recessive gene. The possible allelic relationship of this gene tentatively designed ps, to MS-1 and other MS mutants, will be considered in the next chapters.

Table 5. Segregation of male sterility in sib-mated families from PS plants and selfed families of pollen fertile (PF) plants.

Generation	Source[z] of families	Segregation: Pollen fertile	Segregation: Pollen Sterile	Chi-Square[y]: 3:1	Chi-Square[y]: 1:1
SB_1		14	---	---	---
F_2		23	7	0.04	---
F_3	PF_A selfed	25	9	0.04	---
	PF_B selfed	28	0	---	---
	PF_C selfed	28	6	0.98	---
SB_2	1 x PF_C	22	14	---	1.78
	2 x PF_C	21	19	---	0.10
	5 x PF_C	11	17	---	1.29
	2 x PF_A	17	20	---	0.24
	2 x PF_B	24	0	---	---
	5 x PF_B	36	0	---	---
	7 x PF_B	32	0	---	---

[z] SB-1 derived from sib-mating the original PS plant with a PF sister; F_2 derived from selfing SB_1 plants; F_3 derived from selfing three PF F_2 plants; SB_2 progenies derived from sib-mating PS F_2 plants 1, 2, 5, and 7 with PF F_2 plants PF_A, PF_B, or PF_C. Each combination listed in the SB_2 represents seed from an individual fruit.

[y] All X^2 are non significant at 0.05 probability. --- means not tested.

male sterility is controlled by an allele of ms-1 or ms-2. The relationship among these male sterile mutants and their response to environmental factors will be described in the later chapters.

Study II. Genetic Relationship of PS to Ap and MS-1 Mutants

Because the MS-2 mutant could not be obtained for the study, and because there were no seeds to be obtained from the recent crosses derived from PS heterozygous plants (Psps) and MS-1 plants with partially fertile pollen in the greenhouses due to inadequate crosses made, only the relationship between PS and AP could be studied. (Additionally, further study of the behavior of the MS-1 mutant was possible and is reported as study VI). Each of the three test crosses (psps ApAp X psps Apap) produced F_1 plants that were all male fertile (Table 6). A total of 370 male fertile : 0 male sterile plants were observed. Based on the scheme shown on page 32, this absence of male sterile F_1 plants indicates that PS is not allelic to AP, but is controlled by a different gene.

Table 6. Test of allelism of PS to Ap.

Cross			Progeny Distribution	
Female		Male	MF Plants	PS	Ap
ps/ps Ap/Ap	x	Ps/Ps Ap/ap	69	0	0
ps/ps Ap/Ap	x	Ps/Ps Ap/ap	112	0	0
ps/ps Ap/Ap	x	Ps/Ps Ap/ap	74	0	0
Ps/Ps ap/ap	x	Ap/Ap Ps/ps	44	0	0
Ps/Ps ap/ap	x	Ap/Ap Ps/ps	38	0	0
Ps/Ps ap/ap	x	Ap/Ap Ps/ps	33	0	0

Study III. Relationship of PS Mutant to Sex Types

The objective of the experiment was to determine whether PS performance is dependent upon sex types which are sensitive to some environmental and chemical factors. A test of this hypothesis is provided by the significance of the fertility x sex types (Gy, M and H) interaction in the F_1 and F_2 generations. The F_1 performance of eight crosses between different sex types and the PS line is reported in Table 7. No PS plants appeared in any of the F_1 populations. Therefore, none of the sex types (Gy, M or H) could be used to maintain the PS mutant. A few male sterile flowers were found at the first four nodes after silver treatment of 4 plants in the F_1 families derived from Gy-PS x H and M-PS x H. This special expression of male sterility of F_1 plants from the crosses of PS plants and H plants will be included in the discussion chapter.

