The soybean [Glycine max (L.) Merrill] is an annual legume that originated in China and has been cultivated for centuries. The crop is grown throughout much of the world with the largest production in the United States, Brazil, People's Republic of China, Mexico, Indonesia, and Argentina. Soybean seed contains about 40% protein and 20% oil and is used for human consumption, livestock feed, and industrial purposes.

I. PARENTAL MATERIAL

Cultivated soybean belongs to the family Leguminosae, subfamily Papilionoideae, and genus Glycine. The genus presently includes six perennial species in the subgenus Glycine Wild. and two annual species in the subgenus Soja (Moench) F. J. Herm. The annual species, consisting of the cultivated type max and the wild type soja Sieb. and Zucc., have a chromosome number of 2x = 40 and can be readily intercrossed. The F₁ hybrids from some G. max × G. soja crosses are semisterile due to chromosome translocations (Hadley and Hymowitz, 1973). No successful crosses have been reported between G. max and the six species of the subgenus Glycine (Hadley and Hymowitz, 1973).

More than 5,000 strains of G. max and a smaller number of strains of the other Glycine species are maintained by the U.S. Department of Agriculture at Urbana, Ill., and Stoneville, Miss. Germplasm collections also are maintained by the Applied Genetics Laboratory of the Korea Atomic Energy Research Institute in Seoul, Korea, the National Institute of Agricultural Sciences at Hiratsuka, Japan, and the Asian Vegetable Research and Development Center in Taiwan.

II. PLANT CULTURE

A. Field

Soybeans are adapted to a wide range of soil types, but grow best in well-drained soils with a pH of 6.5 to 7.0. Mineral nutrients required to produce 2,600 kg/ha of seed include 164 kg/ha of N, 16 kg/ha of P, and 45 kg/ha of K (DeMooy et al., 1973). Soybeans can fix symbiotically much of their own N if adequate numbers of rhizobia [Rhizobium japonicum (Kirchner) Buchanan] are applied at planting or are present in the soil. Soybeans respond equally well to direct application of P and K or residual from the previous crop. Secondary elements and micronutrients are adequate in most areas, but deficiencies can occur on light textured, acid, or calcareous soils.

With adequate mineral nutrition, vegetative and reproductive development usually are proportional to the available moisture supply (Howell, 1963). Moisture stress, one of the principal reasons for flower and seed abortion, is associated with inadequate soil moisture and high evapotranspiration (Shibles et al., 1975). Plants may wilt when evapotranspiration is high, even if the soil moisture supply is adequate. The interrelationship between evapotranspiration and flower and seed abortion may explain the common observation of soybean breeders that successful hybridization may be difficult on sunny, windy days when the temperature exceeds 35 C and relative humidity is low. Irrigation can be valuable for successful hybridization and for high yields of self-pollinated seed.

The soybean is a short-day plant, but there is considerable genetic variation for sensitivity to photoperiod (Hamner, 1969; Criswell and Hume, 1972). The critical day length for flowering ranges from about 13 hours for genotypes adapted to tropical latitudes to 24 hours for photoperiod-insensitive genotypes grown at higher latitudes (Shibles et al., 1975). Soybeans seem to be insensitive to day length for 9 days after emergence. Photoperiods shorter than the critical day length are required for 7 to 26 days to complete flower induction (Borthwick and Parker, 1938; Shanmugasundaram and Tsou, 1978).

Sensitivity to day length is an important consideration when genotypes are grown outside of their area of adaptation. When genotypes adapted to tropical latitudes are grown in the field at higher latitudes, they may not mature before frost occurs. They can be induced to flower and mature earlier by creating artificially short days or by grafting (Section IV D). Soybeans frequently are grown in winter nurseries located at sea level in tropical latitudes where day lengths are much shorter than their critical photoperiod. The short day lengths and warm temperatures encourage early flowering and seed maturation, and genotypes can produce a seed crop in 90 days or fewer after planting. Early flowering is useful for generation advance when only a few self-pollinated seeds per plant are needed, but not for artificial hybridization because the flowers self-pollinate before they are large enough to manipulate for hybridization. Artificial lighting is used to extend the natural day length to about 14.5 hours to obtain flowers suitable for hybridization and to increase yields of self-pollinated seed.
The effect of short photoperiod on flowering and seed yield can be partly offset by altitude, probably due to the effects of cool temperature (Major et al., 1975). At tropical latitudes, cultivars adapted to the northern U.S. perform more like those adapted to the southern U.S. at high altitudes than they do at sea level.

