The cultivated sugar beet (*Beta vulgaris* L.) has been developed as a commercial crop within only the past 110 years. Nonetheless, by 1980, sugar production from sugar beets had increased to about 42% of world supply. Sugar beets are grown on an estimated 8.1 million ha in 41 countries throughout the five continents of the world. In North America, sugar beets are grown in the hot climates of Arizona and the Imperial Valley of California and in the cold climates of North Dakota, Minnesota and the Canadian provinces of Quebec, Manitoba, and Alberta. In the United States, about 0.4 million ha are used for growing sugar beets in 17 states. Russia is the largest producer of sugar beet, with about 4.8 million ha. Poland and France each produce over 0.5 million ha, West Germany produces over 0.4 million ha, and Italy and England each produce over 0.2 million ha. In most European countries, sugar beets are important for the protein supplied in the beet foliage, as well as the carbohydrates in sugar beet pulp.

The discovery of sugar beet as a source of sweetener was made in 1747 by a German chemist, Andreas S. Marggraf. He extracted a substance from fodder beets that when crystallized had the same properties as sugar from sugarcane. Some 50 years later, a former student of Marggraf, Franz Carl Achard, discovered that it was possible to extract sugar from white-skinned Silesian beets. These beets are believed to have contained about 6% sugar, from which Achard recovered about one-half. Achard began mass selection for increased sugar content in 1786, which was continued by the von Kopy family until about 1830. The selection resulted in a cultivar with about 9% sugar and was known as the white Silesian cultivar. The first beet sugar factory was erected in Cunern,
Silesia, in 1802 and demonstrated that sugar could be economically produced from beets.

**TYPES OF CULTIVARS**

**Mode of Propagation**

Sugar beet is a herbaceous dicotyledon which normally completes its life cycle in 2 years. This biennial habit is variable and, under certain conditions, the plant may function as an annual or even as a perennial. The plant normally develops a fleshy taproot the first year and a seedstalk the second year. To ensure complete reproductive development, the plant must be exposed to a period of moderately low temperatures (4 to 7°C is ideal), followed by long day length.

Sugar beet is naturally cross-pollinated, with pollen movement achieved by wind and occasionally by insects. Wind-borne pollen may be distributed horizontally at least 4500 m and has been observed up to 5000 m vertically. The sugar beet has a perfect flower that commonly consists of a tricarpellate pistil surrounded by five stamens and a perianth of five narrow sepals. Petals are absent. Flowers are located on the terminal portions of the main axes and on the lateral branches (Fig. 15-1). Flowers are sessile and occur singly or in clusters of two to seven. Each flower is subtended by a slender green bract.

The terms monogerm and multigerm are commonly used to describe sugar beet seed. However, botanically speaking, they are fruit. The ovary forms a fruit which is embedded in the base of the perianth of the flower. Each fruit contains a single seed whose shape varies from round to kidney-shaped. The ovaries are enclosed by the common receptacle of the flower cluster; therefore, a multiple fruit is formed by the aggregation of two or more flowers. The multigerm beet seed is formed by aggregation of two or more enclosed fruits. A monogerm seed is formed when a flower occurs singly. Flowers generally reach anthesis about 5 to 6 weeks after the initiation of reproductive development.

**Past and Current Cultivar Types**

After the middle of the nineteenth century, the sugar beet industry spread rapidly in Europe. Commercial seed-producing firms that handled seed production and nearly all of the genetic improvement were founded to supply the expanding industry. A very few publicly supported institutions conducted plant breeding research. All of the early cultivars grown in Europe were open-pollinated and multigerm.
Figure 15-1  Sugar beet inflorescence and flowers: (A) complete inflorescence of sugar beet plant, (B) branch of monogerm sugar beet with opened flowers (note bracts, sepals, and dehiscing anthers), (C) branch with ripening fruits, (D) open male-fertile flower with anthers dehiscing, (E) open male-sterile flower (note shrunken anthers). (From Hybridization of Crop Plants, p. 605, by permission of the Crop Science Society of America and American Society of Agronomy.)

From the advent of the first successful sugar factory in the United States at Alvarado, California, in 1870 and for about 60 years thereafter, European cultivars with names such as 'Vilmorin' and 'Kleinwanzleben' were used in the United States. These early imported cultivars were open-pollinated and multigerm. They often were not well-adapted to the climatic conditions of the United States. This problem frequently was intensified by their poor seed quality.

It was commonly believed that the climate and labor costs were not conducive to establishment of a sugar beet seed industry in the United States. By the end of World War I, however, U.S. sugar companies were
able to produce most of their seed requirements. Most of this seed was from reproduction of European cultivars. Following removal of trade barriers at the end of World War I, U.S. companies discontinued seed growing and once again imported European seed. European open-pollinated cultivars imported during the 1920s and 1930s formed the foundation of germplasm in the United States and, along with further germplasm accessions, were the source of U.S.-developed open-pollinated cultivars.

Sugar beet cultivar development in the United States was begun in the 1920s by the U.S. Department of Agriculture (USDA). The curly top virus, leaf-spot diseases, and black root were causing catastrophic losses at that time. 'US 1,' the first sugar beet cultivar developed in the United States, was released by the California Agricultural Experiment Station in 1931. 'US 1' was an open-pollinated, multigerm cultivar which displayed some resistance to the curly top virus. Over 50,000 ha were planted to the new cultivar by 1935. 'US 1' was succeeded by 'US 33,' 'US 12,' and 'US 22,' each with increased levels of resistance to curly top compared with the cultivar it replaced. The newest cultivars frequently had combined resistance to several diseases.