Sex expression in these F_1 populations appeared to follow Kubicki's principle (1965) that monoecious is dominant to gynoecious, and both, M or Gy are dominant to the hermaphroditic type. More stable Gy populations can be obtained by using H pollen parents for maintenance than by selfing Gy plants. The same results can be expected when PS monoecious or PS gynoecious types are crossed with H plants.

Table 7. Occurrence of pollen sterility and sex type in F_1 populations derived from crossing male sterile and fertile plants of different sex types

Cross[z]	Number of Plants observed		
	M	Gy	PS or Ap
M-PS x M	44	0	0
Gy-PS x M	30	5	0
M-PS x Gy	38	0	0
Gy-PS x Gy	2	27	0
M-PS x H	30	0	1 PS flower on 1st male node of one plant
Gy-PS x H	0	35	2 MS flowers on 1st male node of 2 plants; 2 Ms flowers on 1st two male nodes of one plant
Ap x M	32	6	0
Ap x Gy	17	10	0

[z] M-PS indicates monoecious PS plants; Gy-PS indicates gynoecious PS plants with anther-aborted male flowers after treatment with silver ions; Ap indicates apetalous male sterile plants. M, Gy, and H are monoecious, gynoecious, and hermaphrodite plants, respectively.

When the data shown in Table 8 were analyzed (Table 11), there were no significant differences in ratio of MS/(MS + MF plants) among 9 F_2 populations derived from crosses of among sex types (Gy, M, H, and Ap). Also, there were no significant differences between replicated or between unreplicated F_2 populations, and between replicated and unreplicated F_2 populations in the ARCB experiment (Table 10). When only three sex types(GY, H and M) were considered as the main influencing factors on male sterile expression and the effect of each factor was separated by contrasting in the SAS GLM procedure, the same results as in Table 9 and 10 were obtained. The effect of each Gy, H and M parent on PS in the F_2 generation was not significantly different (Table 11 and 12). The results indicated that although PS occurs in male flowers(a sexual character), the occurrence of male sterility is not related to any kind of sex expression.

Table 8. Occurrence of male sterility (PS and Ap type) in the F_2 generation of crosses between cucumber sex types in the ARCB experiment[z].

Pedigree[x]	Ratio of Pollen Sterile Plants[y]	
	Sunseeds-GH	OSU-GH
(M-ps x M)-M SELF	12/53	8/33
(Gy-ps x M)-M SELF	10/37	8/29
(Gy-ps x Gy)-Gy SELF	10/42	16/72
(M-ps x H)-M SELF	11/34	8/44
(Ap x Gy)-M SELF	8/29	7/26
(M-ps x Gy)-M SELF	8/39	
(Gy-ps x H)-M SELF		21/66
(Ap x M)-M SELF		5/26
(Ap x Gy)-Gy SELF	13/53	

[z] ARCB indicates Augmented Randomized Complete Block design.

[y] Ratio of Pollen Sterile Plants = Number of pollen sterile plants/(number of pollen sterile plants + number of pollen fertile plants).

[x] Gy, M and H indicate gynoecious, monoecious and hermophroditic sex types, respectively; Ap indicate apetalous male sterile mutant.

Table 9. Analysis of variance of the replicated F_2 populations from cucumber MS (PS and Ap) crosses with related but sexually different parents in the ARCB experiment[z]

Source of Variation[y]	DF	Sums of Squares	Mean Square	F[x]
F_2 Pop.	4	0.0044	0.0011	0.39NS
Block	1	0.0027	0.0027	0.98NS
Error	4	0.0112	0.0028	

[z] ARCB indicates Augmented Randomized Complete Block design.

[y] Block means the two greenhouse here, Sunseeds and OSU greenhouse; F_2 Pop indicates the replicated F_2 populations.

[x] NS means not significant at 5% probability.

Table 10. Analysis of variance of the replicated and unreplicated F_2 populations from cucumber MS (PS and Ap) crosses with related but sexually different parents in the ARCB experiment[z].

