

(1) Hybrid seed production in vegetables

Tomato and Pepper

Samuel Contreras
Departamento de Ciencias Vegetales
Pontificia Universidad Católica de Chile
Santiago – Chile
scontree@uc.cl

(2) Introduction

Solanaceae Family

The Solanaceae family includes more than 2,600 species, many of which are important for humans. Some common uses of these species include food, medicine (e.g. *Atropa belladonna*), ornamental (e.g. *Petunia hybrida*), and industrial applications (e.g. *Nicotiana tabacum*). There are several important vegetables included in this family, such as tomato (*Lycopersicon esculentum*), potato (*Solanum tuberosum*), sweet pepper (*Capsicum annuum*), chilli pepper (*Capsicum frutescens*), and melon pear (*Solanum muricatum*). In contrast, other examples such as *Datura* spp. represent important noxious weeds in agriculture.

During seed production, it is important to be aware of the presence of many of these species because they may host viruses that can infect the mother plant, affect yield and contaminate the seeds.

A general characteristic of this family is that many are herbaceous plants of tropical origin, which makes them susceptible to chilling or freezing injury.

In this lecture, hybrid seed production of tomato and pepper species, with emphasis on the hybridization process, will be presented. In the case of pepper, we will focus on *Capsicum annuum* species that include cultivars of chilis, red, green, yellow, and sweet peppers as well as paprika (George, 1999)

(3) Reproductive structures

Tomato:

Tomato has perfect flowers grouped in compound inflorescences known as a cyme. Each inflorescence usually includes 4 to 8 flowers, and each plant may produce as many as 20 or more successive inflorescences during its life cycle (Tigchelaar, 1986).

In the hermaphroditic tomato flowers, five or more anthers are united forming a cone that encloses the pistil, which is usually shorter than the anthers, especially in commercial cultivars. This characteristic facilitates self-pollination of the flowers.

In this picture, the whole cone of anthers is observed that may be removed from the perfect flowers, leaving just the pistil.

(4) Pepper:

Pepper flowers are usually borne singly at the intersection between the stem and leaves at points where the stem splits into two. The pedicel length varies among cultivars, ranging from 3 to 8 cm (Berke, 2000). *Capsicum annuum* usually has white petals and 5 to 7 individual stamens with pale blue to purple anthers. The pistil is composed of an ovary containing 2 to 4 carpels or locules, a style that is 3.5 to 6.5 mm long and a stigma with a diameter slightly greater than the style (Berke, 2000). The length of the style and relative position of the stigma and the anthers vary among genotypes, and it is an important factor determining the level of natural cross pollinations of the flowers.

(5) Natural Pollination Mechanisms

As previously discussed, species may be classified either as self-pollinated or cross-pollinated. However, among and within species, there is a continuum of variation in the relative frequency of self- or cross-pollination.

Tomato is a species that may be classified as self-pollinated or autogamous; however, classification of pepper is more difficult, because of the differences observed among genotypes, and even within a genotype depending on the environment.

(6) In the case of tomato, flower morphology usually favors self-pollination. The anthers surround the pistil and pollen is shed when the stigma is receptive. Rates of natural cross-pollination in temperate zones have been reported to vary between 0.5 and 4% (Tigchelaar, 1986). Some of the reasons that may contribute to cross-pollination are (George, 1999):

- i) The possibility of insects introducing foreign pollen to the flower. This chance increases in flowers that have been emasculated for hybrid seed production. Additionally, in areas close to the native habitat of the species, i.e. west side of South America, insects able to cross pollinate the plants are more likely to be found.
- ii) Despite the trend of breeding by developing short-styled cultivars and high rates of self-pollination, there are some cultivars with flowers possessing longer styles, which facilitate cross-pollination.

Additionally, high temperature and low irradiation may favor elongation of the style, increasing the chance of cross-pollination (Peñaloza, 2001).

(7) In the case of pepper, the frequency of cross-pollination is greater than for tomato. For example, cross pollination occurs between 8 and 37% of the time, and as great as 68% has been reported (Greenleaf, 1986; George, 1999). A factor that affects the percentage of cross pollination is the relative position of the stigma and anthers, which depends on the genotype. The presence of pollinating insects and receptivity of the stigma 2 to 3 days before pollen shed in some cultivars is another factor (George, 1999).

(8) Fruits and seeds

Tomato

The tomato fruit is a berry, with fleshy pericarp that encloses two or more locules. Most commercial cultivars have three to five locules. The tomato in this picture has three locules.