Sugar beet cultivars currently in use or those under development can be divided into two groups: hybrid and open-pollinated. Cultivars can be further classified according to ploidy level. Open-pollinated cultivars can be divided into diploid (2n = 2x = 18), tetraploid (2n = 4x = 36), or anisoploids. Anisoploid cultivars are a mixture of approximately 10 to 20% tetraploid, 60 to 80% triploid, and 10 to 20% diploid plants. Open-pollination of a mixture of 10 to 20% diploid plants and 80 to 90% tetraploid plants will result in an anisoploid cultivar with the foregoing described percentages. Hybrids based on the use of male sterility are diploid, tetraploid, or triploid (2n = 3x = 27).

In the United States and Western Europe, all new sugar beet cultivars are monogerm hybrids. In North America, most of these hybrids are diploid, whereas in Western Europe the majority of hybrids are triploid. Tetraploid cultivars and hybrids are very rarely used. In areas where multigerm seed is still used, most cultivars are anisoploid.

Three types of sugar beet hybrids have been developed: single crosses, double crosses, and three-way crosses. Three-way cross hybrids have become the predominant sugar beet cultivars. The parents of these hybrids have the desired disease resistance, yield, and quality factors required to produce a high-yielding hybrid.

European sugar beet breeders have developed three rather distinct classes of cultivars which have been used to match the particular climatic or ecological areas where the cultivars are grown. Zucker, or Z-type, cultivars have been bred for high sucrose. Ernte, or E-type, cultivars have been bred for high tonnage. Normal, or N-type, cultivars have been bred
for a balance of tonnage and sucrose percentage. In the United States, breeders have simultaneously improved both root yield and sucrose content in most of their cultivars.

EXTENT AND NATURE OF BREEDING PROGRAMS

Sugar beet breeding and related genetic research in North America has been and continues to be a cooperative effort of the sugar beet industry, the state agricultural experiment stations, and the USDA. Originally, the USDA was the principal supplier of research information and cultivars. Today, cultivar development is done primarily by private companies that are owned by beet-processing companies or are independent seed companies. In developing these cultivars, the private breeders utilize inbred lines, disease-resistant cytoplasmic male-sterile lines (CMS), or pollinators developed by the USDA. The private breeders either use these lines directly as components of hybrid cultivars or incorporate desired characters from these lines into their own breeding lines. Cooperative efforts of private industry, USDA, growers' associations, and state experiment stations have accelerated the development of new cultivars.

In Western Europe, cultivar development is accomplished by private companies and by government agencies. Private companies range in size from small family-owned firms, which develop and market only seed, to larger firms, which develop cultivars and also process and market sugar.

BREEDING OBJECTIVES FOR CULTIVAR DEVELOPMENT

Sugar yield per unit area of land is the primary objective in sugar beet breeding. All other breeding objectives directly or indirectly affect the primary objective of improving sugar yield. Among these objectives are disease resistance, bolting resistance, monogerm seed, storage ability of roots, and beet processing quality. Other miscellaneous characters that receive attention by breeders include earliness, cold tolerance, root shape, seed yield, and herbicide tolerance.

Sugar Yield

Factors that directly affect sugar yield are (a) the tonnage of beets per hectare; (b) percentage of sugar in the beet; and (c) the quality (purity) of the juice, which affects the loss of sugar during processing. These interrelated yield factors are quantitative in nature. Smith et al. (1973)
presented a literature review and data for partitioned genetic variances for root yield, sugar content, recoverable sugar, purity, and six nonsucrose components known to directly affect extractable sucrose. Nonadditive genetic variance is of prime importance in controlling root yield and recoverable sugar. Additive genetic variance is predominant for sucrose percentage and for six nonsucrose juice components.

Sugar beet breeders must be aware of several interactions among the factors that affect sugar yield. Generally, increases in weight or tonnage of beets is accompanied by a reduction in sugar percentage; and when breeding efforts have increased sugar percentage, tonnage has fallen. This inverse relationship can be further complicated by soil nitrogen (N) fertility. High N fertilization reduces sugar content.

Combining ability assessment is an integral part of sugar beet breeding programs. It provides a means of predicting the performance of a line when used as a parent in a hybrid. Combining ability tests for quantitative traits provides a reliable basis for predicting the performance of crosses. Detailed methods of predicting sugar beet hybrid cross performance also have been presented for three-way and four-way hybrids (Smith and Hecker, 1971; Skaracis and Smith, 1984).

Disease Resistance

The problem of disease infection provided the major impetus for the development of sugar beet breeding programs in the United States. European cultivars used by the early U.S. sugar industry generally had little disease resistance and frequently were devastated by such diseases as curly top, cercospora leaf spot, or black root. Breeding for disease resistance has been very successful and may have even saved the sugar industry in California and in the intermountain area of the United States between the Sierra Nevada Mountains of California and the Rocky Mountains.

Curly Top. The curly top virus is a complex of strains which vary in their virulence. Curly top is an extremely devastating disease which causes severe stunting and even death of young plants. Plants infected with the virus show leaf curling, discoloration, and stunting. The curly top virus is transmitted by the beet leafhopper (Circulifer tenellus). Curly top occurs throughout the western United States, but has been extremely prevalent in the intermountain areas, Texas, and California.

