Male sterility (MS) is of practical importance in cucumber (Cucumis sativus L.) breeding because it can facilitate F₁ hybrid seed production without hand pollination. Five MS forms are known: 1) gynoecious (G) flowering; 2) an apetalous sterile mutant (ap); 3) a pleiotropic pollen-aborted mutant (ms-1); 4) an aborted male flower type (ms-2); and 5) a closed-flower type.

The G character is under the control of several loci. Usually, G is considered to be a form of sex expression rather than MS because it is one of several sex segregates (Lower and Edwards, 1986; Pierce and Wehner, 1990). In ap, the corolla lacks staminate and pistillate flowers, and anders become sepal-like (Grimbly, 1980).

The recessive pleiotropic gene ms-1 determines MS in which failure of stamate flower anthesis and pollen sterility (PS) varies from 30% to 90% (Shifriss, 1950). Grimbly (1980) and Hutchins (1936) each reported that ms-1 conditions sterility in which stamineate flowers are devoid of pollen, and fertility of pistillate flowers is decreased.

In ms-2, the MS plants are characterized by abortion of the staminate blossoms. In rare instances when the flowers matured to anthesis, only rudimentary anthers that contained no pollen were present (Barnes, 1960; Miller and Quisenberry, 1978; Whelan, 1974).

The closed-flower variant is controlled by a recessive gene cl. Both staminate and pistillate flowers fail to open at maturity (Groff and Odland, 1963).

Except for G, these forms of MS have not been used in hybrid cucumber seed production because their inheritance is determined by nuclear genes and because they are associated with undesirable traits such as missing corolla, malformed ovary, and closed female flowers (Grimbly, 1980; Hutchins, 1936).

Many inbred lines were observed for sex expression and fertility at the SunSeeds Co. in Brooks, Ore. In 1988, a single MS plant was found in an inbred line, pedigree 23B-X26, which is of pickling type and is segregating for G flowering. The corolla of staminate and pistillate flowers of this mutant is normal and pistillate flowers are fertile. The stamineate flowers are generally normal in appearance but have rudimentary anthers and produce no pollen. These mutant PS plants produce a few aborted flower buds only after they are loaded with fruit or they are physiologically mature. In the search for a suitable technique to maintain the PS mutant, it was hypothesized that it might be similar to certain sex types [G, monoeccious (M), or hermaphroditic (H)] in its sensitivity to some external factors, and that it might be maintained by a certain sex type or by selfing after restoring fertility by changing external factors.

The purpose of the present study was to determine 1) the genetic control of the PS mutant and possible allelic relationships with other known MS types (except G flowering and the closed-flower type), and 2) if the PS mutant could be induced to produce pollen in different sex expression backgrounds or by using chemical treatment or environmental modification.

Materials and Methods

Inheritance of the PS mutant. When the original PS plant was found, it was sib-mated (SB) with a male fertile (MF) plant of the same inbred line. Fourteen seeds (SB) from this SB were obtained from the mutant plant and were planted in the greenhouse. Each of the 14 seeds produced an MF plant. Each of these was selfed, and seeds from the 14 plants were bulked. Thirty-five progeny plants from this bulk population were grown, the PS segregates were SB.
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 SB mutant 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 Summer 1989, all of the SB families were grown in the greenhouse. Forty seeds of each family were planted in seven 1.4-liter pots filled with a standard commercial soil mix consisting of 70% bark materials, 30% soil, lime, complete fertilizer, and a wetting agent. The 10 families were randomly arranged in a greenhouse. Plants were irrigated and fertilized with an automatic drip system. No supplemental lighting was used.

Plants were classified as PS or fertile at anthesis according to the external appearance of anthers in the staminate flowers. A plant was classified as PS if it had open staminate flowers with aborted anthers on the first 10 nodes. Also, anthers periodically were observed without staining under a light microscope (×40) for presence of pollen.

Allelism tests. Initially, tests for allelism with PS mutants were to include ap, ms-1, and ms-2. As described below, tests with ap and ms-2 were successfully completed. Tests with ms-1 were not possible because, in making the initial crosses to obtain plants heterozygous for this mutant, the sterility of the pistillate flowers of MS plants of the ms-1 parent (SunSeeds M line 22676-6) was much higher than that of staminate flowers, and no viable seeds were obtained.

For the test of allelism with ap, heterozygous Ap ap plants [obtained by crossing normal (Ap Ap) plants with SunSeeds ap line 23BX75], were crossed with the PS mutant. Additionally, Ap Ap plants homozygous for PS were crossed with plants heterozygous (MF) for PS. To test for allelism with ms-2, PS mutant plants were crossed with plants heterozygous for ms-2 (obtained by crossing normal Ms-2 Ms-2 with SunSeeds ms-2 line 18849 x 23BX114-A). The F_{1} progenies from these crosses were planted in the greenhouses and fields of SunSeeds Co., Oregon State Univ. (OSU), and Amsa Seed Co. in Modesto, Calif., during Spring 1991 to Fall 1992.

