CHAPTER THIRTEEN

Techniques for Artificial Hybridization

Successful and efficient artificial hybridization is an important aspect of plant breeding. It requires knowledge of the reproductive structure and development of the species, the conditions needed to promote flowering and seed development, and the procedures for emasculation and pollination. There is extensive variation among species for all aspects of artificial hybridization; therefore, it is only possible in this chapter to review some of the general principles that are involved. Specific procedures used in artificial hybridization of plant species are provided in Bassett (1986), Fehr and Hadley (1980), and Fehr (1987).

REPRODUCTIVE STRUCTURE AND DEVELOPMENT

The location of the male and female reproductive organs and the timing of their development are major considerations in artificial hybridization. The flowers may be bisexual and contain both the male and female organs, or the male and female organs may occur in separate unisexual flowers (Lersten, 1980) (Chap. 2). In monoecious species, the male and female organs are located in separate unisexual flowers on the same plant. In dioecious species, unisexual male and female flowers are located on separate plants. Lengths of the styles and stamens are similar in most species with bisexual flowers; however, there are species with pin flowers, in which the style is longer than the stamen, or thrum flowers, in which the stamen is longer than the style.

The female organ in a bisexual flower generally is receptive to pollination at the time pollen is shed, and fertile male pollen must be eliminated to avoid self-pollination. The process, referred to as emasculation, may require manual removal of the anthers, or a more indirect procedure may be adequate.

The reproductive organs of bisexual flowers in leguminous species may be protected by a calyx, corolla, or both. In grass species, the reproductive organs of bisexual flowers may be enclosed by glumes, a lemma and palea, or both. The protective structure surrounding the reproductive organs may have to be partially or entirely removed during artificial hybridization.

The number of anthers in a flower varies among most species from 3 to 10. The anthers within a flower may be independent, fused, or both. Their size ranges from less than 1 mm to several millimeters in length. Procedures for manipulation of the anthers during emasculation and pollen collection vary widely among species.

FLORAL INDUCTION

The simultaneous flowering of parents to be mated and the suitability of the flowers for successful artificial hybridization are major considerations in artificial hybridization. The environmental conditions that prompt floral induction and adequate flower development may vary widely among genotypes within a species, as well as among species. The primary environmental factors considered for the development of suitable flowers and successful seed set are light, temperature, moisture, and soil fertility (Major, 1980).

Light

Induction of flowering in many species is controlled by the duration of the dark period. By tradition, however, it is referred to as a day-length rather than a night-length response. The response of genotypes to day length is divided into three categories: short-day, long-day, and day-neutral. A short-day plant flowers when the day length is equal to or less than a critical duration, referred to as the critical photoperiod. A long-day plant flowers when the day length is equal to or greater than the critical photoperiod. Floral induction of a day-neutral plant, also referred to as a day-length-insensitive plant, is not controlled by day length.

Genotypes within a species often vary in the critical day length required to induce flowering. Their response generally reflects the day-length conditions present during the growing season in the geographical area to which they are adapted. A genotype of a short-day species grown at a low latitude where day length is short would have a much shorter critical photoperiod than a genotype adapted to higher latitudes. The reverse would be true for a long-day species.

Photoperiod, light level, and quality can influence floral induction of some species when artificial lighting is used to delay or induce flowering. Light level refers to the intensity present at the plant surface. Flowering generally can be delayed or induced at light levels far below that of sunlight. Light quality refers to the relative amount of different wavelengths emitted by the light source. An incandescent lamp has a higher percentage of the red wavelength than does a fluorescent lamp. There is variation among genotypes within a species and among species for the quality of light that will most effectively control flowering; therefore, a single type of lamp is not suitable for all species.

Experiments on the effect of light on flowering generally have considered only the presence of flowers, regardless of their suitability for artificial hybridization. The use of extremely short days for a short-day species may promote flowering, but the flowers may be too small to manipulate for artificial hybridization.

Temperature

A well-known effect of temperature on flowering is the vernalization requirement of some species. Vernalization is the exposure of plants to a period of cold temperature to enhance their sensitivity to stimuli that promote flowering. Winter wheat is an example of a species with a vernalization requirement. When sown in the fall and exposed to winter temperatures, the species will flower and mature during the following summer. The same wheat sown in the spring will not flower normally or produce adequate seed in the summer.

