(1) **Hybrid Cucumber Seed Production**

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(2) Introduction

Cucurbitaceae family

The Cucurbitaceae family includes numerous species of economic importance. Some examples are presented in this table:

(3) Citrullus lanatus is the scientific name for Watermelon

(4) The genus *Cucumis* includes the species:
Cucumis sativus (cucumber) and
Cucumis melo (melon).

(5) The genus *Cucurbita* includes the species
Cucurbita pepo (summer squash)
Cucurbita maxima, Cucurbita mixta, and Cucurbita moschata known as pumpkin or winter squash.

(6) These species share the common characteristics of annual plants susceptible to chilling or freeze injury. Most of them are important outdoor crops and some, such as cucumber, are also important as a protected crop (George, 1999).

(7) Reproductive structures and natural pollination mechanisms

In this table is seen the wide range of flowering habits found in Cucurbitaceae species. The presence of male and female flowers in the same plant or monoecious flowering is the most common habit in many of these species.

In melon, the presence of perfect and male flowers in the same plant or andromonoecious habit is the most common.

Several gynoecious genotypes have been developed for cucumber. In this case, plants have predominantly female flowers.

Morphological studies show that male, female and perfect flowers are formed from an anatomically similar 'presexual' primordium. Subsequent differentiation and regulation of the flower sex is under genetic, environmental and hormonal control (Wien, 1997).

Different flowering habits along with regulation of flower sex expression have been extensively studied in the last 60 years, primarily because of their importance for breeding and potential use in hybrid seed production.

(8) Under natural conditions, most cucurbit cultivars are cross pollinated by insects, mainly bees. However, self-pollination may occur depending on the genotype, environmental conditions, plant population and insect activity. In many cases, for commercial production of cucurbit seed, hives are incorporated in the field to ensure adequate pollination and good yield. This practice is also performed in seed production of open-pollinated seed or hybrids that are not hand pollinated.

(9) The flowers of Cucurbitaceae species are borne in leaf axis, generally solitary. They are bell shaped and have five fused yellow or white petals. Female flowers have an inferior ovary that forms the fruit called a *pepo*. Male flowers have five stamens.

In picture A, a cucumber female flower is seen on which the inferior ovary is forming a fruit or *pepo*. Picture B shows a male cucumber flower.

(10) Here, cucumber flowers are seen in more detail. In the female flower, the inferior ovary is observed which will form the *pepo* fruit.

(11) Fruit and seeds

The cucumber fruit is formed from an ovary with three fused carpels as seen in this picture. The seeds begin development inside the locular cavities attached to the placenta of the ovary. The thick, fleshy wall of the fruit is the mesocarp.

(12) This picture shows a fruit with developing seeds at the left and a parthenocarpic fruit without seeds at the right. In the case of parthenocarpic fruits, no pollination is required for fruit development and the fruit does not have any seeds.

(13) Cucumber seeds are non-endospermic, flattened and relatively large. Inside, there is a large embryo with two large, flat cotyledons.

(14) Use of hybrids in the Cucurbitaceae family

Heterosis or hybrid vigor has been reported in several cucurbit species. Earliness, yield and fruit quality are some of the most frequent traits influenced by heterosis. Additionally, several disease resistance genes have been introduced in F1 hybrids. Today, most commercial summer squash, cucumber, melon, and watermelon cultivars are F1 hybrids (Robinson, 2000).

Some of the reasons for the increased popularity of hybrid cultivars in species of this family are that numerous seeds are produced per fruit and there are a relatively low number of seeds required per hectare for crop establishment. In addition, different techniques to achieve more cost-efficient hybrid seed production have been developed.

(15) Among these techniques used for hybrid cucurbit seed production are (Robinson, 2000):

Hand emasculation and pollination. This technique is frequently used for melon seed production. In this species, andromonoecious lines are common and they must be emasculated and hand pollinated if used as the female parent for producing hybrid seed. This method has also been used for some watermelon and cucumber hybrids.

Flower removal or manual removal of male flowers from the female parental line. This method has been used for hybrid seed production of summer squash. It consists of planting the male and female lines in the field, removing the male flowers from the monoecious female line and then allowing pollination by bees. This method has been replaced by the use of ethephon as a growth regulator that suppresses formation of male flowers in the female line.

Gynoecy or the use of lines with only female flowers as female parents of hybrids. This method has been used in cucumber. Hybrid seed is produced by growing the gynoecious female line in the same field as the male line producing the pollen. Pollination is performed by bees.

Use of growth regulators to modify sex expression. Ethephon is the most common growth regulator used. This compound is mainly used in commercial hybrid seed production of summer squash and some monoecious cucumber hybrids.