(9) The seeds are located inside the locular cavities, attached to the placenta. Each fruit may have 100 to 500 seeds depending on the cultivar and environmental conditions. Tomato seeds form a thick, outer epidermal layer of cells that partially break down forming the “hairs” that surround the seeds. These hairy structures collect the gelatinous material that gives the seeds a “gooey membrane” (Rost, 1996). After harvest, during the fermentation process, seeds are removed from this membrane.

As may be seen in this scheme, the seeds are composed of the testa, the embryo that curves inside the seed and includes a root apex and two cotyledons, and an endosperm surrounding the embryo.

(10) Pepper

Pepper fruits are berries of shape, color, and sizes that vary among cultivars. As with tomato, they present a fleshy pericarp that encloses two or more locular cavities. For example, the green peppers in this figure have four locular cavities.

Numerous seeds are attached to the placenta tissue. Unlike tomato, the locular cavity of pepper fruits is not filled with a gelatinous material surrounding the seeds.

(11) Similar to tomato, pepper seeds have a curved and flattened embryo surrounded by an abundant endosperm and testa. Pepper seeds contain 22% lipids and 18% proteins.

(12) Advantages of hybrid cultivars in tomato and peppers.

For tomato and pepper both standard and hybrid cultivars are available. Standard or open-pollinated seed is much less expensive than hybrid seed; the reason is because of the type of production, specifically the hybridization process, which is very expensive. Despite their higher cost, the use of hybrid seed is increasing in popularity. In general, hybrid cultivars are preferred because of their vigor, uniformity, disease resistance, stress tolerance and excellent horticultural traits such as long shelf-life. From the breeder’s perspective, development of hybrid cultivars allows better control of intellectual property rights including control and protection of parental lines (Tay, 2002).

(13) Seed production under protected structures

Commercial production of tomato and pepper seeds may be performed in the open field or under protected structures, such as greenhouses. Both systems have advantages and disadvantages that must be considered. Factors that affect this decision include the area of production and the type of seed to produce. For example, OP seed probably will be produced in the open field because it is of lower commercial value that does not justify a high investment in resources during production. On the other hand, hybrid seed is of much higher value which justifies its production under protected structures. In areas with favorable weather (i.e. absence of rain and low temperatures), low disease pressure and no isolation problems, hybrid seed production can be performed in the open field without sacrificing seed yield or quality. But, in most cases, it is preferable that hybrid seed production occurs under some type of protection. This can vary from a simple net covering of one row or a group of plants in the field to sophisticated greenhouses with accurate control of environmental conditions, watering and plant nutrition.

The following pictures show different tomato and pepper production in the open field. In this case, it is important to select areas with favorable climate, availability of irrigation and low weed, pest and disease pressure. Optimal temperatures depend on the species and genotypes, but, in general, temperatures around 18-27°C during the day, and 15-18°C during the night are preferred. Higher temperatures cause flower abscission and problems in pollination, fecundity and fruit set. Relative humidity should be greater than 70% in order to favor pollination, but not too high because it could cause pollen agglutination and pollination problems as well as favor disease development.

(14) In picture A of this slide, pepper seed production is seen with plants under a nylon net in order to isolate the plants from insects that could cause cross-pollination. Also note that the plants are grown over a plastic mulch.

Pictures B and C show a field in the extreme north of Chile where hybrid seeds of solanaceous species are produced. Production is performed under screenhouses or areas protected by a white fine net. This protection allows an excellent isolation from insects that may cause pollen contamination as well as from insects that could transmit viral diseases. This enables the production of genetically pure and pathogen-free seeds. Picture C also shows soil fumigation before planting the seedlings.

(15) In this picture, tomato plants growing inside high tunnels are shown. This hybrid seed production is in the central zone of Chile, close to Santiago. In this case, the use of high tunnels extends the growing season and attains higher seed yields.

(16) These two pictures show hybrid seed production of sweet pepper under high tunnels, also in the central zone of Chile. Again, these structures extend the growing season and isolate the plants from possible sources of pollen or disease contamination. In order to attain this last objective, the integrity of the plastic covering the tunnels is important, as well as blocking any possible point of access for insects.

(17) In general, seed production under protected structures offers several advantages such as a more favorable environment for plant growth and extending the growing season,

along with protecting plants from pollen and/or disease contamination. On the other hand, these structures are a significant cost that may be justified only in cases of producing high value seed, such as hybrid seeds.

