Cultivated lentils (Lens culinaris Medik.) had their origin in the Near East arc where they were cultivated with other vegetables as early as the seventh millenium B.C. (Zohary, 1972; Williams et al., 1974). Evidence for the Near Eastern origin comes from archaeological remains. The mountainous region between Hindu-Kush and the Himalayas was first suggested as the center of origin (Barulina, 1930), but evidence acquired later supports the Near East (Zohary, 1972).

Lentil was one of the first plants to be cultivated by man, and it has long since been an important food crop in Eurasia and the subcontinent of India (Youngman, 1968; Zohary, 1972). Lentils are a high-protein pulse crop for direct human consumption. They are valuable when grown in rotation with cereals because they have the capability of fixing atmospheric nitrogen in symbiosis with Rhizobium spp. In the Western Hemisphere, the Pacific Northwest of the United States, followed by South America and Canada, is the largest producer of lentils.

I. PARENTAL MATERIAL

Lens culinaris Medik. belongs to the Order Rosales, Sub-order Rosineae, Family Leguminosae and Sub-family Papilionaceae. Within Papilionaceae, Lens holds an intermediate position between Vicia and Lathyrus, but it is closer to Vicia. Lens contains the cultivated lentil, L.
culinaris (synonym L. esculenta Moench.), and four wild species; L. ervoides Brign., L. montbretii Fisch. and Mey., L. nigricans Bieb., and L. orientalis Boiss. (Williams et al., 1974). Synonyms for the wild species have appeared in the literature, but they are too numerous to be mentioned. Similarities in plant type and pollen-grain morphology suggest that L. culinaris was derived from L. orientalis (Zohary, 1972; Williams et al., 1974). Lens culinaris is diploid, with 2n = 14 chromosomes, as is L. orientalis and L. nigricans. No chromosome numbers have been reported for L. ervoides or L. montbretii.

Slinkard (unpublished data, 1977) crossed L. culinaris and L. nigricans and produced progeny that segregated for seed size and seed coat color. Other attempts to intercross any of the five Lens species have not been reported.

Genetic collections of Lens for breeding purposes are maintained in the United States, India, and Syria. The U.S. Department of Agriculture Plant Introduction Station, Pullman, Washington, maintains a collection of over 500 accessions that includes material collected in 31 different countries. The International Centre for Agricultural Research in the Dry Areas (ICARDA), located in Aleppo, Syria, has recently established a germplasm collection that now stands at about 2,000 entries (Hawtin, 1977). The Indian Agricultural Research Institute, New Delhi-12, India maintains a collection of over 3,000 lentil lines collected from all lentil growing countries of Asia, Africa, Europe, and America (Sharma and Kant, 1975).

II. PLANT CULTURE

A. Field

Lentil nurseries for field crossing should be located on sites that have not been seeded to lentils for 2 to 3 years. Hard seed dormancy can result in volunteer plants that can cause seed mixtures in parental material or, more seriously, could be used inadvertently as parents in crosses. In the temperate regions, crossing blocks are located on well-drained soil on south-facing slopes to insure rapid emergence and good stands.

In Idaho, Oregon, Washington, and the western provinces of Canada, lentils are planted as early in the spring as possible in a seedbed with a minimum of straw residue on the surface. Soil temperatures rise quickly under cleanly tilled surfaces, and germination, emergence, and growth are enhanced. Delayed seeding usually results in a reduced plant growth, a rapid and brief flowering period, reduced time available for crossing, and greatly reduced seed set. In dry areas, improved germination and emergence can result if the plot area is rolled after seeding to improve contact of the soil with the seed. Under humid or wet soil conditions, this practice is avoided because it may cause soil crusting and reduce seedling emergence.

Plant densities in lentil nurseries are much lower than the density used in commercial production. For crossing purposes, lentils are planted in single-row plots 2 to 5 m long and 60 to 100 cm apart, with a within-row spacing of 5 to 15 cm between plants. The additional space given to individual plants promotes branching, increases flower numbers, and pro-
longs flowering; all of these factors are beneficial to a lentil crossing program. Nurseries for evaluation of bulk populations or selections are planted similarly. The space between rows provided by this system allows individual plant selection within rows and greater seed yields from the selected plants or rows. However, the effects of intergenotypic competition and its influence on selection must be considered in low plant densities.

Lentils apparently are not excessive users of water. Some moisture stress seems to increase flowering and seed set. This effect is often observed on the dryland Palouse hills of eastern Washington and northern Idaho where higher seed yields occur on the slopes and hilltops where moisture is limiting than in the low areas where moisture and nutrients are not limiting. In the low areas, excessive vegetative growth sometimes occurs at the expense of seed yield. Irrigation benefits lentil yields, but must be carefully managed to avoid over-watering.