Source of Variation[y]	DF	Sums of Squares	Mean Square	F
Block	1	0.0027	0.0027	0.98NS
F_2 Pop.	8	0.0199	0.0025	0.89NS
Between Replicated F_2 Pop.	4	0.0143	0.0036	1.28NS
Between Unreplicated F_2 Pop.	3	0.0115	0.0038	1.37NS
Replicated Versus Unreplicated F_2 Pop.	1	0.0006	0.0006	0.21NS
Error	4	0.0112	0.0028	

[z] ARCB indicates Augmented Randomized Complete Block design.

[y] Block means the two greenhouse here, Sunseeds and OSU greenhouse; F_2 Pop indicates F_2 populations.

[x] NS means not significant at 5% probability.

Table 11. Analysis of variance of the replicated F_2 populations from cucumber PS crosses in the ARCB experiment by contrasting sexually different parents; only Gy, H, and M included[z].

Source of Variation[y]	DF	Sums of Squares	Mean Square	F
Block	1	0.0031	0.0031	0.87NS
F_2 Pop.	3	0.0030	0.0010	
Female Parents				
(Gy vs. M)	1	0.0020	0.0020	0.55NS
Male Parents				
(Gy vs. M vs. H)	2	0.0028	0.0014	0.18NS
Error	3	0.0011	0.0036	

[z] ARCB indicates Augmented Randomized Complete Block design.

[y] Block means the two greenhouse here, Sunseeds and OSU greenhouse; F_2 Pop indicates the replicated F_2 populations; Gy, M and H indicate gynoecious, monoecious and hermophroditic sex types, respectively.

[x] NS means not significant at 5% probability.

Table 12. Analysis of variance of the replicated and unreplicated F_2 populations from PS crosses in the ARCB experiment by contrasting sexually different parents; only Gy, H, and M included[z].

Source of Variation[y]	DF	SS	MS	F[x]
Block	1	0.0031	0.0031	0.87NS
F_2 Pop.	5	0.0117	0.0007	
Replicated vs. Unreplicated				
Female Parents	1	0.0081	0.0081	2.25NS
Replicated vs. Unreplicated				
Male Parents	1	0.0001	0.0001	0.01NS
Replicated Female Parents				
(Gy vs. M)	1	0.0081	0.0081	2.25NS
Replicated Male Parents				
(Gy vs. H vs. M)	2	0.0105	0.0053	1.46NS
Unreplicated Female				
Parents(Gy vs. M)	1	0.0081	0.0081	2.25NS
Unreplicated Male				
Parents(Gy vs. H)	1	0.0105	0.0105	2.91NS
Error	4	0.0112	0.0028	

[z] ARCB indicates Augmented Randomized Complete Block design.

[y] Block means the two greenhouse here, Sunseeds and OSU greenhouse; F_2 Pop indicates F_2 populations; Gy, M and H indicate gynoecious, monoecious and hermophroditic sex types, respectively.

[x] NS means not significant at 5% probability.

Study IV. Effect of Hormone Treatment on the Flowering Behavior of PS Mutant

The flowering behavior of the PS mutant when treated with kinetin, IAA, and GA is shown in Table 13 and 14. Mean squares and sources of variation for the expression of PS are listed in Table 13. There were no significant main effects of the kinetin, IAA and GA_3 on the occurrence of PS plants, but there was a significant interaction between IAA and GA_3 ($p=0.05$). There was no evidence that other interaction effects, kinetin by IAA, kinetin by GA_3, and kinetin by IAA by GA_3, significantly influenced the expression of PS. These results indicate that PS character is quite stable, and that the hormones used offer no possibility for causing useful changes in PS for F_1 hybrid production.