(18) 1.3 Hand pollination as hybridization process

Hand pollination is the preferred method for hybrid seed production of many vegetables, including tomato and pepper. Other vegetable species where hybrid seed is produced primarily by hand pollination are eggplants, melon, pumpkin, watermelon and zucchini. The method is simple since it involves the manual emasculation of the anthers from the flowers of the female parent, followed by hand pollination with pollen from the male parent thus preventing the introduction of foreign pollen which may contaminate the pollinated flowers. However, the process is labor intensive and requires skilled growers and pollinators. To be cost-effective, this system only works for species where a single pollination of the female flower will produce many seeds, as is the case of solanaceous and cucurbit crops (Tay, 2002).

In this module, hand pollination to accomplish hybridization will be illustrated for tomato.

(19) For tomato hybrid seed production two parental lines are required. The male line provides the pollen. Fruit production is not important in the male line, so after pollination, male plants can be destroyed. On the other hand, the female line is responsible for the seed development after pollination. These plants produce the fruits containing the hybrid seed, so adequate agronomic management is fundamental to obtaining high crop yield and seed quality. Both lines may be grown separately in different fields or in different sections of the same field.

The ratio of female to male plants is usually four to six females to one male, depending on the flowering ability, pollen productivity and fertility of the male (Tay, 2002).

Depending on the timing of flowering of each line, the planting date may be adjusted. Usually the male line is planted 1 to 3 weeks earlier in order to produce enough pollen when female plants are ready for pollination. An additional advantage of early male planting is that it provides more time for expression of off-types plants, allowing more accurate roguing.

All plants for both lines should be checked for trueness-to-type before starting hybridization. Any off-type plant should be removed to ensure a high genetic purity.

(20) This is a scheme of the work required during hand pollination for tomato hybrid seed production. The first step in the process is the emasculation of the female-plant flowers. Second, the pollen is extracted from the male plants. Third, the pollen is stored, and fourth the pollen is transferred manually to the emasculated flowers.

(21) In these two pictures, the emasculation process is seen. This is critical during hybrid seed production, because it affects both yield and seed quality. If emasculation is performed too early, the pistil of the flower could be damaged. Because the ovary of the pistil is the organ that develops into the fruit and is where the seeds are produced, any damage to this structure influences yield. On the other hand, if emasculation is done too late, pollen from the anthers could reach the stigma causing self-pollination. Seed produced in this way is not a F1 genotype but possesses the same genotype as the mother line, so it results in contamination and reduced genetic purity of the seed.

In tomato, anthesis within each inflorescence is not uniform, which is a problem in seed production. The optimal moment for emasculation must be determined for each flower and is generally one to two days before anthesis, when the flower is still green, the sepals have begun to separate and the petals have not reached their final dark yellow color. The whole anther cone can be removed with forceps or directly by hand. Usually, emasculation is done in the afternoon and the emasculated flowers are pollinated the following day.

An emasculated flower is susceptible to contamination by foreign pollen, so proper isolation is important. When female plants are under protected structures, it is important to ensure that no insects are present. When produced in the open field, spatial isolation of 50 to 200 m from other genotypes may be required.

(22) Pollen is extracted from open dark yellow flowers of the male line. After male flowers are collected, their anther cones are removed and dry. Once the anthers split, pollen can be shaken into a closed container. The pollen is then separated from other flower parts by sieving through a 200-300 mesh screen (Tay 2002).

(23) Once extracted, tomato pollen can be stored in a cool (0 to 5 °C) and dry place for several (30-60) days. This picture shows an example in which pollen is stored inside moisture proof containers containing silica gel. Each container is properly labeled with the parental line information and day of collection and stored in a refrigerator.

(24) Pollination is performed 24 to 72 hours after emasculation. The timing depends on environmental conditions at each production area.

Normally, pollination is accomplished early in the morning. There are different techniques to place the pollen onto the stigma surface. Alternatives include dipping the stigma into a small pollen container or on a finger containing the pollen. Another possibility is using a brush. Regardless of the technique used, it is important to introduce enough pollen onto the stigma to ensure high seed set, because pollination is done only once on each flower and one pollen grain is responsible for the formation of one seed. Once pollination is done, two to three sepals are cut with a small scissor to document that the flower has been pollinated and identify the hybridized fruit during harvesting (Tay, 2002).

After pollination and before harvest, those fruits that have not been hand-pollinated, are removed from the plant in order to avoid contamination at harvest and reduce competition for nutrients from the hand-pollinated fruits.