Breeding for resistance to curly top began with mass selection within European cultivars under natural field epidemics. The first curly top resistant cultivar, 'US 1,' was released by the USDA in 1931. Cyclic mass
selection and later selections from inbred lines followed by progeny testing led to cultivars with enough resistance to maintain yield in most seasons.

Inheritance of resistance to the curly top virus seems to be quantitative. Breeding for resistance now is accomplished by manually spreading viruliferous leafhoppers in field nurseries. These leafhoppers are reared in the greenhouse on virus-infected mother beets and are released in the field. The planting of a very susceptible cultivar to be infected as a source of virus also is effective in such nurseries. Individual plant selections also are made by placing a cage containing viruliferous leafhoppers over the plants. Highly resistant inbred lines have been developed by this method and are used in synthesizing resistant hybrid cultivars.

*Cercospora Leaf Spot.* Cercospora leaf spot, incited by the fungus *Cercospora beticola* Sacc., is the most widespread and destructive foliar disease of sugar beets. The pathogen infects the leaves, which not only reduces root size and sucrose content, but also decreases purity of the juice derived from diseased beets. Spores of the fungus overwinter on the debris of beet foliage from previous years and, under ideal humidity and temperature conditions, can infect the succeeding year’s beets, which results in an epidemic. Areas subject to high humidity, such as those east of the Rocky Mountains in the United States and in southern Europe, are subject to epidemics. Areas in California, notably the Sacramento Valley, also are subject to sporadic epidemics.

Breeding for resistance to leaf spot began as early as 1910 in Italy and 1920 in the United States. Progress in breeding for resistance to this disease has been slow and difficult. This is likely due to the complex inheritance and of low heritability of resistance. Smith and Gaskill (1970) reported that resistance to *Cercospora* was controlled by four or five pairs of genes, and Smith and Ruppel (1974) reported the narrow-sense heritability for resistance to be about 0.25. Mass selection for resistance to *Cercospora* has not been successful. Progeny testing of selected inbred plants under intense epidemic conditions led to resistant inbred lines which were used as parents in crosses that resulted in the first resistant cultivar. ‘US 217,’ developed in 1936, was essentially a synthetic cultivar developed from a combination of five inbred lines (McFarlane, 1971). Italian cultivars developed from breeding lines of the Italian plant breeder, O. Munerati, proved to be high in leaf-spot resistance and sucrose content. One such cultivar, ‘Mezzano 71,’ was the source of resistance for the synthesis of a highly leaf-spot-resistant line, US 201, developed by the USDA. Highly resistant, monogerm male-sterile lines and their maintainer lines have been developed at both the diploid and tetraploid level (Smith and Ruppel, 1979; 1980; 1985).
**Black Root.** Black root causes seedling damping-off, or if infected plants are not killed, feeder roots are damaged and plants are stunted and yield is reduced. Black root most often is incited by a complex of causal organisms, the principal agent being *Aphanomyces cochlioides* Drechs. Other organisms associated with the complex include *Pythium* spp., *Rhizoctonia solani*, and *Phoma betae* Frank. Black root is most prevalent in areas with higher rainfall and humidity and in soils with pH of less than 7. Mass selection and recurrent selection with progeny testing have been the major methods of breeding for resistance. Screening of sugar beet seedlings in the greenhouse using artificial inoculation techniques has been most effective in developing resistant breeding lines (Schneider, 1954).

**Nematodes.** The sugar beet nematode (*Heterodera schachtii* Schm.) is a serious or potentially serious pest in most areas where sugar beet is grown. It is one of the major reasons why sugar beets are grown in rotation. Avoidance through crop rotation with 3 to 5 years between beet crops and soil fumigation currently are the major controls. Nematodes feed and multiply on the smaller lateral roots, which results in wilting, stunting, and reduced stands. Breeding programs for nematode resistance are conducted in both Europe and the United States. The best sources of resistance are wild relatives of sugar beet, specifically *Beta procumbens*, *B. patellaris*, and *B. webbiana*. Some progress has been made in crosses of these wild species, especially *B. procumbens*, to *B. vulgaris*. Recently, diploid plants with an incorporated chromosome segment from *B. procumbens* have been recovered. These plants apparently carry the gene or genes for resistance. Further breeding work is needed to incorporate this resistance into hybrid cultivars. A greenhouse method of inoculating seedlings with nematode cysts or larvae has been developed.

**Rhizoctonia.** Rhizoctonia root and crown rot incited by *Rhizoctonia solani* Kuehn is a soil-borne fungus disease which occurs sporadically in specific fields within production areas. Sugar beet plants attacked in the seedling stage may be cankered or even killed. The disease causes reduced stands and damaged roots, which may cause further loss in storage piles and problems in processing. Sugar beet lines resistant to the disease have been developed by mass selection and recurrent selection with progeny testing (Hecker and Ruppel, 1977).

**Rhizomania.** Rhizomania, a virus disease (beet necrotic yellow vein virus), is carried and transmitted by a soil fungus, *Polymyxa betae*, which is widespread in the world and has recently been reported in Alberta, California, Nebraska, Ontario, and Quebec. The disease is characterized by root stunting, constriction of the storage root below the soil, frequent root rotting, and proliferation of lateral rootlets on the main taproot.
Losses in Europe of 20 to 50% are common and range as high as 80% in some European fields. Study of the nature of the disease complex and breeding efforts associated with development of tolerance or resistance are under way.