Table 13. Analysis of variance of the effects of hormonal factors on arcsine transformed percent PS plants in an "AB line"[z]

Source[y] of Variation	DF	Mean Square	F Value[x]	Pr > F[w]
Block	1	0.0005	0.67NS	0.4206
A	2	0.0008	1.13NS	0.3373
B	2	0.0004	0.55NS	0.5861
A*B	4	0.0003	0.44NS	0.7775
C	2	0.0018	2.55NS	0.0974
A*C	4	0.0039	0.54NS	0.7052
B*C	4	0.0028	3.91*	0.0128
A*B*C	8	0.0009	1.27NS	0.3009
Error	26	0.0007		

[z] An "AB line" indicates a male sterile line, half plants of which are male sterile and half are fertile plants with heterozygous genotype.

[y] A, B, and C indicate the effect of kinetin, IAA, and GA_3, respectively.

[x] *: significant at 5% probability; NS: not significant.

[w] Pr > F means probability that F values are bigger than the critical F value.

Table 14. Main effects of three hormone treatments on percent PS plants in a cucumber "AB line"[z]

Main Effect[y]		N[x]	Mean of Percent PS Plants[w]
Kinetin	a1	18	48.60
	a2	18	49.57
	a3	18	49.89
Mean			49.02
IAA	b1	18	48.93
	b2	18	49.86
	b3	18	49.28
Mean			49.36
GA_3	c1	18	48.33
	c2	18	49.39
	c3	18	50.35
Mean			49.36

[z] "AB line" indicates a male sterile line, half plants of which are male sterile and other half are fertile plants with heterozygous genotype.

[y] a1 = 0ppm, a2 = 500ppm, a3 = 1500ppm; b1 = 0ppm, b2 = 100ppm, b3 = 200ppm; c1 = 0mg/L, c2 = 1mg/L, c3 = 10mg/L.

[x] N indicates the number of observations.

[w] Percent PS plants = [PS/(PS + PF)*100]%.

Study V. Effect of Temperature and Fertilizer Level on Performance of PS Mutant

Incidence of PS in cucumbers grown at two temperature environments and at levels of N, P, and K fertilizer is shown in Table 15 and 16. The analysis of variance of these data are shown in Table 15. Temperature and fertilizer variation does not change the expression of PS although they can influence the pollen fertility of normal fertile plants. There were no significant interactions between fertilizer and temperature level.

Observations of PS plants which were treated with H_3BO_4 (1000ppm) are shown in Table 17. All of the plants studied remained PS. These results indicate that boron is not an effective factor in changing the fertility of PS cucumber plants even though it can increase the pollen fertility in certain genera of Crucifera family (Tan and Chen, 1980).

Table 15. Analysis of variance of the effect of temperature and fertilizer on the incidence of PS plants

Source[z] of Variation	DF	Mean Square	F Value[y]	Pr > F
Block	1	0.0010	**1.14NS**	0.3038
Temp(T)	1	0.0002	**0.25NS**	0.6271
Block*T	1	0.0003	**0.31NS**	0.5862
N	1	0.0025	**2.82NS**	0.1155
T*N	1	0.0001	**0.13NS**	0.7237
P	1	0.0030	**3.44NS**	0.0848
T*P	1	0.0002	**0.22NS**	0.6489
N*P				0.0501
T*N*P	1	0.0038	**4031NS**	0.0567
K	1	0.0001	**0.11NS**	0.7503
T*K	1	0.0006	**0.67NS**	0.4264
N*K	1	0.0021	**2.45NS**	0.1396
P*K	1	0.0016	**1.79NS**	0.2021
N*P*K	1	0.0018	**2.10NS**	0.1691
T*N*P*K	8	0.0018	**2.09NS**	0.1093
Error	14	0.0009		

[z] T indicates the effect of temperatures; N, P, and K indicate the effect of nitrogen, phosphorus, and potassium treatment, respectively.

[y] NS: not significant.