(25) Genetic male sterility as an alternative hybridization technique

As previously mentioned, hand pollination is an expensive labor process during hybrid seed production of tomato and peppers. During this process, flower emasculation is especially critical because it is labor-intensive and affects both seed yield and genetic purity. Male sterility, or the failure of a plant to produce functional pollen, may be used to avoid the emasculation requirement, reducing the amount of labor required for hybridization. Additionally, it reduces the chance of self-pollination or damage to the pistil.

(26) There are two types of male sterility, genetic and cytoplasmic.

Genetic male sterility is used for pepper hybrid seed production. This type is generally controlled by single recessive nuclear alleles. In this figure, this allele is represented by *ms*. Because *ms* is recessive, only the homozygotic genotype “*ms ms*” is sterile, while the heterozygotic genotype “*Ms ms*” and the homozygotic “*Ms Ms*” are fertile.

(27) In this slide, a scheme of how genetic male sterility is accomplished for hybrid seed production is presented. The hybrid seed is produced by crossing a male line that is male fertile and homozygous for the dominant form of the *MS* allele with a female line that is male sterile, i.e. homozygous for the recessive form of *ms*. The hybrid seed is completely heterozygous for *ms*, and therefore 100% male fertile.

(28) An important aspect of this type of hybrid seed production is how to maintain the male sterile line. Obviously it can not be multiplied by self-pollination because it does not produce functional pollen. So, for keeping and multiplying the male sterile line, two isolines are used. These isolines are genotypes almost identical to each other with the exception that one is homozygous for the dominant form of *MS* and the other is heterozygous for this allele. As a consequence, the progeny of the cross between the two isolines is 50% heterozygous for *MS* and 50% homozygous for the recessive form of *ms*.

(29) A disadvantage of this process is that 50% of the progeny, i.e., all the heterozygous genotypes, must be eliminated prior to pollination because these plants are fertile.

In some cases, *ms* alleles are linked with marker traits that may help in early identification of male-sterile plants, so the male fertile genotypes can be eliminated during their early development. **(30)** However, for many of the available male-sterile lines of pepper, the male-fertile individuals are only possible to identify and eliminate at flowering. In these cases, it is necessary to plant the female lines at two times the normal plant density, so when the male-fertile individuals are eliminated, the plant population is close to normal. However, this procedure involves extra cost of production and planting more seedlings, and later identification and removal of male-fertile plants. Plant distribution may be affected as well.

(31) Harvest, seed extraction and drying

Harvest timing

The timing of harvest is an important decision in seed production because it affects both yield and seed quality. During their development, seeds reach a peak of maximum quality and it is important to perform harvest at this moment.

Some studies have attempted to identify the optimal time for tomato seed harvest. In order to have practical significance, these studies have related the stage of seed development with external fruit appearance. In this picture, the different maturity stages that have been described for tomato fruits are presented.

(32) The time of maximum seed dry weight or physiological maturity has been observed between the mature green and breaker stages of tomato fruits.

(33) However, maximum seed quality has been obtained when red fruits are harvested.

(34) In this graphic, the ability of the seed to germinate after storage under unfavorable environmental conditions or when overripe fruits are harvested is presented. Thus, as a general recommendation for tomato, red fruits should be harvested.

(35) Harvest

In hybrid seed production of tomato and pepper, fruits are harvested manually. During this time, only fruits from hand pollinated flowers are picked. Any other fruit must be eliminated because they would be products of self-pollination and, if harvested, they would decrease genetic purity of the seed lot. In the case of tomato, hand pollinated fruits are identified because their sepals were cut at the time of pollination.

(36) In the case of pepper, hand pollinated flowers are labeled with a “twister” placed on the pedicel. Only fruits that have this label are harvested.

For peppers, in general, the optimal moment for harvest is when the whole fruit has reached the color characteristic of each genotype; it could be red, yellow, purple, etc.

(37) Seed extraction

After the fruits have been collected, it is necessary to separate the seeds from the flesh of the fruits. This is done by placing the fruits into a “crusher” that squashes or crushes the fruits. The resulting mix of gelatinous seeds, juice and fruit residue then moves through a revolving cylindrical screen (George, 1999). The juice and gelatinous seed moves through the cylinder screen and is then recollected; the fruit residue passes through the cylinder. These residues may be collected and used in composting.

In these pictures, the extraction of pepper seeds is seen. Because the pepper fruit is dryer than the tomato fruit, it is necessary to add extra water during fruit crushing and in the

screen cylinder to facilitate the separation of fruit and seeds. In the picture on the right, seed collected after it passes through the screen cylinder is seen.

(38) Here is the same process for tomato. The picture on the left shows how the fruit debris is collected and later used in composting.