Other Diseases. Beet yellows and beet western yellows are two principal viruses in the United States, and are most prevalent in California. *Myzus persicae*, the green peach aphid, is the principal vector. Resistant parental lines have been developed by mass selection in self-sterile lines and by inbreeding with progeny testing in self-fertile plants. Powdery mildew is a foliar disease caused by *Erysiphe polygoni* D.C. (*E. betae*). Lines bred for resistance to powdery mildew have responded well to mass selection. *Erwinia* root rot, incited by the bacteria *Erwinia carotovora*, is a sporadic problem. Mass selection has been effective in producing resistant pollinators. The sugar beet root maggot, *Tetanops myopaeformis* Roder, can be a seasonal pest, especially in certain sections of the western United States. Resistance seems to exist in *B. vulgaris*. Yellow wilt, incited by a rickettsia-like organism, is a severe problem in areas of South America and a potentially serious problem in the United States. The disease is carried by a leafhopper vector. Resistance to this disease has been developed by mass selection and recurrent selection with progeny testing. Efforts to breed resistance to *Fusarium* yellows, downy mildew, *Sclerotium* root rot, and storage rot diseases also have been successful.

Nonbolting

Long days accompanied by extended periods of cool weather often induces bolting (flowering) in this biennial crop. Bolting, which is necessary for seed production, is not tolerable in beets grown for sucrose production because yield and sugar content may be reduced by as much as 50%. Bolting resistance is required in northern Europe and in California and Arizona. Mass selection and recurrent selection with progeny testing are effective breeding methods for developing bolting resistance.

Monogerm Seed

The monogerm seed character, which permits improved methods of thinning, reduced labor, and more accurate spacing, revolutionized the sugar beet seed industry. Monogerm seed is now an essential feature of all North American sugar beet hybrids and is predominant in Western Europe. The monogerm trait is conditioned by the homozygous condition of *mm*, which produces plants with single-germ fruit and seeds. Either a single fruit or a lateral branch can be born in the axil of a leaf, but never
both together. Four multigerm alleles, designated $M$, $M^1$, $M^2$, and $M^3$, show different degrees of dominance over $m$. Homozygous $mm$ plants, normally monogerm, are sometimes observed to have double-germ fruits on the central floral axis and are thought to be the result of nonallelic genes. The presence of these modifying genes, which often cause different degrees of monogermness, increases the difficulty of breeding good monogerm cultivars.

**Processing Quality**

Beet processing quality refers to the ability to extract sucrose from the beet. The two main components of quality are sugar content and juice purity. Sugar content is the percentage of sucrose by weight in the fresh beet root, and juice purity refers to the ratio of sucrose to total solids dissolved in the sugar beet juice. The value of the sugar beet sold to the processor depends on both sugar content and juice purity. Many genetic and environmental factors affect extractability and final crystallization of sucrose. Certain nonsucrose substances dissolved in the juice impede crystallization of the sucrose. Sodium, potassium, betaine, and nitrate nitrogen are among the most important nonsucrose constituents in the juice. Additive genetic variance has been shown to condition the factors affecting quality (Smith et al., 1973). Breeding progress has been made in quality improvement in sugar beet, however, the effects of excessive nitrogen fertilizer often have masked genetic improvement. The need for higher quality sugar beets still exists and further breeding efforts are needed.

**Qualitative Traits**

Over 40 qualitative mendelian factors have been identified in sugar beet. These characters, their gene symbols, and their known or proposed linkage groups are presented in Table 15-1. The characters presented are mostly relatively simply inherited, being conditioned by single recessive or single dominant genes.

**STEPS IN CULTIVAR DEVELOPMENT**

**Development of Female and Maintainer Lines**

The female parent of a hybrid must have sterile cytoplasm and be monogerm. Seed of the female (A line) is produced by crossing with its
<table>
<thead>
<tr>
<th>Linkage Group</th>
<th>Genetic Symbols</th>
<th>Authority or Verifier*</th>
<th>Character</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Y,Yr,y</td>
<td>Kajanus (1917), Keller (1936)</td>
<td>Yellow pigment</td>
</tr>
<tr>
<td></td>
<td>R,R',R',R,R',r</td>
<td>Kajanus (1917), Keller (1936)</td>
<td>Hypocotyl color</td>
</tr>
<tr>
<td></td>
<td>CL,cl</td>
<td>Owen and Ryser (1942)</td>
<td>Colored leaf</td>
</tr>
<tr>
<td></td>
<td>Tr,Tr</td>
<td>Owen and Ryser (1942)</td>
<td>Trout or spotted leaf</td>
</tr>
<tr>
<td></td>
<td>Cv,Cv</td>
<td>Deming</td>
<td>Colored vein</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Owen and Ryser (1942)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B,b</td>
<td>Munerati (1931), Abegg (1936)</td>
<td>Annual growth habit</td>
</tr>
<tr>
<td></td>
<td>B,br</td>
<td>Owen et al. (1940)</td>
<td>Annual growth habit but less effect than B, considered allelic to B</td>
</tr>
<tr>
<td></td>
<td>v1,V1</td>
<td>Owen and Ryser (1942)</td>
<td>Variegated foliage</td>
</tr>
<tr>
<td></td>
<td>C,c</td>
<td>Abegg and Owen (1936)</td>
<td>Partially dominant curly top resistance</td>
</tr>
<tr>
<td></td>
<td>cr, Cr</td>
<td>Stewart</td>
<td>Crinkled foliage, reduced plant size</td>
</tr>
<tr>
<td></td>
<td>p,P</td>
<td>Linde-Laursen</td>
<td>Nonproduction of color</td>
</tr>
<tr>
<td>II</td>
<td>m,M,M',M',M',M'</td>
<td>Savitsky (1950, 1952)</td>
<td>Monogerm seed</td>
</tr>
<tr>
<td></td>
<td>lb</td>
<td>Savitsky (1952)</td>
<td>Late bolting</td>
</tr>
<tr>
<td></td>
<td>C2</td>
<td>Savitsky and Murphy (1954)</td>
<td>Partial curly top resistance</td>
</tr>
<tr>
<td>III§</td>
<td>a1,A1</td>
<td>Owen (1952)</td>
<td>Genetic male sterility</td>
</tr>
<tr>
<td>IV§</td>
<td>lu5,Lu2</td>
<td>Theurer (1968a)</td>
<td>Lutescens, green cotyledons followed by death</td>
</tr>
<tr>
<td>V§</td>
<td>Ru,Ru</td>
<td>Owen and Ryser (1942)</td>
<td>Russet root</td>
</tr>
<tr>
<td></td>
<td>ch2, Ch2</td>
<td>Savitsky (1957)</td>
<td>Chlorina cotyledons and all leaves are yellow-green</td>
</tr>
<tr>
<td></td>
<td>ch1, Ch1</td>
<td>Savitsky (1940)</td>
<td>Chlorina, more reduction in root yield than ch2</td>
</tr>
</tbody>
</table>
Table 15-1  continued