Table 16. Main effects of three hormone treatments on percent PS plants in a cucumber "AB line"[z]

Main Effect[y]		N[x]	Mean of Percent PS Plants[w]
Temperature	T1	16	49.61
	T2	16	50.13
Mean			49.87
Nitrogen	N1	16	49.00
	N2	16	50.75
Mean			49.88
Phosphorus	P1	16	50.84
	P2	16	48.91
Mean			48.38
Potassium	K1	16	50.04
	K2	16	49.71
Mean			49.88

[z] An "AB line" indicates a male sterile line, half plants of which are male sterile and half are fertile plants with heterozygous genotype.

[y] N1 = 0ppm, N2 = 173ppm; P1 = 0ppm, p2 = 39ppm; K1 = 0ppm, K2 = 280ppm.

[x] N indicates the number of observations.

[w] Percent PS plants = [MS/(MS + MF)*100}%.

Table 17. Observations from ten PS plants treated with H_3BO_4

PS Plant No.	Times of Treatment	Duration of Observation	Number of Male Flowers	Number of PS Flowers
1	8	Oct.-Feb.	94	94
2	8	Oct.-Feb.	147	147
3	8	Oct.-Jan.	87	87
4	8	Oct.-Fec.	91	91
5	8	Oct.-Feb.	66	66
6	8	Oct.-Feb.	89	89
7	8	Oct.-Feb	105	105
8	8	Oct.-Feb.	83	83
9	8	Oct.-Feb.	55	55
10	8	Oct.-Feb.	91	91

Study VI. Elucidation of the Behavior of MS-1 and the Genetic Relationship of MS-1 to Ap

Table 18 presents the segregation data of progeny from the nine selfed single plants from MS-1 line Sunseeds 22676-6. These data show that source plants 22676-6-3, 22676-6-5, and 22676-6-11 were homozygous (MS1 MS1), and that the remaining six source plants were heterozygous (MS1 ms1). Analysis Of variance showed significant differences in the percentage of male sterile-1 plants among the six segregating families ($P < 0.01$) and among environments ($P < 0.05$) (Table 19). The percentage of MS plants in the field was significantly higher than in the greenhouse. However, there was no difference in male sterile expression of MS-1 between OSU and Sunseeds greenhouses (Table 20). Figure 3 further shows the tendency in which field planting significantly increased the proportion of progeny plants which were male sterile. However, the male flower buds of MS-1 plants from the tenth to the 15th node, opened normally in the greenhouse, but continued to abort in the field (Table 22). Pollen from these opened male flowers was observed under the light microscope (20X and 40X). The percent of sterile pollen ranged from 75% to 100%. During the observations of Sunseeds 22676-6 progenies, more than hundred MS-1 plants were crossed with wild type and

heterozygous PMS plants. Only 4 seeds were obtained from 43 fruit. No seeds were produced from 31 fruit derived from the two MS-1 test crosses (Table 23). Even though the female flowers on MS-1 plants appear to be normal, they have a greater tendency to be sterile than the male flowers.

The allelic relationship of MS-1 to Ap was tested by studying progeny populations of test crosses of MS-1 X Ap fertile plants (Apap) and MS-1 plants (with partially fertile pollen). The F_1 progenies produced 98 fertile and no sterile plants (Table 24). Thus, Ms-1 is non allelic to Ap.

Table 18. Segregation of male-sterile plants in progenies from self pollinated single plants of Sunseeds 22676-6 in three environments[z]

Source Plant	Numbers of Plants					
	OSU GH		Sunseeds GH		Field	
	MF	MS	MF	MS	MF	MS
22676-6-1	46	7	108	13	80	24
22676-6-2	33	4	103	10	93	17
22676-6-3	26	0	206	0	117	0
22676-6-4	35	5	116	18	43	13
22676-6-5	41	0	107	0	88	0
22676-6-7	41	6	170	18	124	36
22676-6-9	36	6	152	20	128	43
22676-6-10	44	7	252	55	174	51
22676-6-11	46	0	309	0	173	0

[z] Numbers of male fertile (MF) and male sterile (MS) plants were obtained from nodes 10-15 of each plant grown. If all of male flower buds on a plant aborted or had only few opened sterile flowers in field, or If all of opened male flowers were sterile and semi-sterile in greenhouse, the plant was classified as MS.