Genetic male sterility has been reported in cucumber, melon, squash, and watermelon. However, this principle has not been used extensively. It has importance for winter squash (*C. maxima*) and watermelon hybrid seed production in the United States and China, respectively.

(16) Production of cucumber hybrid seed

Cucumber is the species most extensively studied in the Cucurbitaceae for the production of hybrid seed. The physiology of cucumber growth and productivity has been investigated as a requirement for optimizing greenhouse production (Wien, 1997). Additionally, understanding the genetic and hormonal control of flower sex expression has contributed to improving hybrid seed production of this species.

Among the many types of cucumber cultivars, it is possible to find gynoecious genotypes, i.e. plants that only have female flowers. Parthenocarpy, or the development of fruits without fertilization and seed formation, is another important trait available for cucumber breeding. Gynoecious cultivars with parthenocarpic fruits are usually preferred for greenhouse production because of their higher yields and ease in crop management.

(17) Use of gynoecious lines in hybrid seed production

The gynoecious trait is determined by a single dominant gene: “F”. (18) Because these plants have only female flowers, hybrid seeds may be produced using gynoecious maternal lines without the requirement for male flower emasculation. In the field, the ratio between female and male line plants is usually 3:1 and pollination is performed by bees.

(19) There are different systems that have been proposed to produce hybrid cucumber seed using a gynoecious line (Robinson, 2000). They differ in the flower type of the line used as pollen donor.
Gynoecious x Monoecious hybrids
Gynoecious x Gynoecious hybrids
Gynoecious x Hermaphroditic hybrids, and
Gynoecious x Andromonoecious hybrids.

From these systems, the Gynoecious x Monoecious and Gynoecious x Gynoecious hybrids are most commonly used and will, therefore, be discussed in more detail.

(20) Gynoecious x Monoecious hybrids

This system became popular for the development of stable inbred lines for the gynoecy trait during the late 1950s. The hybrids produced by the cross of a gynoecious and monoecious line resulted in hybrid vigor and a high degree of female sex expression, with uniform and concentrated fruit formation, which was especially advantageous for mechanical harvest (Robinson, 2000).

Because of its tendency to produce mostly female flowers, seed from this type of hybrid usually is blended with seed from a monoecious cultivar added in a proportion of about 10%. This practice improves pollination, which is required for fruit set in genotypes that are not parthenocarpic. However, it has the disadvantage of affecting uniformity, which is one of the principal advantages of hybrid cultivar production (Robinson, 2000).

When the hybrid is parthenocarpic, blending is not required because fruits can develop without pollination. This is the case for many cucumber hybrids used in greenhouse production.

(21) Gynoecious x Gynoecious hybrids

When two gynoecious inbred lines homozygous for the gene F are crossed, the resulting F1 hybrid is homozygous for F. These hybrids are more stable for gynoecious sex expression compared to hybrids produced by crossing gynoecious and monoecious lines. In the case of hybrids heterozygous for the gene F, some environments such as high

temperature and long days may promote the development of male flowers, which is less likely in gynoecious x gynoecious hybrids.

The stability of gynoecious sex expression in these types of hybrids is especially important for parthenocarpic cultivars used for greenhouse production. These cultivars produce long, seedless fruits in the absence of pollination. However, when female flowers are pollinated, the formation of seeds enlarges the fruits at the blossom end, affecting their shape and quality. Using homozygous gynoecious hybrids reduces the chance of pollination and misshapen fruit because these plants produce no pollen (Robinson, 2000).

(22) Flowering model in cucumber

The use of gynoecious lines in hybrid cucumber seed production has many important advantages. The most obvious is the absence of male or hermaphroditic flowers that require emasculation or flower removal. Additionally, female lines are more productive and seed yield is higher. (23) Despite these advantages, there are problems that must be addressed. For example, how are the gynoecious inbred lines maintained if no pollen is produced? Another question is how to use a gynoecious line as the male parent if it produces only female flowers?

In cucumber and other cucurbits, the use of growth regulators to modify sex expression of flowers has been extensively studied. This knowledge has been used to improve hybrid seed production and the multiplication of inbred gynoecious lines. A model that explains the determination of flower sex expression in cucumber has been proposed and we will review this model later in the discussion on how different growth regulators are used for hybrid seed production of cucumber and other cucurbits.