<table>
<thead>
<tr>
<th>Linkage Group</th>
<th>Genetic Symbols</th>
<th>Authority or Verifier*</th>
<th>Character</th>
</tr>
</thead>
<tbody>
<tr>
<td>vi₄, Vi₄</td>
<td>Stander and Theurer (1970)</td>
<td>Virescens, pronounced delay in chlorophyll production in first true leaves, white to light green leaves</td>
<td></td>
</tr>
<tr>
<td>X,X</td>
<td>Owen (1945)</td>
<td>Restores male fertility in sterile cytoplasm</td>
<td></td>
</tr>
<tr>
<td>Z,Z</td>
<td>Owen (1942, 1945)</td>
<td>Produces partial fertility in sterile cytoplasm</td>
<td></td>
</tr>
<tr>
<td>Rf₁</td>
<td>Theurer and Ryser (1969)</td>
<td>Pollen restorer</td>
<td></td>
</tr>
<tr>
<td>Rf₂</td>
<td>Theurer (1971)</td>
<td>Annual pollen restorer</td>
<td></td>
</tr>
<tr>
<td>Rf₃</td>
<td>Roundy and Theurer (1974)</td>
<td>Weak pollen restorer from a yellow leaf mutant</td>
<td></td>
</tr>
<tr>
<td>Sh,sh</td>
<td>Hogaboam (1957)</td>
<td>Enhances pollen production</td>
<td></td>
</tr>
<tr>
<td>yl,Yl</td>
<td>Roundy and Theurer (1974)</td>
<td>Yellow leaf</td>
<td></td>
</tr>
<tr>
<td>bl,Bl</td>
<td>Munerati and Costa (1930)</td>
<td>Black root</td>
<td></td>
</tr>
<tr>
<td>d,D</td>
<td>Theurer (1968b)</td>
<td>Dwarf plants. Seedlings have thick hypocotyls</td>
<td></td>
</tr>
<tr>
<td>fl,Fl</td>
<td>Theurer (1984)</td>
<td>Feather shape, no petiole, laminae extends entire length of leaf</td>
<td></td>
</tr>
<tr>
<td>pl,Pl</td>
<td>Abegg (1936)</td>
<td>Plantain leaf; semiparallel veination</td>
<td></td>
</tr>
<tr>
<td>Pl₃</td>
<td>Theurer (1984)</td>
<td>Plantain leaf; semiparallel veination dominant</td>
<td></td>
</tr>
<tr>
<td>Sˢ,Sᶠ</td>
<td>Owen (1942)</td>
<td>Self-fertility</td>
<td></td>
</tr>
<tr>
<td>Sⁿ,Sᵇ</td>
<td>Owen (1942)</td>
<td>Self-sterility</td>
<td></td>
</tr>
<tr>
<td>Linkage Group</td>
<td>Genetic Symbols</td>
<td>Authority or Verifier*</td>
<td>Character</td>
</tr>
<tr>
<td>---------------</td>
<td>----------------</td>
<td>------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>$S_a, S_b, S_c, S_d$</td>
<td>Larsen (1977)</td>
<td>Self-incompatibility, four gametophytic loci</td>
<td></td>
</tr>
<tr>
<td>$w, W$</td>
<td>Stewart†</td>
<td>Albino lethal</td>
<td></td>
</tr>
<tr>
<td>$v_2, V_2$</td>
<td>Abegg (1940)</td>
<td>Variegated cotyledons</td>
<td></td>
</tr>
<tr>
<td>$v_3, V_3$</td>
<td>Abegg (1940)</td>
<td>Variegated foliage and root flesh</td>
<td></td>
</tr>
<tr>
<td>$f, F$</td>
<td>Stewart†</td>
<td>Flaccid leaf</td>
<td></td>
</tr>
<tr>
<td>$n, N$</td>
<td>Stewart†</td>
<td>Nana plants, leaves thick and leathery</td>
<td></td>
</tr>
<tr>
<td>$m, M$</td>
<td>Abegg (1940)</td>
<td>Miniature, lethal, conflicts with monogerm</td>
<td></td>
</tr>
<tr>
<td>$re, Re$</td>
<td>Savitsky†</td>
<td>New leaves of mature plants reduced in size and lanceolate shaped</td>
<td></td>
</tr>
<tr>
<td>$w_1, W_1$</td>
<td>Savitsky (1957)</td>
<td>Albino seedlings</td>
<td></td>
</tr>
<tr>
<td>$Au, au$</td>
<td>Savitsky (1957)</td>
<td>Golden-yellow cotyledons, semilethal heterozygote, lethal homozygote</td>
<td></td>
</tr>
<tr>
<td>$lu, Lu$</td>
<td>Savitsky (1957)</td>
<td>Lutescens, leaves produced after second pair become progressively yellow, some survival</td>
<td></td>
</tr>
<tr>
<td>$v_{i_1}, v_{i_2}, v_{i_3}$</td>
<td>Savitsky (1957)</td>
<td>Virescens, golden-yellow seedlings, varying degrees of delay of chlorophyll production</td>
<td></td>
</tr>
</tbody>
</table>