Table 19. Analysis of variance of arsine transformed percent of MS-1 plants for six segregating families from selfed plants of Sunseeds 22676-6 grown in three environments

Source of Variance	DF	Mean square	F	Pr > F
Environment	2	0.0331	41.20**	0.0001
Family	5	0.0029	3.63*	0.0392
Error	10	0.0008		

** Significant at 0.01 level, and * at 0.05 level.

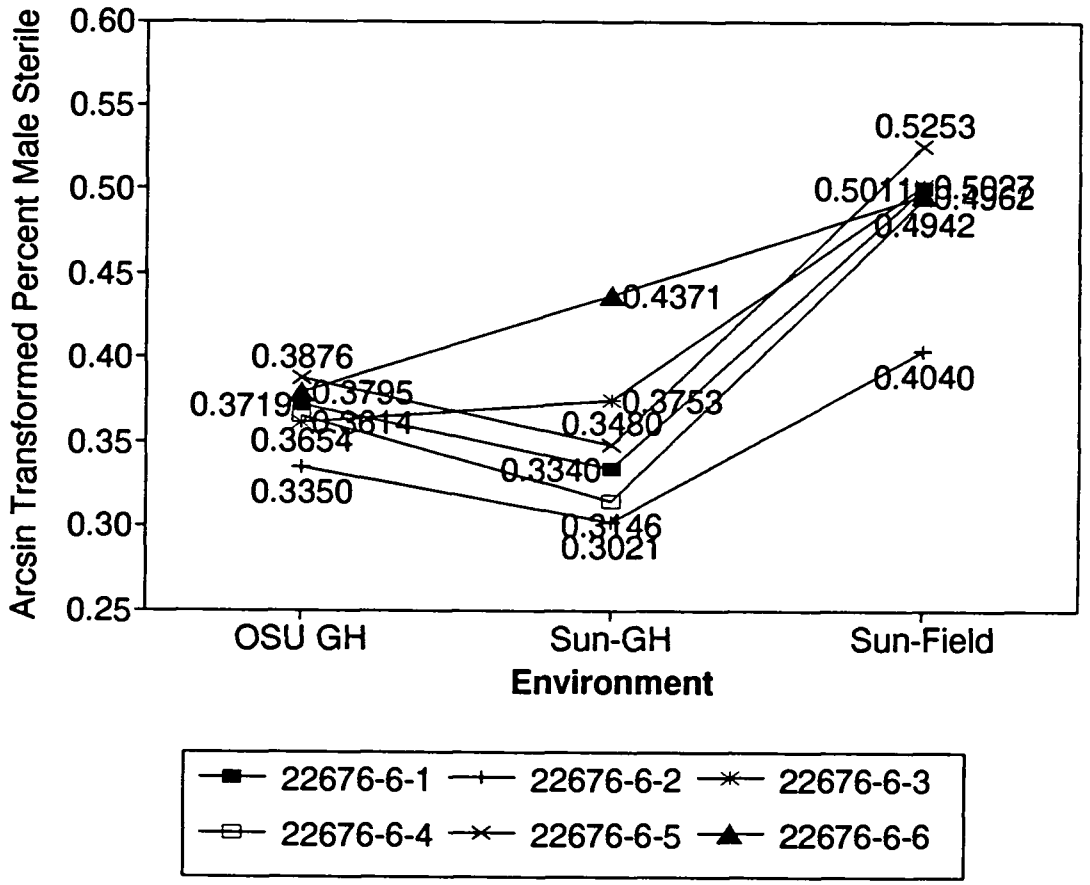


Figure 3. Arcsine transformed percentage of male sterile plants in progenies of Sunseeds 22676-6, from the first to fifteenth node, in three environments.