The light level required to delay flowering is dependent on the quality of light emitted from the source and the genotype being grown. Blue light with a wavelength of about 480 nm requires more than 30 times the energy to inhibit flowering as red light with a wavelength of about 640 nm (Parker et al., 1946). Flowering of genotypes that possess the e gene can be delayed by incandescent light, but not by cool-white fluorescent light (Buzzell, 1971). The level of light required to control flowering was estimated as 5.4 lux by Borthwick and Parker (1938) with the cultivar ‘Biloxi’ adapted to the southern U.S. My experience at Isabela, Puerto Rico, using quartz-ioidine lamps to extend the day length is that genotypes adapted to the northern U.S. frequently self-pollinate before they are large enough to manipulate for hybridization if the light level is less than 32 lux. However, a level of 5.4 lux in Puerto Rico will prolong vegetative growth so that plants are taller and produce more seed than those grown without an extended day length.

Temperature can also play a significant role in the flowering and development of soybean (Major et al., 1975). It can influence the time of flowering and suitability of flowers for hybridization. Temperatures below 21 C or above 32 C can reduce floral initiation or seed set (Hamner, 1969; van Schaik and Probst, 1958). Artificial hybridization is most successful between 26 and 32 C because cooler temperatures reduce pollen shed and result in flowers that self-pollinate before they are large enough to manipulate. Warmer temperatures frequently are associated with increased flower abortion caused by moisture stress; however, successful crosses are possible at about 35 C if soil moisture is adequate (Hartwig, 1978).

B. Growth Chamber and Greenhouse

Soybeans can be grown in an assortment of media ranging from a mixture of 1 soil:1 sand:1 compost to hydroponic solutions. Mineral nutrients can be provided as fertilizer in soil media or by watering with a solution containing 150 ppm N, 20 ppm P, and 60 ppm K (Summerfield, 1976). Seeds commonly are inoculated with rhizobia to encourage N fixation.

Plants for hybridization or for production of a large amount of self-pollinated seed commonly are grown in 25-cm diam pots. The genotype may be overplanted and thinned to two plants per pot, or seedlings may be transferred from a germination medium. When only a few self-pollinated seeds per plant are needed, a density of nine plants or more is common in a 15-cm pot and growth is controlled by a short photoperiod and an anti-gibberellin, such as Amo-1618 (Wilcox, 1974).

Artificial lighting in growth chambers and in winter greenhouses is needed for adequate photosynthesis and control of flowering. Adequate photosynthesis and control of flowering occurs with a light level of about 21 klux provided by a mixture of cool-white fluorescent and incandescent lights or wide-spectrum fluorescent lights (Tanner and Hume, 1976). For
hybridization, a photoperiod of adequate length is required. A 12-hour photoperiod is used for early flowering and seed production (Wilcox, 1974). Temperature should be maintained at not less than 21 C, with 26 to 32 C being most desirable. High relative humidity seems to improve the success of artificial hybridization (Byth, 1966). Automatic humidifiers may be used or floors may be kept wet.

Soybeans often have a thin stem and long internodes and grow abnormally tall in growth chambers and greenhouses. Excessive plant height is encouraged by long photoperiods, high temperature, excessive N, low light levels, and inappropriate light quality (Tanner and Hume, 1976). When photoperiod is reduced, the first flowers to develop may be satisfactory for hybridization, but the later ones usually self-pollinate before hybridization is possible.

Successful hybridization in growth chambers or greenhouses is difficult, even when all environmental requirements seem to have been met. Some genotypes are more difficult to use as parents than others. ‘Corsoy’ is an example of a highly productive cultivar that is used readily as a parent for artificial hybridization in the field, but may not have normal seed set in the growth chamber or greenhouse. Sensitivity to light level and quality seems to be involved.

III. FLORAL CHARACTERISTICS

Soybean flowers typically are self-pollinated on the day when the corolla opens. The amount of natural crossing is approximately 1% for adjacent plants within a row and 0.5% between plants in adjacent rows (Weber and Hanson, 1961). Natural crossing is associated with insect vectors, primarily honeybees (Apis mellifera L.) (McGregor, 1976).