The influence of temperature on the induction of flowering can involve minimums, maximums, cumulative amounts of heat, or a combination of the three factors. In maize and sorghum, for example, the expected flowering date of a genotype can be defined by the number of growing-degree-days that it must accumulate during the growing season. The number of growing-degree-days for a 24-hour period is computed from the equation [(daily maximum temperature + daily minimum temperature)/2] − 10°C. Temperatures above the maximum or below the minimum do not enhance the initiation of flowering. In maize and sorghum, 30°C is considered the maximum and 10°C the minimum temperature. If the maximum for a day were 33°C and the minimum 8°C, there would be $[(30 + 10)/2] - 10 = 10$ growing-degree-days. If the maximum daily temperature were 29°C and the minimum 12°C, the number of growing-degree-days would be $[(29 + 12)/2] - 10 = 10.5$.

Temperatures that are adequate for natural hybridization may not be suitable for artificial hybridization. At an excessively low or high temperature, pollen shed in bisexual flowers may be too limited for artificial hybridization, even though there are adequate amounts of pollen for natural self-pollination.

Moisture

Inadequate or excessive moisture during flowering can reduce the success of artificial hybridization. Whenever possible it is desirable to control by irrigation the availability of soil moisture. The relative humidity of the air can influence the tendency of exposed flowers to become excessively dry and abort, regardless of the soil moisture available. In some species, the relative humidity of the air surrounding a flower that is used as a female in artificial hybridization will be kept as high as possible by enclosing the pistil with the outer parts of the flower or placing the entire flower in an appropriate bag.

Soil Fertility

The principal objective in soil fertilization is to obtain plants that are vigorous and healthy. Such plants are more likely to have normal flower development, pollen shed, and seed set, which enhance the success of artificial hybridization. Nitrogen levels are important for nonleguminous species or for legumes that are not properly inoculated. Phosphorus and potassium levels should be maintained at adequate levels, and special nutrients should be provided as needed.

TECHNIQUES FOR ARTIFICIAL HYBRIDIZATION

Synchronization of Flowering

The first step in artificial hybridization is to obtain receptive flowers of the female parent at the time when suitable quantities of fertile pollen are available from the male (Fehr, 1980; Major, 1980). Special techniques may be required to synchronize the flowering of parents that normally would not bloom at the same time.

Multiple Planting Dates. The most common method for synchronizing flowering is to plant one or more of the parents on different dates. Three different techniques are used in the field depending on the planting arrangement employed: (a) A separate block is used for each date of planting for unpaired parents. (b) The row for each parent is subdivided into sections for different planting dates. (c) Plantings for different dates are interspersed within a row.

The advantage of using separate blocks for each planting date is the ease of mechanical land preparation and planting. A heavy rain after one planting date could necessitate additional tillage before the next date of sowing. The tillage could be done mechanically if separate areas of a field were available, but hand labor would be required if plants on different dates were placed in a single row for each parent. One disadvantage of separate blocks is the time required to walk between different areas of the field to check for flowers and to make the necessary hybridizations. A second disadvantage is that the method cannot be used conveniently for paired-parent arrangements.

The advantages and disadvantages of use of the same row for multiple planting dates are the reverse of those described for use of separate blocks. There are two advantages of subdividing a row into sections in comparison with an interspersed planting. First, it is easier to till and plant a section of row than to make individual plantings at regular intervals within a row. Second, plants from the first date of an interspersed planting may suppress growth of adjacent plants from later dates; this is not a problem when separate sections of row are used. The primary advantage of interspersed planting on different dates is realized in a testcross nursery when open-pollination is used to obtain hybrid seed. Multiple-date plantings are interspersed along the rows of the male parent to obtain a more uniform distribution of pollen than can be realized when distinct sections of row are used for different planting dates.

The interval between planting dates depends on the difference in maturity among the parents and the average environmental conditions that occur at a location, particularly light and temperature. Practical experience is needed to arrive at the appropriate interval.

Day Length. There are two ways in which day length is used to synchronize flowering of parents. One procedure is to artificially shorten or increase the day length of some parents but not others. Shortening the day length can be accomplished by covering plants with a lightproof container. Increasing the day length requires some type of artificial lighting.