The relationship of PS to sex types was studied as a component in an AB line consisting of half homozygous MS (A) and half heterozygous fertile (B) plants. Cucumber lines of three sex types—M-F; G (SunSeeds 23-116), M-ff; M (SunSeeds 23-113), and mmmF; H (MSU-669H)—were also used.

Initially, the AB line was planted in 70 1.4-liter pots. When the flower buds could be identified, G plants were tagged and sprayed with a 500 ppm solution of silver thiosulfate. The PS plants in the AB line were classified into two types: 1) M–PS, which have anther-aborted male flowers and 2) G–PS, which have anther-aborted sterile flowers after treatment with silver thiosulfate. After these PS plants were tagged, both types were moved to the greenhouse and crossed with G, M, and H plants to give the following combinations: 1) M–PS x M, 2) G–PS x M, 3) M–PS x G, 4) G–PS x G, 5) G–PS x H, and 6) M–PS x H. Seeds from each cross were bulked and planted in the greenhouse in Winter 1990. The G F_{1} plants were treated with silver thiosulfate and the percentage of G plants in each F_{2} was recorded. The plants were also checked daily to determine if PS plants were present. During Spring 1991, F_{2} populations from each cross were planted in the SunSeeds and OSU greenhouses (as two blocks) to determine the segregation of fertility types. F_{2} seed was produced by selfing F_{1} plants of the sex type shown in the parentheses in the following: M–PS x M (M), G–PS x M (M), M–PS x G (M), G–PS x G (G), G–PS x H (G), and M–PS x H (M). The number of F_{2} seeds from crosses M–PS x G and G–PS x H was not adequate for replication.

Therefore, an augmented randomized complete-block design (Federer, 1956), which involves replicated and unreplicated populations, was used for the sex F_{2} populations. A replication for each treatment consisted of five pots and each pot contained 10 plants. The G plants were treated with silver thiosulfate to produce male flowers, and the MS plants were observed at anthesis. The percentage of PS plants were transformed by arcsin and tested by Fisher’s protected LSD.

Hormone treatment and the flowering behavior of PS. Three levels for each of the hormones (kinetin, IAA, and GA_{3}) were applied as a factorial set of 27 combinations arranged in a completely randomized block design with two replications. For each treatment combination and replication there were 50 plants in five pots. The bulked experimental seed from the AB line was divided into 54 parts and sprouted in petri dishes. Concentrations used were 0, 500, and 1500 ppm for kinetin; 0, 100, and 200 ppm for IAA; and 0, 1, and 10 mg·liter⁻¹ for GA_{3}. The sprouted seeds were immersed in the assigned hormone solution for 24 h, sown in pots, and moved to a 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 thiosulfate to promote male flowers on the G plants. The proportion of PS plants was recorded when the fertility of male flowers could be determined.

Temperature and fertilizer level and performance of PS. To estimate the effects of temperature and fertilizer on PS, two levels of temperature, N, P, and K were tested on bulked seed from the AB line. Test plants were grown in two greenhouses: A) maintained at 13C night and 24C day, and B) maintained at 24C night and 32C day. Two replications of eight factorial combinations of two levels each of N (0 and 173 ppm), P (0 and 39 ppm), and K (0 and 280 ppm) were used in each greenhouse. For each replication of each treatment, 50 seeds were planted in five sand-filled pots (10 seeds per pot), which were fertilized with the respective fertilizer combination in water solution. All plants were sprayed with silver thiosulfate three times to promote staminate flower production on the G plants. The flowers at the first few nodes of each plant were observed for the expression of MS.

Results and Discussion

Inheritance of the PS mutant. All offspring of the original PS plant from SB were MF (Table 1). Segregation among the 30 F_{2} plants produced from these SB plants closely fit the expected 3 MF : 1 MS ratio.

Among the three families from selfed MF F_{2} plants, the families from plant MF_{A} and MF_{B}, segregated 3 MF : 1 MS, while the family from plant MF_{C} had only MF progeny.

Four F_{3} families derived from test crossing MF plants MF_{A} and MF_{B} with sister PS plants segregated in the expected 1 MF : 1 MS ratio. The families involving plant MF_{B} as pollen parent produced only MF plants. These data indicate that MF_{A} and MF_{B} in the F_{2} generation were heterozygous for MS and that MF_{B} was homozygous.

Additional genetic data were provided by the crosses made to determine the relationship between PS and sex types. There were no PS plants in the F_{2} progenies when M and G PS plants were crossed with MF–M plants or G plants treated with silver thiosulfate (Table 2). Ratios of MF : MS plants in the F_{2} progenies of these crosses closely fit the 3:1 ratio expected for recessive monogenic inheritance (Table 3).
When six F1 progenies obtained by crossing M–PS or G–PS plants with M, G, or H were observed, no typical PS plants were found. M–PS x M, M–PS x G, and M–PS x H F1 plants were all M; G–PS x M F1 plants were 30 M : 5 G; G–PS x G F1 plants were 2 M:27 G; and G–PS x H F1 plants were all G (Table 2). A few (one to four) MS flowers were found on the first four nodes of four plants in the F1 progenies from G–PS x H (after silver-ion treatment) and M–PS x H. These results indicate that there is no possibility of using any of the three cucumber sex types to maintain the PS character.