The structure of soybean flowers is similar to that of other legume species and consists of a calyx with five sepals, a corolla with five petals, 10 stamens, and a pistil (Carlson, 1973). The calyx encloses the corolla until the day before anthesis (Fig. 1A). The corolla emerges and unfolds to expose a standard, two wing petals, and two keel petals (Fig. 1D). An open flower is about 7 mm long from the base of the calyx to the tip of the standard and 6 mm wide across the standard.

The pistil consists of a single ovary that contains one to five ovules, a style that curves toward the standard, and a club-shaped stigma (Fig. 1C). The stigma is receptive to pollen about 1 day before anthesis and remains receptive for 2 days after anthesis, if the flower petals are not removed (Kuehl, 1961). Filaments of nine stamens are fused, and the one nearest the standard is free. The stamens form a ring below the stigma until about 1 day before anthesis (Fig. 1C), then their filaments begin to elongate rapidly and elevate the anthers around the stigma. The anthers dehisce on the day of anthesis, pollen grains fall on the stigma, and within 10 hours the pollen tubes reach the ovary and fertilization is completed (Johnson and Bernard, 1963).

Soybeans have been classified as indeterminate, semi-determinate, and determinate based on the abruptness of stem termination after flowering begins (Bernard and Weiss, 1973). When grown at their latitude of adapta-
Fig. 1—Preparation and pollination of a soybean flower. (A) Flower at the stage for preparation and pollination, (B) removal of the corolla from the female flower after the calyx has been removed, (C) ring of 10 anthers and the stigma, (D) flower with pollen available, (E) pollination of the stigma, and (F) a pod 7 days after pollination with the calyx scar differentiating it from self-pollinated pods. (Photographs A, B, D, E, and F by C. J. Deutsch and P. A. Krumhardt, Iowa State Univ., Ames, Iowa; photograph C by G. L. Berkey, Ohio Agric. Res. and Devel. Ctr., Wooster, Ohio).
tion, indeterminate genotypes flower when about one-half of the nodes on the main stem have developed. They have short racemes with few flowers, and their terminal node has only a few flowers. Semi-determinate genotypes also flower when about one-half of the nodes on the main stem have developed, but node development and flowering on the main stem stop more abruptly than on indeterminates. Their racemes are short and have few flowers, except for the terminal one, which may have several times more flowers than those lower on the plant. Determinate cultivars begin flowering when all or most of the nodes on the main stem have developed. They usually have elongated racemes that may be several centimeters in length and may have a large number of flowers. Stem termination and flowering habit are reported to be controlled by two major genes (Bernard and Weiss, 1973).

IV. ARTIFICIAL HYBRIDIZATION AND SELF-POLLINATION

Self-pollination occurs naturally in soybean with no manipulation of the flowers. The following discussion pertains only to artificial hybridization.

A. Equipment

 forceps with fine tips and a smooth interior surface are required to manipulate the small flower. Plastic tags about 7 × 15 mm and wired with a flexible copper strand often are used to identify pollinated flowers. Plastic tags with a snap-on design can be attached quickly to the plant, but can be knocked or blown off more readily than those with a wire attachment (Ivers, 1977). Paper tags have been used successfully, but they are more susceptible to weather and insect damage than plastic tags.

Some breeders use magnifiers mounted on a headband or on a pair of glass frames. A magnification of 2.5 × provides satisfactory enlargement of the flower. Petri dishes or envelopes are used to collect male flowers. Desiccators containing calcium chloride crystals are used in some environments to dry male flowers to obtain adequate pollen shed.

B. Preparation of the Female

Flowers that are expected to open the following day are selected on the female parent. The buds are swollen and the corolla is just visible through the calyx or has begun to emerge (Fig. 1A). Usually no more than two buds on a raceme are prepared, and all self-pollinated flowers or immature buds are removed with forceps. Special care is required to remove immature buds that are hidden under the stipules at the leaf axil, and could develop into flowers at a later date.

The flower is grasped between the thumb and index finger and the location of the stigma determined by examining the sepals. A long, curved
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sepal covers the keel, and the stigma is on the opposite side of the flower (Fig. 1A). The calyx is removed by grasping a sepal with the forceps, pulling it down and around the flower, and repeating the procedure until the five sepalts are removed. The exposed corolla is removed by grasping it just above the calyx scar, then lifting and wiggling the forceps simultaneously (Fig. 1B). Practice is required to grasp the corolla low enough to remove the keel petals without injuring the stigma. The ring of anthers is visible after the corolla is removed, unless the anthers were removed with the petals (Fig. 1C).