Lentils respond to nitrogen and phosphorus on soils that have low residual levels of these nutrients. Lentils also respond to potassium, especially on sandy soils. All fertilizer applications should be based on soil tests.

In field nurseries, methods of weed control include use of herbicides before seeding, before seedling emergence, and after emergence. The herbicide used and time of application are determined by the range of weed species present. Although herbicides are available for weed control, hand weeding and cultivation is safer from the standpoint of avoiding injury from herbicides.

The introduction and spread of plant viruses in nurseries by aphids can be controlled by insecticides. At least one virus, pea seedborne mosaic virus (PSbMV), is seedborne in lentils and can drastically affect lentil growth and yield (Hampton and Muehlbauer, 1977). Virus-free seedstocks and good insect control are the only methods currently used to prevent this virus from infecting the nursery and jeopardizing breeding stocks. Resistance to the virus is controlled by a single recessive gene, sbv, but the gene has not been incorporated into any known cultivar (Haddad and Muehlbauer, 1977).

B. Growth Chamber and Greenhouse

Lentils can be grown in a standard growth medium of equal parts of soil, sand, and peat moss with the nutrient levels adjusted according to soil tests. During the growth period, adequate amounts of nutrients are maintained in the growth media by addition of nutrient solutions or controlled-release fertilizers applied to the surface. Because lentils fix nitrogen through symbiosis with *Rhizobium leguminosarum*, it is important that rhizobia are either present in the soil or are added with the seed at the time of planting. Overwatering in growth rooms often promotes the growth of damping-off organisms, especially *Pythium ultimum* Trow., which also hastens maturity. Soil fungi can be suppressed by fumigation or steam sterilization of the soil before seeding, seed treatment with a fungicide, careful watering procedures, or drenching the soil with a fungicide. Soil fumigation to control diseases and soilborne insects and weeds seems to give better results than
steam sterilization, because root and stem-rotting fungi do not usually re-colonize fumigated soil as rapidly as steam pasteurized soil. Maximum seed production on F, hybrid plants can be obtained by hydroponic culture (A. Slinkard, unpublished data, 1977).

Flowering and seed set on lentils are enhanced by a photoperiod of 16 hours or longer, with a light level of 10 to 15 klux. Artificial light is used to extend the photoperiod. Lentils are considered to be either long-day or day-neutral plants (Shulka, 1955; Moursi and Gawad, 1963; Saint-Clair, 1972). Regardless of the day-length response, an extended photoperiod up to continuous illumination is conducive to earlier flowering and a reduced life cycle (F. J. Muehlbauer, unpublished data, 1977).

In greenhouses and growth chambers, temperatures of 27 C during the day and 21 C during the night are optimum for lentil growth and seed set (Saint-Clair, 1972); however, night temperatures as low as 15 C are not detrimental (A. Slinkard, unpublished data, 1977). Seed set in greenhouses

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Fig. 1—Lentil flower at the appropriate stage for emasculation, and the preparatory steps in the emasculation procedure. (a) Female flower before emasculation, (b) removal of sepals, (c) removal of a notch from the standard petal, and (d) removal of the wing petals.
or growth rooms is improved by keeping relative humidity at about 50% (Wilson, 1972). Relative humidity can be maintained at this level with a commercially available greenhouse humidifier.

III. FLORAL CHARACTERISTICS

Lentils are naturally self-pollinated and have an extremely low level of natural cross-pollination estimated to be less than 0.8% (Wilson and Law, 1972). This low level of cross-pollination is presumed to be caused by pollen transfer by small insects, such as thrips. Wind and honeybees are ineffective for cross-pollination.

The individual lentil flower is complete and has a typical papilionaceous structure (Fig. 1a). Lentil flowers are borne singly or in multiples on peduncles that originate from the upper nodes of the lentil plant. The flower has a calyx that consists of five equally elongated sepals that equal or exceed the length of the corolla of the unopened flower. The corolla has a standard, two wings, and two lower petals that lie internal to the wings and are united at their lower margins to form the keel. There are 10 stamens, 9 of which are usually joined in a staminal column surrounding the pistil and one stamen remains free (Fig. 2a). The anthers open lengthwise and shed the pollen directly onto the stigma. After the anthers dehisce, the brush of hairs

Fig. 2—Completion of emasculation and pollination. (a) Staminal column and stigma before emasculation, (b and c) removal of the stamens with forceps, (d) flower chosen for source of pollen, (e) pollination showing dehisced anthers and pollen-laden stigma of male parent being brushed against the stigma of the female flower.
on the stigma sweep the pollen out of the tip of the keel as the style and ovary elongate. The style usually develops at a right angle to the ovary and is flattened on the outer side. The ovary is flat and glabrous and normally contains one ovule or two that alternate along the margin. The small size and delicate structure of the flower are major factors preventing rapid and readily successful hybridization of lentil.