*For complete reference citation, see Smith (1980).
†As cited by Abegg (1940).
‡Putterfuss (1968) classes this as linkage group II.
§Tentative linkage group assignments based on the fact that they were good marker genes not associated genes in groups I and II.
maintainer (O-type) line. The development of a female line involves the synthesis of an O-type line that contains the characteristics desired in the female parent of the hybrid. O-type plants are identified by crossing individual plants to a known CMS line and evaluating the progeny for pollen fertility. This process is called O-type indexing. A lines are produced by converting an O-type line to a CMS equivalent by repeated backcrossing.

Development of Male-Fertile Pollinator Parents

Male-fertile pollinator parents are almost exclusively multigerm. Multigerms are better pollen producers and provide better pollen dispersal and seed production in hybrid matings with CMS lines than monogerm lines. Recurrent selection with progeny testing has proved to be an effective method of population improvement for the identification of good pollinator parents. Pollinator parents ideally have good general combining ability and good specific combining ability with the female parent used in the hybrid.

Evaluation of the Hybrid for Yield, Quality, Disease Resistance, and Other Traits

Replicated yield tests of experimental hybrids are conducted under field conditions that emulate commercial production as closely as possible. Multiple year and location testing is needed, and disease evaluation must be done under uniform epidemic or artificial inoculation.

Seed Increase of the Parental Components of the Hybrid

The CMS female, O-type maintainer, and pollinator lines must have adequate seed to be used in final crosses for commercial hybrid seed production. On the average, 2 years are required to increase the parental components of a newly developed cultivar.

SOURCES OF GENETIC VARIABILITY

Types of Parents and Populations

Wild Relatives. Sugar beet has several wild relatives, some of which may be hybridized with sugar beet to obtain genes for disease resistance or
other desired characters. The genus *Beta* and the origin of species therein has been extensively studied and the species classified into four sections (Table 15-2). The section Vulgares contains the common cultivated sugar beet. All five species of this section are diploids (2n = 2x = 18) and all produce fertile hybrids when crossed with the cultivated beet. All cultivated beets may have originated from *B. maritima*, which is a notable member of this section. Considerable genetic diversity is suggested by the wide variation for nearly all characters in the segregating generations from hybridizations among the species within the Vulgares section. Several characters, including high *Cercospora* resistance, have been transferred from *B. maritima* to *B. vulgaris*.

The section Patellares includes three species that are characterized by hard-coated monogerm seed and a viney growth habit. One of these three species is tetraploid (2n = 4x = 36) and the other two are diploid (2n = 2x = 18). Plants of this section offer the breeder a valuable source of genes for disease and nematode resistance. Intensive hybridization has been conducted between *B. vulgaris* and the species of the section Patellares because these are the only *Beta* species that are immune or highly resistant to the sugar beet nematode (*Heterodera schachtii*). Successful crossing and gene transfer have been difficult because F1 hybrids gener-

| Table 15-2 Sections and Species of *Beta* and Their Normal Chromosome Numbers* |
|------------------------------------------|-------------------|-------------------|
| Section  | Species          | Chromosome Numbers |
| Vulgares | *B. vulgaris* L. | 2n = 2x = 18       |
|          | *B. maritima* L. | 2n = 2x = 18       |
|          | *B. macrocarpa* Guss. | 2n = 2x = 18   |
|          | *B. patula* Ait.  | 2n = 2x = 18       |
|          | *B. atriangularis* Rouy | 2n = 2x = 18 |
| Patellares | *B. patellaris* Moq. | 2n = 4x = 36   |
|          | *B. procumbens* Chr. Sm. | 2n = 2x = 18 |
|          | *B. webbiana* Moq. | 2n = 2x = 18       |
| Corolliniae | *B. macrorhiza* Stev. | 2n = 2x = 18   |
|          | *B. trigyna* Wald. et Kit | 2n = 4x = 36, 5x = 45, 6x = 54 |
|          | *B. foliosa* (sensu Haussk.) | 2n = 2x = 18   |
|          | *B. lomatogona* Fisch. et Mey. | 2n = 2x = 18, 4x = 36 |
|          | *B. corolliflora*   | 2n = 4x = 36       |
| Nanae    | *B. nana* Bois. et Held. | 2n = 2x = 18     |