Recent evidence indicates that emasculation is unnecessary to prevent self-pollination of a flower when artificial pollination is unsuccessful (Walker et al., 1979). When emasculation is not used, the anthers near the stigma frequently are removed to make it clearly visible for pollination. I recommend that inexperienced persons use complete emasculation initially to familiarize themselves with a good female flower. They learn that the anthers are difficult to remove if the flower is immature or that the anthers surround the stigma if self-pollination has already occurred.

C. Pollination

The female flower usually is hand-pollinated immediately after it is prepared; although a delay of several hours does not seem to reduce seed set. The time of pollen shed is primarily a function of temperature. Pollen shed may begin at 0700 hours and end by 0900 hours when early morning temperatures are above 30°C, or may begin at 1000 hours and continue throughout much of the day with more moderate temperatures.

Pollen is available from a flower with a recently opened corolla, but the degree of corolla opening associated with pollen shed may vary during the day (Fig. 1D). In many environments, it is possible to collect male flowers and use them immediately without storage. In the southern U.S. and other humid climates, pollen shed occurs in the morning when female flowers are more immature and difficult to manipulate than in the afternoon, and the flowers may be damp from heavy dew. In those circumstances, male flowers are collected into envelopes or petri dishes at about 0800 hours and the open container is placed in a desiccator for about 4 hours at a room temperature of about 25°C. The desiccator is taken to the field in the afternoon and kept in the shade to prevent excessive temperatures from developing within it.

Pollen viability can be maintained in flowers for up to 2 days when stored at about 5°C. Kuehl (1961) demonstrated that flowers can be stored successfully for several weeks in a desiccator at 3°C; however, cultivars differed in the percentage of pollen that germinated after long-term storage. He found that flowers stored over calcium chloride for several weeks did not dehisce, but when they were placed over water in a closed container for about 30 min, the anthers would dehisce.

Hand pollination is carried out by removing the stamens and pistil with a forceps from a flower of the male parent and gently brushing the anthers against the stigma of the female flower (Fig. 1E). Access to the stamens can be achieved by removing the front sepal and keel petals, or piercing the keel with the closed forceps and allowing them to open to push the petals away. Brushing the anthers on the stigma causes them to rupture, and the highest
The percentage of successful crosses is obtained when pollen is clearly visible on the stigma. Some breeders prefer to tap the anthers on their thumbnail to check pollen shed before brushing the stigma. Several male flowers may have to be used to obtain suitable pollen shed when conditions are unfavorable, or the same male may be used to pollinate several flowers when pollen shed is excellent. Some breeders prefer to leave the stamens of the male flower hooked over the style of the female, but there are no data to suggest that it improves the percentage of successful pollinations. The forceps are cleaned after pollination by placing the tips in one's mouth or dipping them in 95% ethanol.

Pollinated flowers are identified with a tag attached to the internode or to the raceme below the pollinated flower. Information recorded may include the number of crosses, number of consecutive nodes above the tag where crosses were made, date, breeder, and parentage. The pollinated flowers are not protected.

There seems to be a considerable difference in the efficiency of hybridization for indeterminate and determinate cultivars. Indeterminate cultivars usually have short racemes in the leaf axil and the flowers are difficult to reach and manipulate. Determinate cultivars usually have long racemes at the top of the plant that are well above the leaf axil. An experienced breeder can hybridize about 20 flowers per hour with an indeterminate cultivar compared with about 50 flowers per hour with a determinate (Hartwig, 1978).

An experienced breeder has an expected success of near 50%, which is equivalent to one hybrid seed per cross, assuming two seeds per pod. Inexperienced individuals may have no success. The most common causes of failure include use of female flowers that are too immature, damage to the stigma, and inadequate pollination. Some breeders have indicated a tendency for success to be less when the first flowers on a plant are used as female.

D. Factors Affecting Efficiency

Efficiency of artificial hybridization can be increased when pollen transport and information written on the tag after pollination are minimized. When male flowers do not have to be collected and dried in a desiccator, it is useful to plant the parents of a cross adjacent to each other (Chapter 5).