VI. ARTIFICIAL HYBRIDIZATION OR SELF-POLLINATION

A. Equipment

A dissecting microscope, sharply pointed forceps, alcohol, and small tags are the only equipment needed for lentil hybridization. The dissecting microscope with 5× to 7× magnification is used in the laboratory to view parts of the small lentil flower during the steps in emasculation and pollination. In the field, magnifying glasses may be substituted for microscopes. The sharply pointed forceps used for emasculation and pollination are immersed in 95% alcohol between crosses to prevent contamination by foreign pollen. The tags are used to record the parents, the date, and the person making the cross. The tag is affixed to the internode directly below the cross-pollinated flower.

B. Preparation of the Female

Emascation of lentil flowers is necessary to prevent self-pollination (Wilson, 1972). Female flowers are chosen for emasculation when the tips of the petals reach 50 to 75% of the length of the sepals (Fig. 1a). At that stage of development, almost none of the flowers will have pollinated. Nearly all of the flowers will have pollinated when the petal length is greater than 75% as long as the sepals (Fig. 2c) (Wilson, 1972). Lentil flower petals expand about 25% of the length of the sepals every 24 hours; therefore, pollen is normally shed less than 24 hours after the optimum time for emasculation.

During the emasculation process, the selected flower bud is held between the thumb and forefinger with the suture of the keel facing the operator (Fig. 1b). Care should be taken not to bend or twist the peduncle. Sharply pointed forceps are used to remove the sepals closest to the keel. Several alternative methods of removing the anthers can be used. The easiest method seems to be to remove a notch from the standard (Fig. 1c) followed by removal of the wings (Fig. 1d). Removal of the notch makes it easier to fold back the standard to gain access to the wings. The standard and keel are then folded away to expose the staminal column and stigma (Fig. 2a). The stamens are removed by grasping the filaments with forceps (Fig. 2b) and breaking them free from the staminal column (Fig. 2c). The female flower is now emasculated and ready for pollination.

Some operators do not remove the notch from the standard, but immediately fold it back. They feel that by keeping the standard intact, a higher relative humidity is maintained within the flower after pollination;
thus promoting a higher percentage of seed set. In another procedure, the keel is removed at the same time as the wings to facilitate access to the stamens. However, removal of the keel often results in injury to the ovary and may result in a higher percentage of flower abortion.

C. Pollination

The highest percentage of crossed seed is produced if the lentil stigma is pollinated immediately after emasculation (Malhotra et al., 1978). The stigma retains its receptivity for at least 24 hours under conditions of high relative humidity and low temperature; however, reduced pod set is readily apparent if the stigma is exposed more than 12 hours. Immediate pollination while the emasculated female flower is in hand eliminates the time-consuming task of finding emasculated flowers within the lentil canopy.

Flowers in which the corolla has elongated to three-fourths the length of the sepals are selected as sources of pollen (Fig. 2d). Most flowers at that stage have anthers that have recently dehisced their pollen. Viable pollen suitable for transfer is identified by its bright orange-yellow color and is contained in a flower with a turgid appearance to the keel and wing petals. Pollen retains its viability for several days if flowers are collected immediately after anthesis, placed in petri dishes, and stored in a cool place.

Pollination is accomplished by first removing or folding back the keel and standard of the male flower to expose the 10 dehisced anthers and the pollen-laden stigma. The anthers and pollen-laden stigma are held with the forceps and brushed lightly against the stigma of the emasculated flower (Fig. 2e). The keel and standard of the pollinated female flower are carefully returned to their original position to protect the stigma. In an alternative method of pollen transfer, a spear-pointed needle is inserted through the keel and pollen is collected on the needle point. Pollen is transferred from the pollen-laden needle to the stigma of the emasculated female flower (Malhotra et al., 1978). Another method of pollination used successfully involves removing the standard and wing petals and opening one side of the tip of the keel with sharply pointed forceps to locate the pocket of pollen. The stigma of the emasculated female flower is dipped into the pollen pocket to achieve pollination. Regardless of the method of pollination used, enough pollen should be applied to the stigma to make it visible to the unaided eye, even if this requires the use of several male flowers. Pollinated flowers do not need protection from foreign pollen.

After pollination has been completed, a tag is affixed to the internode directly below the pollinated flower to identify the cross. Tags should not be affixed to the peduncle because of its delicate structure and susceptibility to injury. An alternative method of identifying crosses is to use colored thread looped around the peduncle, with a different color for each parental combination (Malhotra et al., 1978).