(24) This model proposed by Yin and Quinn (1995) assumes that one hormone, probably ethylene, has male and female receptors and is able to regulate both sexes by inducing one and independently inhibiting the other. The ethylene effect would inhibit development of male structures in the flower, while inducing formation of female structures. This model was tested in cucumber by the application of different hormones and their inhibitors. (25) When gibberellic acid (GA), an inhibitor of ethylene synthesis, was applied, promotion of maleness and reduction of femaleness was observed. (26) Application of silver nitrate, an ethylene inhibitor, had similar effects. (27) On the other hand, when ethrel, an ethylene release agent, was applied, reduction of maleness and promotion of femaleness was observed. (28) This model explains the use of growth regulators to induce or suppress the formation of male flowers in cucumbers and other cucurbits, a practice that have been routinely adopted for seed production since the 1970s.

(29) Use of growth regulators to modify sex expression of flowers

Use of growth regulators to promote maleness

Self-pollination is required to maintain inbred gynoecious lines, but these genotypes do not produce pollen. The solution to this problem came with the discovery of growth regulators able to induce formation of male flowers in these lines.
(30) The first compound used to induce maleness in gynoecious lines was GA. Some recommendations were three applications of GA_3 at 1000 ppm or $GA_{4/7}$ at 50 ppm beginning at the two leaf plant stage and spraying biweekly (George, 1999).

(31) A problem observed with GA use is that different gynoecious lines vary in response to GA application and, in some cases, the number of induced male flowers was not sufficient for hybrid seed production (Robinson 2000). Additionally, GA applications typically cause excessive stem elongation or malformed male flowers.

(32) Because silver ions inhibit ethylene action, an alternative to GA is silver nitrate. This compound induces male flowering in gynoecious lines for extended periods and is often more effective than GA (Robinson, 2000). A single application of silver nitrate solution at 600 mg per liter before the first flower opens has been recommended (George, 1999). Silver thiosulfate is another alternative and appears to be less toxic than silver nitrate.

(33) Use of growth regulators to promote femaleness

There are different compounds that induce of femaleness in cucurbits. However, (34) ethephon, a compound that releases ethylene gas, has been used the most in hybrid seed production. The main practical advantage of ethephon is its persistent effect on some species. The response to ethephon varies among species and cultivars. For example, in monoecious summer squash and cucumber, it can induce the formation of only female flowers for enough time to permit hybrid seed production by open pollination. In *Cucurbita maxima,* using ethephon dramatically reduces the labor required for removing male flowers in hybrid seed production. Ethephon is less effective in promoting femaleness in melon and watermelon.

The most important use of ethephon in commercial hybrid seed production is in summer squash (*Cucurbita pepo*).

(35) When used in hybrid seed production of monoecious cucumbers, ethrel, the trade name for ethephon, is applied in concentrations of 250 to 500 ppm when the plants have 1, 3 and 5 true leaves (Peñaloza, 2001). These applications suppress formation of male flowers during the pollination period. Because of the importance of avoiding self-fertilization in hybrid seed production, the plants should be checked periodically and any male flowers that appear should be removed before anthesis.

Once development of male flowers in the female line has been suppressed, bees can be used for pollination. Five to six hives per hectare are recommended to ensure successful pollination. In the field, the proportion of female and male plants is usually 3 to 4 female plants per each male plant.

(36) An important practical implication of using ethephon in hybrid seed production of cucumber or summer squash is that the plants must be very uniform when the product is applied. This requires good agronomic management of the crop, which includes adequate soil preparation, sowing, irrigation, fertilization, and weed and pest control. If the plants are uneven in growth at the time of ethephon application, some plants will receive the product at differing stages of development causing those plants to produce male flowers that results in undesired pollen contamination.

(37) Isolation

When hybrid seeds are produced in the field, an isolation distance of at least 1000 m from other cucumber plants is required. In the case of foundation seed production, isolation should be at least 1500 m. These isolation requirements may be avoided when seeds are produced inside insect-proof structures (George, 1999), such as shown in this picture.

(38) Harvesting

Ideally, fruits should be harvested manually at the moment they are fully mature and seeds reach their maximum physiological quality. This moment is often determined by the color of the mature fruit, which may vary among cultivars, but usually is a clear brownish color.

The maturity of the seeds may be confirmed by cutting the fruit longitudinally and determining that the seeds separate easily from the interior flesh (George, 1999). For gynoecious and parthenocarpic cultivars, acidity of the fruit pulp may also be used as a harvest index (Peñaloza, 2001).

After harvest, seeds are extracted by scraping the open fruit manually or using a crusher and seed extractor (George, 1999). The extracted seeds can be fermented for about one day and then washed. Hydrochloric acid at a concentration of 10% may be used to accelerate fermentation in high quality seed lots (Peñaloza, 2001).

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