Table 20. Percentage of MS plants segregating in progenies from selfed single plants of Sunseeds 22676-6

		Percentage of MS Plants	
Environment	N	Natural Mean	Transformed Mean
Sunseeds Field	6	22.01	0.4873 A
OSU Greenhouse	6	12.88	0.3668 B
Sunseeds Greenhouse	6	12.02	0.3518 B

[z] Means bearing the same letters were not significantly different at the 5% level. Mean separation by LSD derived from arsine transformed data.

Table 21. Mean percentage of MS plants segregating in progenies from selfed single plants of Sunseeds 22676-6 across three environments.

		Percentage of MS Plants	
Family No.	N	Natural Mean	Transformed Mean
22676-6-10	3	18.10	0.4376 A
22676-6-9	3	17.02	0.4203 A
22676-6-4	3	16.38	0.4131 A
22676-6-1	3	15.68	0.4023 A
22676-6-7	3	14.95	0.3914 AB
22676-6-2	3	11.70	0.3470 B

[z] Means bearing the same letters were not significantly different at the 5% level. Mean separation by LSD derived from arsine transformed data.

Table 22. Effect of three environments on the number of MS segregates having open staminate flower after node 15[z]

Family No.	Number of Male Sterile Segregates					
	OSU GH[y]		Sunseeds GH		Field	
	Total	WOS-Flw[x]	Total	WOS-Flw	Total	WOS-Flw
22676-6-1	7	7	13	13	24	1
22676-6-2	4	4	10	10	17	0
22676-6-4	5	5	18	18	13	0
22676-6-7	6	6	18	18	36	1
22676-6-9	6	6	20	20	43	0
22676-6-10	7	7	55	55	51	0

z Progenies obtained by field selfing single plants obtained from Sunseeds 22676-6 in Sunseeds field at Brooks, Oregon.

y GH: Greenhouse.

x WOM-Flw: with opened staminate flowers.

Table 23. Seed production from test crosses of MS-1 with plants heterozygous for MS-1, PS, and Ap.

Cross				
MS-1		Male Parent	Fruit No.[z]	Seed No.[y]
ms-1/ms-1	X	MS-1/_	43	4
ms-1/ms-1	X	PS/ps	24	0
ms-1/ms-1	X	Ap/ap	7	0

[z] Fruit No. indicates the number of total fruit obtained from pollinated flowers.

[y] Seed No. indicates the number of total seed obtained from the total fruit above.

Table 24. Test of allelism of MS-1 to Ap[z]

Cross			Progeny Distribution[y]		
			Numbers of Plants		
Female		Male	MF No.	MS-1 No.	Ap No.
+/+ +/ap	x	ms-1/ms-1 +/+	55	0	0
+/+ +/ap	x	ms-1/ms-1 +/+	43	0	0

[z] Ap means apetalous male sterile mutant.

[y] MF No., MS-1 No. and Ap No. indicate the number of male fertile plants, the number of MS-1 plants and the number of apetalous plants, respectively.

DISCUSSION

These experiments were initiated to investigate the genetic mechanism of the new PS mutant. After it was found that the PS characteristic was under the control of a single recessive gene, the possibility of its application to commercial seed production was considered. Gynoecious lines have been utilized successfully in hybrid seed production of cucumber for a long time. The following hypotheses were proposed for test: 1.) the PS character of cucumber is related to other mutants or it can be combined together with other mutants for use in hybrid seed production; 2.) it can be maintained by combining it with a certain sex type; and 3.) it is as easily converted to fertility by environmental or chemical factors as any types of sex expression. If the above hypotheses are tenable, PS plants can easily be developed into male sterile lines and maintained by the effect of other mutants or certain sex types, or by treating MS plants with environmental and chemical factors.