The percentage of successful pollinations varies widely by individuals and environments. Wilson (1972) reported 46, 61, and 82% successful manual pollinations by three experienced operators in the greenhouse. Success depends upon an environment with at least 50% relative humidity, high
light level, and temperatures between 15 and 25 C. The percentage of successful crosses declines rapidly when the relative humidity drops below 35%, primarily because viable pollen is difficult to gather and transfer. Malhotra et al. (1978) reported 27 to 43% successful pollinations under field conditions in India. Under controlled environmental conditions with 21 day/15 C night temperatures, successful pollinations approaching 90% can be obtained (A. Slinkard, unpublished data, 1977).

D. Factors Affecting Efficiency

Crossing block layouts for lentils are similar to those used for other pulse crops. The layout used most often is to plant 4 to 5 m parental rows 60 to 70 cm from each other with the plants spaced 5 to 15 cm apart within rows. Paired rows of each parental combination facilitate crossing and reduce the movement of personnel within the block. In a variation of this layout, a male parent row is planted with female parent rows on each side, thereby making three-row, two-parent or three-parent crossing blocks. The two female rows in this system can be the same or different parents or can be used for different dates of planting of the same female parent. In another design, a group of parents is planted in adjacent rows across the nursery, with a parent common to all crosses planted perpendicular to those rows. In most crossing-block layouts, provision is made for second and sometimes third plantings close to the first planting to coordinate flowering of the parents or to extend the period of time for crossing.

Cotyledon color is a valuable genetic marker in identifying F1 hybrids. F1 hybrid seeds from crosses between yellow cotyledon female parents and red cotyledon male parents can be identified by their red cotyledon color; seeds with yellow cotyledons are discarded as selves (Wilson, 1972). Yellow cotyledon color also can be used to identify F1 hybrids in crosses between green cotyledon and yellow cotyledon parents, but care must be taken because green cotyledon color bleaches to yellow with age (A. Slinkard, unpublished data, 1977). Cotyledon color is controlled by the genes Yc for color and i for the recessive color inhibitor so that the genetic constitution of red cotyledons is II YcYc, yellow cotyledons II ycyC, and green cotyledons ii Yc Yc or ii ycyC (Slinkard, 1978).

The most reliable genetic marker in use to identify F1 hybrid seeds is red cotyledon color. The classification of lentil seeds into red and yellow cotyledon color groups is facilitated by the use of ultraviolet irradiation. Under ultraviolet irradiation at a wavelength of 366 m, cotyledon colors can be distinguished through the seed coat by the fluorescent properties of red cotyledons. For seeds with black seed coats, a portion of the seed coat must be removed; however, such damage to the seed coat often lowers the germination percentage. Metal shields should be used to protect workers from direct exposure to ultraviolet irradiation.

Genes for flower color can be used as genetic markers. Flower color is presumably controlled by P for the major pigmentation gene and V for violet flower color. The genetic constitution of different flower colors was suggested as violet, VV PP; pink, vv PP; white, VV pp; and rose, vv pp (Lal and Srivastava, 1975).
If cotyledon color is not used, true hybrids can be separated from selves by the presence of obvious segregation in the F₁ generation. Segregation for seed coat color can be observed and used to identify hybrid populations. This method can be misleading because various seed coat colors and patterns are often borne on the same plant even though the seed coat is maternal and should not segregate. The solution to this problem must await a definitive study on the inheritance of seed coat coloration.

V. NATURAL HYBRIDIZATION

Natural hybridization is not used to make lentil hybrids.

VI. SEED DEVELOPMENT, HARVEST, AND STORAGE

Pod and seed development can be observed within 3 days after pollination. When cross-pollination is successful, the development of the ovary is rapid and seed within the pod can be seen readily within 7 days. When cross-pollination is unsuccessful, the ovary often will enlarge, but abortion will occur at a later date when the developing pod may have reached as much as 50% of its normal size.

Seed losses from pod dehiscence can be avoided by harvesting crosses as soon as the pods take on a yellow-brown color. Harvested pods should be allowed to dry completely in envelopes or bags before the seeds are removed. Harvesting and separating seeds from the pod are always performed by hand.

Lentils, like most large-seeded legumes, lose viability rapidly when stored at high temperature and high relative humidity. Usually hybrid seeds can be kept at room temperature, provided they are sown in a reasonable period of time. For periods of 3 years or more, lentil breeding material should be stored at about 10°C with 30% relative humidity or in a freezer at −20°C.

VII. TECHNIQUES FOR SPECIAL SITUATIONS

Post-harvest dormancy problems with lentil seeds often are encountered when the seeds are not allowed a storage period of at least 3 months. Scarification effectively insures good moisture imbibition of the seeds, but seeds often do not germinate with scarification only. Nearly 100% germination of recently harvested seeds can be obtained by soaking the scarified seeds in an aerated water bath for 4 hours at 23°C (N. Haddad, unpublished data, 1977). When using the aerated soaking technique, individual crosses or lines are identified separately in cloth bags or screened containers. Best results are obtained if the seeds are planted immediately after treatment without drying.
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