*According to Coons (1975), *Beta trigyna*, as collected in Hungary and the Crimea and distributed to various herbaria, is a hexaploid. The plants collected in the Caucasus and having 2n = 36 were named *corolliflora* by Zossimovitch, but considered by Coons to be *B. trigyna*. A pentaploid (5x) form of *B. trigyna* found to be apomictic also has been identified.
ally do not form secondary roots and most of the few F₁ hybrids that have survived are sterile. Several methods have been used to recover viable crosses between species of the section Patellares and those of section Vulgares. Bridge crosses have been used to overcome the lethal condition of interspecific F₁ seedlings. For example, Swiss chard (*Beta vulgaris* L.) is closely related to sugar beet, crosses readily with it, and the hybrids are vigorous and fertile. Hybrids between Swiss chard and wild species of the section Patellares have been crossed with sugar beet. Fodder and red garden beet (*Beta vulgaris* L.) also have been used in successful bridging crosses. Another method of overcoming the interspecific crossing problem between section Patellares and Vulgares is by grafting small interspecific F₁ seedlings on to the root system of young sugar beet seedlings (Coe, 1954; Johnson, 1956).

The section Corollinae includes five species that are characterized by a corolla-like perianth. Species of this section are only distantly related to cultivated sugar beet. Chromosome numbers within this section range from 18 to 54. Usually, hybrids between species of Corollinae and sugar beet display little homology between chromosomes and are sterile. Solving the chromosome homology problem would be particularly useful to breeders because there are several characters of potential use in the Corollinae section, including monogerm seed, apomixis, and curly top resistance.

The section Nanae contains a single species, *B. nana*, which is characterized by small plants with rosettes of leaves, generally not more than 10 cm across. Plants have single flowers and hard nut-like monogerm seeds. Hybridization with sugar beet has not been reported.

Germplasm collections available to breeders and geneticists are maintained in the United States at the USDA sugar beet research stations at Fort Collins, Colorado; Salinas, California; East Lansing, Michigan; Beltsville, Maryland; and the Plant Introduction Station at Ames, Iowa.

*Types of Populations Used for Parental Line Development.* Among the most important decisions that a plant breeder must make is the selection of parents for population development. The breeder must identify the characters to be improved, identify parental germplasm sources, understand the inheritance of the characters, and determine the best methods of synthesizing the desired population. The development of modern-day sugar beet hybrids depends on the selection or development of genotypes that combine well in crosses. Identification of genotypes with good combining ability may begin with inbred lines, or even open-pollinated cultivars. Source populations are synthesized by intercrossing lines or populations with the desired characteristics, such as disease resistance and root sucrose content. Several generations of intercrossing allows adequate genetic recombination, and the resulting synthetic can be utilized
for cyclic selection. The type of population synthesized and the type of lines used for the original cross depends on whether the new lines to be derived from the population will be male or female parents in a hybrid. Although male and female lines can be derived from the same source population, they usually are developed separately. Because breeding true monogerm lines with good O-type characteristics is difficult, female source populations are generally obtained by recombining sources that already have these characteristics. Thus, the use of a line as a male or female is determined early in its development.

All of the species within the section Vulgares have the normal chromosome number \(2n = 2x = 18\). This chromosome number can be converted to the autotetraploid condition \(2n = 4x = 36\) by colchicine treatment of dry, pregerminated or germinated seed. This conversion is necessary and routinely done in programs where triploid hybrids are the desired end products. Colchicine concentrations of 0.3 to 0.5% for dry and pregerminated seed and 0.1 to 0.5% for germinated seed are effective in inducing high percentages of tetraploid plants (Savitsky, 1966). Treatment of seed for 16 hours at 30°C is most effective for obtaining the highest number of tetraploids. Such plants are transplanted, photothermally induced, and checked for chromosome number before flowering.

In summary, the sources of parental germplasm range from the wild relatives of *Beta* to commercial cultivars and inbred lines of hybrid cultivars, lines from advanced stages of testing, pollinator lines known to have good combining ability (especially for sucrose and root yield), and inbred lines with specific desired characters.

**Population Development by Hybridization**

*Procedures for Artificial Hybridization*

Floral Induction and Greenhouse Hybridizations. The term photothermal has been used to describe the initiation of seedstalks and flowering of biennial beets brought about mainly by the cumulative effect of low-temperature exposure, followed or accompanied by the effect of long photoperiod. The main difference between biennial and annual beets for photothermal induction is the longer period of low temperature exposure required for flowering in biennials. Most commercial cultivars of sugar beet require 90 to 110 days of exposure to inductive temperatures for initiation of reproductive development.

Hybridization and self-pollination of sugar beet in the greenhouse is an integral part of most sugar beet breeding programs. Greenhouse hybridization and seed increases facilitate the low-cost production of small quan-
tities of seed with the assurance of no pollen contamination. Facilities used for greenhouse hybridization range from large compartmentalized glass houses to small plastic isolation chambers. The essential features of these indoor isolators include a filtered air system to exclude foreign pollen, a heating and cooling system for year-round use, and a supplemental incandescent light source. In addition to these requirements, photothermal induction chambers are essential in overcoming the biennial nature of sugar beet.