A second procedure that is successful for some species is to use environments in which the day length is short or long enough to promote flowering of all parents simultaneously. The natural day length at higher latitudes changes during the summer, which promotes differential flowering among short-day genotypes. Simultaneous flowering may occur, however, if the parents are grown in an off-season field nursery at a lower latitude, where day length is shorter than the critical photoperiod for all genotypes. A similar response may be observed for genotypes of a long-day species by artificially increasing the day length beyond the critical duration.

Temperature. Temperature can be manipulated to synchronize the flowering of parents in some species. The number of growing-degree-days can be altered artificially by maintaining the temperature of some parents higher or lower than that of other parents.

Grafting. The growth factors responsible for initiating flowering can be transferred from one parent to another by grafting. The scion of a parent with delayed flowering may be grafted to a parent with an earlier flowering tendency. The growth factors from the earlier parent pass to the scion and initiate flowering in the later parent sooner than if grafting were not used.

Pruning. Removal of the growing point from a plant frequently causes the initiation of tillering or branching. The onset of flowering on the new vegetative growth may be delayed compared with that of unpruned plants. The delay caused by pruning may be sufficient to synchronize the flowering of parents.

Flower Removal. Some species have immature flower buds that do not fully develop unless older flowers on the plant are removed. The systematic removal of flowers from parents of such species can extend their normal flowering duration, a delay that may be adequate to permit hybridization with a later-flowering genotype.

Plant Population. Flowering on tillers and branches of some species may be delayed compared with flowering on the main stem. Use of a low plant population (density) for some parents can promote the tillering or branching necessary to obtain flowers at the appropriate time for mating with later-flowering parents.

Growth Factors. Application of growth factors to plants is a potential means for artificial induction of flowering. The use of such factors may become more widespread when they have been properly isolated and characterized.

Hybridization

Choice of Female and Male Parents. In a cross between two parents, one must be used as the female and the other as the male. It may make no difference which parent is used as female, or the choice may need to be carefully planned.

One common reason for careful selection of the female parent is to permit the differentiation of progeny that are true hybrids from those that are the result of accidental self-pollinations (Fehr, 1980). To make this distinction, the female parent must have recessive alleles and the male parent dominant alleles for a qualitative character controlled by nuclear genes. Seeds or plants obtained after artificial hybridization are hybrids if they have the dominant character of the male, but are the result of accidental self-pollination if they exhibit the recessive character of the female.

The success of artificial hybridization may be influenced by the parent chosen as female, particularly for interspecific hybridization. Reciprocal crosses often are advisable when matings are attempted between parents of species that may be difficult to cross.

Emasculation of Bisexual Flowers. The elimination of fertile pollen is necessary if bisexual flowers are to be used as the female for artificial hybridization. The pollen in the anthers may be rendered inviable or inactive by appropriate treatments or the anthers may have to be removed manually.

Emasculation Without Anther Removal. Emasculation of the bisexual flowers of some species is unnecessary if the stigma is receptive before the anthers are able to shed pollen. The structure enclosing the reproductive organs is removed, pollen from the male parent immediately is placed on the receptive stigma, and the flower is not covered after pollination. If fertilization does not occur, the exposed stigma loses its receptivity before the anthers in the flower are able to shed pollen.

Treatment of the anthers with heat, cold, or chemicals has been used to inactivate pollen. Hot-water emasculation consists of soaking flowers at a water temperature high enough to kill the pollen without injuring the stigma. High and low air temperatures have been successfully used to temporarily or permanently inactivate pollen. Soaking flowers in alcohol has been successful for inactivating pollen without injuring the stigma. The proper percentage of alcohol and duration of soaking are important variables for successful alcohol emasculation.

High relative humidity has been used to delay pollen shed without destroying fertile pollen. Although dehiscence control is not a form of emasculation per se, it does permit artificial hybridization of bisexual flowers of some species without anther removal.

Emasculation by Anther Removal. Removal of immature anthers from a bisexual flower is a direct method for eliminating male-fertile pollen. One procedure is to open or remove the structures enclosing the reproductive organs and remove the anthers with a forcep, pencil, or other appropriate instrument. Vacuum pressure sometimes is used to remove the anthers by suction. Removal of the floral structure enclosing the reproductive organs can simultaneously remove the stamens when the two are fused.

Partial removal of anthers by clipping them with a scissors has been used as a rapid method of emasculation. A flower is cut low enough to at least partially remove the anthers without injuring the stigma. With removal of part of the structures that enclose the flower, the cut anthers generally dry up before they can shed pollen. Accidental self-pollination is more of a risk with scissor emasculation than with removal of the entire anther.