Unfortunately, none of the experimental results supported acceptance of the above hypotheses. The PS character was found to be independent (non allelic) of the apetalous character, and it was not possible to complete a study of the possible allelism of PS with MS-1 and MS-2 during the course of the study. Expression of the PS

character was not affected by environmental or chemical factors in the study. Although the results in STUDY IV showed that the interaction between IAA and GA_3 had a significant effect on PS plants, there is no reasonable explanation for it. Most of the results of these experiments are negative. Therefore, in spite of the successful use of male sterile lines in other crops the utilization of PS in commercial seed production appears at this time to be uneconomical.

A striking phenomenon was found with MS-1 materials when they were grown in the greenhouse. These MS-1 plants produced some naturally opened staminate flowers with 0-25% fertile pollen on the later nodes. The reasons for this occurrence of opened staminate flowers is unknown. One possible explanation is that in the greenhouse there was a smaller difference between day and night temperatures than in the field. Due to the degree of sterility in both staminate and pistillate flowers of MS-1 in cucumber, the utilization of MS-1 in commercial seed production is not possible. However, it might be possible to combine MS-1 and PS into an "AB line" (assuming that MS-1 and PS are independent alleles). If ps is dominant to ms-1, the following procedure may be used:

Season	Procedure
1	PS and MS-1 populations are planted in the

same greenhouse. After the fertility of the plants has been determined, all fertile plants are rouged out. PS plants are pollinated with opened staminate flowers with partially fertile pollen on male sterile plants of MS-1 by hand or bees. Fruit on PS plants are harvested, and seeds are extracted and bulked when they are dried.

2 Bulked seeds are planted in a greenhouse again for an additional production of the "AB line" by the procedure described in 1. Part of the bulked seed could be used for testing combining ability for hybrid seed production in the field.

3 Repeat the above procedure.

If ps is recessive to ms-1, the "AB line" would contain one hundred percent MS-1 plants and this line would be useless due to the sterility of its female flowers.

The hypothetical procedure described above needs more work to demonstrate feasibility and practicality. If it is feasible, the newly created "AB line(s)" would be an ideal male sterile line(s) which consist(s) of one hundred percent PS plants. If 100% PS lines could be contained, the utilization of "AB lines" would be another prospective way to produce commercial hybrid seed in cucumber.

During the process of producing "AB lines", the main

consideration would be the limited amount of pollen available on MS-1 plants. An insufficient supply of fertile pollen could make this procedure uneconomical.

The sterility of pistillate flowers in the MS-1 mutant may change under certain conditions. If pistillate flowers are fertile, the MS-1 mutant will have more potential value for commercial hybrid seed production in cucumber because the male sterile line can be maintained in a greenhouse through selfing. This type of male sterile line could be used as a seed parent in a hybrid production field when the staminate flowers would abort.

SUMMARY AND CONCLUSIONS

Based on the results of analysis of selfed and sib-mated progenies derived from PS plants, it can be concluded that the PS character is controlled by a single recessive gene.

An allelic analysis of PS and the Ap mutant indicated that PS is not allelic to Ap. Ap and the new male sterile (PS) are controlled by independent recessive genes.

PS was not affected by sex types gynoecious, monoecious, or hermaphroditic. In other words, PS lines can not be maintained by hermaphrodite lines as is possible with the gynoecious character.

Environmental, hormonal, and chemical factors did not significantly influence the expression of PS. Unlike the sex types of cucumber, which can be changed by environmental, chemical or hormonal factors, the PS character can not be maintained by treatment with these factors.

From additional observations of the MS-1 mutant, it was found that MS-1 actually is not only semi-male sterile, but strongly female sterile. Male flower buds on MS-1 plants can open normally in the greenhouse even though they abort continuously in the field. The result of allelic tests indicates that MS-1 is non allelic to Ap mutant. MS-1 is not of any value in commercial seed production because of its

female sterility. In response to the new observation that staminate flowers may open normally and produce pollen in a greenhouse, it might be possible to combine the MS-1 and PS characteristic for hybrid seed production.

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