When the F2 generations were observed under greenhouse conditions, the percentage of PS plants ranged from 18 to 32 (Table 3). All comparisons, which included blocks (two greenhouses), crosses, male parent effects, and female parent effects, were nonsignificant. F2 results support the conclusions that PS is not related to the known kinds of cucumber sex expression.

The allelism test crosses involving ap plants produced F1 plants that were all MF (Table 4). The absence of MS F1 plants indicates that our PS mutant is not allelic to ap.

Test crosses between the PS mutant and Ms-2 ms-2 produced 126 MF : 118 MS plants (Table 4), which closely fits a 1:1 ratio and indicates that our mutant is allelic to ms-2. The allele controlling the occurrence of PS was designated as ms-2PS.

Table 2. Occurrence of pollen sterility (PS) and sex type in F1 populations derived from crossing male sterile (MS) and fertile plants of different sex types [monoecious (M), gynoecious (G), hermaphroditic (H)].

| Cross                  | Total | Male fertile | Male sterile | χ² 3:1 | (P)    |
|------------------------|-------|--------------|--------------|--------|--------|
| M–PS x M               | 86    | 66           | 20           | 0.14   | 0.90–0.50 |
| G–PS x M               | 66    | 48           | 18           | 0.18   | 0.90–0.50 |
| M–PS x G               | 39    | 31           | 8            | 0.35   | 0.90–0.50 |
| G–PS x G               | 114   | 88           | 26           | 0.29   | 0.90–0.50 |
| G–PS x H               | 66    | 45           | 21           | 1.63   | 0.50–0.10 |
| M–PS x H               | 78    | 59           | 19           | 0.12   | 0.90–0.50 |

Relationship of PS to sex types. When six F1 progenies obtained by crossing M–PS or G–PS plants with M, G, or H were observed, no typical PS plants were found.

M–PS x M, M–PS x G, and M–PS x H F1 plants were all M; G–PS x M F1 plants were 30 M : 5 G; G–PS x G F1 plants were 2 M:27 G; and G–PS x H F1 plants were all G (Table 2). A few (one to four) MS flowers were found on the first four nodes of four plants in the F1 progenies from G–PS x H (after silver-ion treatment) and M–PS x H. These results indicate that there is no possibility of using any of the three cucumber sex types to maintain the PS character.

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Effect of hormone treatment on the expression of PS. Treatments of the AB line with 27 factorials of three combinations each of IAA, GA3, and kinetin had no effect on the occurrence of PS in the progeny of the AB line (data not presented). All three levels of each hormone (including the 0 ppm control) produced ≈ 50% PS plants (main effects), the expected ratio of PS plants from the AB line. It was not possible to determine whether there was an
interaction between hormone treatments and silver thiosulfate applied to induce male flowering in G plants.

**Effect of temperature and fertilizer level on the expression of PS.** The analysis of variance of the results obtained from two temperature levels and two levels each of N, P, and K showed no individual treatment effects or interactions (data not shown). The percentage of PS was always very close to 50.

The lack of any influence of the hormone and fertilizer levels applied indicate that PS is very stable and unlikely to be influenced in its expression by external factors. Thus, it cannot be made fertile by environmental manipulation for maintenance. PS was not affected by sex type, so it cannot be maintained by H lines, as is possible with the G character.

The use of AB lines possessing PS is not practical for F1 hybrid seed production and as such is not an economical management tool. While PS lines could be maintained by tissue culture, this method is not as economical as the use of G lines. Thus, it seems that the PS mutant described here, as is the case with other cucumber MS forms reported, has little practical use at this time.

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#### Table 4. Tests of allelism of the pollen sterile (PS) mutant with *Ap* (apetalous) and *Ms-2* (male sterile-2) in cucumber.

| Cross description | No. and genotype of F1 plants | \( \chi^2 \) |
|-------------------|-------------------------------|-------------|
| PS \((Ap\; Ap\; ms^{-2}\; ms^{-2})\) | Male fertile (MF) | Male sterile (MS) | |
| \(x\) heterozygous apetalous \((Ap\; ap\; Ms-2\; Ms-2)\) | 255 | 0 | 1:1 |
| Apetalous \((ap\; ap\; Ms-2\; Ms-2)\) | 115 | 0 | |
| PS \(ms^{-2}\; ms^{-2}\) | 126 | 118 | 0.26<sup>y</sup> |
| \(x\) heterozygous MS \((Ms-2\; ms^{-2})\) | \(ms^{-2}\; ms^{-2}\) | MS-2 \(ms^{-2}\) | |

<sup>z</sup>Genotypes of parents and F1 progenies and the symbol \(ms^{-2}\) for PS are based on the results of the tests.

<sup>y</sup>The \(\chi^2\) value of 0.26 indicated a nonsignificant deviation from the expected 1 MF : 1 MS ratio at \(P = 0.05\).