Protection of Unisexual Female Flowers. Emasculation is not a concern for flowers that contain only the female organ, that are male-sterile, or that exhibit self-incompatibility. It may be necessary, however, to cover the flowers to prevent undesired pollinations. The same protection may be necessary for flowers that are artificially emasculated but not pollinated immediately after emasculation.

Pollination. Pollination may be done immediately after emasculation or may be delayed for up to several days, depending on the species. Although emasculation and pollination are separate operations, they must be properly synchronized to achieve success. Pollen may be collected and applied manually to the stigma or pollination may be accomplished indirectly.

Direct Pollination. Pollen must be collected from flowers that have not been contaminated by viable pollen from other parents. For some species, the flowers to be used as male are covered for a period of time before pollen is collected, so that any contaminating pollen on the flowers dies.

There are many different methods of pollen collection and application. Entire flowers may be removed and taken to the female parent. Pollen may be collected by placing the male flowers in a bag and tapping the bag to cause pollen shed. Mechanical vibrators may be used to rupture the anthers so that pollen can be collected in a vial. Vacuum pressure may be used to remove undehisced anthers from flowers. Plants may be cut off and placed in a container until pollen is shed naturally and is collected.

Loose pollen may be applied directly on the stigma by pouring it from a container; placing the stigma into the pollen; or applying the pollen with a brush, piece of cotton, or other instrument. An anther or a pollen-laden stigma from the male flower may be manually brushed against the stigma of the female flower to cause pollination.

Indirect Pollination. Pollination can be accomplished without directly applying pollen to the stigma of a female flower. For indirect pollination, the structures enclosing the female organ are completely or partially removed. An inflorescence of flowers shedding pollen can be swirled around the female, causing pollen to fall on the stigma, a method referred to as the go-go or swirl method. The approach method consists of placing an inflorescence of male flowers above the female flowers. Over a period of time, pollen shed from the male falls onto the stigmas of the female parent.

Mutual pollination is the reciprocal pollination of parents, each of which has a high level of self-incompatibility. Inflorescences of the parents are placed together in a bag to allow pollen to pass readily from one to the other and to protect the parents from undesired sources of pollen.

Protection of a Pollinated Flower. A newly pollinated flower may be subject to desiccation and contamination from undesirable pollen. The female organ may be covered by the floral parts that normally enclose it, unless these parts were removed while preparing the flower. Artificial enclosures include such items as bags and soda straws. Flowers of some species require no protection of any kind after pollination.

Labeling of Flowers. Female flowers used for artificial hybridization generally are identified by a tag, wire, bag, or other appropriate label. The label may be placed on the female at the time it is prepared for pollination or after pollination is completed. Information on the label may include the date of female preparation, date of pollination, position of the female flower, name of the female parent, name of the male parent, and name of the person who did the work. The differentiation of female flowers used for hybridization from those that were not used also can be aided by the removal of distinguishing floral parts during hybridization.

Obtaining Seed. The amount of hybrid seed obtained by artificial hybridization varies from one to several hundred per pollination, depending on the species. For some species, inexperienced persons can successfully carry out the work. Successful artificial hybridization for other species requires considerable practice and skill.

REFERENCES

Bassett, M. J. 1986. Editor. *Breeding vegetable crops.* AVI Publishing Co., Westport, Conn.

Fehr, W. R. 1980. Artificial hybridization and self-pollination. pp. 105–131. *In* W. R. Fehr and H. H. Hadley (eds.), *Hybridization of crop plants.* American Society of Agronomy, Madison, Wis.

Fehr, W. R. 1987. Editor. *Principles of cultivar development.* Vol. 2. *Crop species.* Macmillan, New York.

Fehr, W. R., and H. H. Hadley. 1980. Editors. *Hybridization of crop plants.* American Society of Agronomy, Madison, Wis.

Lersten, N. R. 1980. Reproduction and seed development. pp. 17–43. *In* W. R. Fehr and H. H. Hadley (eds.), *Hybridization of crop plants.* American Society of Agronomy, Madison, Wis.

Major, D. J. 1980. Environmental effects on flowering. pp. 1–15. *In* W. R. Fehr and H. H. Hadley (eds.), *Hybridization of crop plants.* American Society of Agronomy, Madison, Wis.