(39) In the case of tomato, once the seeds are extracted from the fruit, they must be separated from the covering gelatinous material.

(40) There are several alternatives to achieve this objective:

- For **natural fermentation**, the pulp containing the seeds is left to ferment for up to 3 days at temperatures between 18 and 25C (George, 1999; Peñaloza 2001). The time of fermentation depends on the temperature. The mix of gelatinous seeds and juice must be stirred frequently in order to achieve a uniform fermentation rate in the container. Fermentation is done when the gelatinous coating of the seeds has broken; exceeding the fermentation time negatively affects seed quality (George, 1999).

- **Separation with sodium carbonate** can be used as an alternative to fermentation to separate small lots of seeds. The seeds and pulp are mixed with an equal volume of a 10% solution of sodium carbonate (George, 1999). This mixture is left at room temperature for 18 to 48 h and the seeds then thoroughly washed. With this method seeds tend to get darker. As a result, this technique is not used in commercial seed lots.

- **Separation with hydrochloric acid** consists in adding around 100 ml of the acid to each 5 kg of pulp and left for 30 minutes (Peñaloza, 2001). This method is usually combined with the late stages of fermentation and has the advantage of producing bright, clean seeds.

There is evidence that both sodium carbonate and hydrochloric acid separation methods inactivate the tobacco mosaic virus (TMV) that may be transmitted in the testa of tomato seeds (George, 1999).

(41) After separation, tomato seeds are washed. This is accomplished by using long and narrow water troughs that have riffles and intervals (George 1999). When placed in the trough, seeds sink because of their density, while impurities float and move off with the transported water.

Pepper seeds are not fermented and after extraction with the crusher and revolving cylindrical screen, they are washed in a similar way as tomato seeds.

(42) Seed Drying

Both tomato and pepper seeds must be dried soon after being washed. This may be accomplished in several ways. Independent of the method used, it is important to quickly remove excess water. This can be done by placing the washed seeds inside fine mesh bags and spinning them. Later the seeds are placed in dryers at temperatures that should not exceed 28-30C because this could affect seed physiological quality.

The drying process may extend for 2 to 4 days. During this time, it is important to stir the seeds several times each day so they dry uniformly.

In this picture, tomato seeds are dried inside nylon bags over a dryer that forces the movement of warm air through them.

(43) This picture shows different seed lots being dried at the same time. Inside each bag, along with the seeds, is an identification label. This emphasizes the importance of keeping good track of parental lines and hybrid seed during all phases of the production process.

Overall, hybrid seed production of tomato and pepper is an expensive process, due primarily to the work force required for emasculation and pollination. Because of this, hybrid seed of these species is significantly more expensive than for open-pollinated cultivars. Some of the requirements to attain high yields and seed quality are: i) having an adequate knowledge of the reproductive biology of the species, ii) performing good agronomic management of the plants, including harvest at the right time and proper seed extraction and drying, and iii) protecting genetic purity of parental lines and hybrid seed during all phases of the the production process.

References

- Berke, T.G. 2000. Hybrid Seed Production in *Capsicum*, p 49- 67. *In* Hybrid Seed Production in Vegetables, Rationale and Methods in Selected Species, A.S. Basra (ed.). Food Products Press. 135 p.
- George, R.A.T. 1999. Vegetable Seed Production. CABI Publishing. 327 p.
- Greenleaf, W.H. 1986. Pepper Breeding, p 67- 134. *In* Breeding of Vegetable Crops, M. Bassett (ed.). AVI Publishing Company, INC. 584 p.
- Peñaloza, P. 2001. Semillas de Hortalizas, Manual de Producción. Ediciones Universitarias de Valparaíso, Universidad Católica de Valparaíso. 161 p.
- Rost, 1996. Introduction to the tomato. <http://www-plb.ucdavis.edu/labs/rost/Tomato/Intro/Intro/PLB.htm>
- Tay, D. 2002. Vegetable Hybrid Seed Production. P 128- 139. *In* Proceedings International Seed Seminar: Trade, Production and Technology. M. McDonald and S. Contreras (ed). Pontificia Universidad Católica de Chile, Facultad de Agronomía e Ingeniería Forestal, Departamento de Ciencias Vegetales. October, 15th and 16th, 2002. Santiago - Chile.
- Tigchelaar, E.C. 1986. Tomato Breeding, p 135- 171. *In* Breeding of Vegetable Crops, M. Bassett (ed.). AVI Publishing Company, INC. 584 p.

About tomato Hybrid seed production:

<http://www.avrdc.org/LC/tomato/hybrid/01title.html>