Plants usually are grown in rows 65 to 100 cm apart to facilitate movement of personnel within the field nursery. Yield of self-pollinated seed from an individual plant may range from a few seeds to more than 1,000 as a function of plant density. A density of 30 plants/m of row can be used when 30 or fewer seeds per plant is adequate, 10 plants/m can be used to obtain about 100 seeds/plant, and 3 plants/m usually results in maximum seed production per plant. Densities of 12 plants/m or less commonly are used for artificial hybridization.

Multiple planting dates about 7 to 14 days apart usually are used to match parents of different flowering dates. When differences in flowering dates are extreme between parents, flowering of the later parent can be hastened by creating an artificially short day or flowering of the earlier par-
ent can be delayed by use of artificially long days or delayed planting. For example, crosses with genotypes adapted to the southern U.S. are made in northern U.S. locations by covering the late genotype with a box, large can, or similar container to create an artificially short photoperiod of about 12 hours for about 15 days beginning when there are three nodes with trifoliate leaves on the main stem. Plants induced to flower early tend to have flowers that self-pollinate when they are small and can be difficult to prepare for hybridization.

Grafting can be used to hasten the flowering of late flowering genotypes. A scion from a late genotype grafted on a stock that has begun to flower will begin to bloom up to 42 days earlier than normal (Kihl et al., 1977). First flowers on the scion appear from 21 to 50 days after the graft.

Many qualitative characters have potential for use as genetic markers in soybeans; however, few are different among cultivars commonly used as parents (Bernard and Weiss, 1973). The most widely used genetic markers are flower color (purple dominant to white), pubescence color (brown dominant to gray), and pod color (brown dominant to tan). The association of purple hypocotyl color with purple flowers and green hypocotyl color with white flowers is commonly used to identify hybrids in the seedling stage. Differences in maturity, height, hilum color, and pest resistance between parents also are used to verify hybrid plants.

V. NATURAL HYBRIDIZATION

Genetic male sterility is available in soybeans and may be useful to facilitate hybridization, particularly for recurrent selection programs (Brim and Stuber, 1973). The distance required for complete isolation of a crossing block is not known; however, outcrossing is less than 0.5% when male-sterile plants are 12 m or more from a foreign pollen source (Boerma and Moradshahi, 1975). Plants on the boundaries of the crossing block probably sustain the most outcrossing with foreign pollen and can be eliminated at harvest to minimize contamination (Brim, 1977).

Little is known about the optimum plant density for natural hybridization. Cross-pollination is more common within rows than between adjacent rows; therefore, Brim (1977) prefers to grow populations with genetic male sterility on a square grid to create rows in all directions. He uses single-plant hills on 50-cm centers, subdivides the area into blocks of an equal number of hills for harvest, and bulks an equal amount of seed from male-sterile plants in each block to enhance random pollination.

VI. SEED DEVELOPMENT, HARVEST, AND STORAGE

Observing pod development 7 days after pollination generally is adequate to identify a successful cross (Fig. 1F). Abortion of pods and seeds can occur several weeks after pollination, but the percentage of abortion usually is low if plant stress is minimized (Shibles et al., 1975). During the check for successful pollinations, any new buds that may have developed at a node should be removed.
Pods that develop from artificial hybridization can be distinguished from self-pollinated pods by the presence of the calyx scar, caused by removal of the sepals (Fig. 1F). The sepals begin to fall off as the pods mature; therefore, harvest should be completed at or immediately before the time the pods reach their mature color. Harvesting pods early also avoids any loss by shattering. Marking the calyx or young developing pod of pollinated flowers with a felt-tip pen containing colored, nontoxic ink minimizes the chance of mistaking a selfed pod for a hybrid one (Ivers, 1977).

Pods are air-dried at not more than 38°C until the seeds contain 13% moisture or less, then the seeds are removed by hand. Each cross may be numbered for future reference using a system similar to that described in Chapter 5.

Seed can be stored satisfactorily at about 25°C for up to a year if relative humidity is 50% or less. In the humid tropics, germination percentage declines rapidly unless the seed is dried to 7% moisture and stored in an air-tight container at room temperature. Long-term storage in any climate is best accomplished by drying seed to 7% moisture and storing it at 10°C or less in a room maintained at 50% relative humidity or in an air-tight container.

REFERENCES


Hartwig, E. E. 1978. Personal communication. Delta Branch Experiment Station, Stoneville, MS 38776.