The sugar beet life cycle, which is characterized by vegetative growth the first year and reproductive or flowering growth the second year, can be effectively reduced by exposing young seedlings to prolonged cool temperature and continuous light. It is even possible, with proper timing, to obtain two generations of sugar beet seed in a 12-month period (Gaskill, 1952). Seedlings are grown in a warm greenhouse (20 to 23°C) in individual pots or tubes for 2 to 8 weeks after planting the seed. The longer period favors greater seed production from larger plants. Continuous illumination is provided from time of emergence with light furnished by incandescent 150-W lamps equipped with reflectors. The lamps are spaced 1.5 m apart and are suspended about 76 cm above the plants. Following this preinduction treatment, flowering is induced by transferring seedlings in their original containers to a chamber with constant temperature of 4 to 7°C and continuous illumination. The light source may be incandescent lamps, fluorescent lamps, or both. Fluorescent lamps are used more commonly because they produce less heat per unit of light output than incandescent lamps. Reflector-type light units, equipped with two 20-W, 61 cm, standard cool-white fluorescent lamps suspended approximately 48 cm above the plants will provide light of sufficient intensity for an area of about 0.4 m². Duration of this photothermal induction depends on the genetic resistance to bolting (flower stalk formation) of the material being prepared for crossing. For genotypes having average bolting tendencies, 10 weeks of the induction treatment is sufficient.

The postinduction treatment consists of transplanting seedlings to the crossing location and providing continuous illumination as described for the preinduction period. The postinduction period requires incandescent light and day/night temperatures of about 26/16°C.

Photothermal induction is frequently reversed in biennial beets, either by increasing the temperature or by decreasing the length of the photoperiods. The temperatures that favor reproductive development in sugar beets are fairly well established. Temperatures above 21°C favor vegetative growth, and temperatures between 4 and 13°C favor initiation of the reproductive phase.

Flowering in the sugar beet is indeterminate and continues until the plant is cut. Flowers begin to open at the base of each stem, and flowering
continues upward as the stem elongates. Flowers generally open in the morning hours, but also open throughout the day. The stigma matures approximately 6 days before the anthers dehisce and is receptive for up to 12 days after anthesis. By the time the stigma becomes functional, the embryo sac is fully formed and the egg cell is capable of being fertilized about 5 days before anthesis occurs.

Emasculation and Pollination. When male sterility or genetic markers are not available to ensure or recognize hybridization, a fine pointed jeweler’s forceps can be used to remove the anthers of the sugar beet flower. Anthers must be removed from flowers just ready to open and before anther dehiscence (Fig. 15-2). Sepals may be teased back to expose the anthers, which may be removed from the flower with the forceps. Extreme care must be taken not to damage the stigma during anther removal. Any unemasculated flowers should be removed from the branch and the branch bagged to prevent any contamination of emasculated flowers with foreign pollen. Pollinations can be made immediately following emasculation or up to 12 days after normal anthesis would have occurred. Pollen is collected by shaking flowering branches in a paper bag or by use of a vacuum aspirator. Pollen may remain viable for several hours or longer at room temperature. Pollen also may be stored at below-freezing temperature (0 to -10°C) for extended periods and still retain partial viability. Pollen collected from desired male-fertile plants may be dusted or brushed over the stigmas of the emasculated flowers.

Success of the pollination can be seen about 1 week after pollination by swelling of the ovary. Fertilization failure may be indicated by the lobes of the stigma remaining open and by a pronounced hairy stigma compared with less pronounced stigmatic hairs of the successfully pollinated flowers.

Inbreeding. The production, maintenance, and use of inbred lines in sugar beets is an integral part of most sugar beet breeding programs. Inbreds may be produced in the field or greenhouse by bagging branches of mother roots or by the use of tent isolators. The sugar beet normally exhibits a high degree of inherent self-incompatibility and is not readily selfed. This self-incompatibility system seems to include four gametophytic S loci with complementary interaction. The system requires that the four S alleles in the pollen be matched in the pistil to cause incompatibility (Larsen, 1977). A self-fertility gene Sf, when introduced into self-incompatible lines, makes it possible to produce self-fertile inbreds (Owen, 1942). Induction of self-pollination of otherwise self-incompatible plants also has been achieved by bagging plants at high altitudes. Selfing is achieved by bagging branches of plants just before flowering using white
paper bags of suitable porosity and wet strength. A wooden stake or bamboo cane 1 to 2 m long is driven into the ground next to the root to provide a support for the bags placed on the plant. Larger leaves are removed from the branch before a bag is placed over it. Bags are fastened to the branch and to the wooden stake by soft wire ties or by string. Less severe inbreeding may be achieved by interpollinating plants of the same line (sib-mating). Advantages of sib-pollination are a reduction of inbreeding depression and production of sufficient quantities of seed for yield trials and disease nurseries.
Procedures for Field Hybridization. Plants intended for hybridization are grown from seed the first year. In breeding programs located in regions that have extensive periods of freezing temperatures in the winter, sugar beet roots grown in the summer are dug and stored indoors during the winter. The beets are prepared for storage by digging the roots and trimming the leaves and petioles without injuring the crown buds. Roots are placed in cold storage without light at a temperature of 5 to 7°C and a relative humidity approaching 100%. The length of storage time required for successful induction depends on the genetic constitution of the line, but may range from as few as 75 to over 140 days. In the spring when temperatures are favorable for growth and when the threat of frost has passed, roots are planted in crossing isolations. These isolations consist of alternating rows of female (usually CMS) and male lines.

The sugar beet has been described as "an extremely versatile crop from a breeding perspective," attributed quite correctly to (a) the kinds of fertility-regulating mechanisms present and (b) the options available for breeding methods (Poehlman, 1979). Cytoplasmic male sterility is one such fertility-regulating mechanism which provides a practical method of emasculation and has facilitated the large-scale production of commercial hybrid seed. In sugar beet, CMS is most correctly described as cytoplasmic-genetic due to the interactions of sterile cytoplasm (S) and normal cytoplasm (N) with two nuclear genes, designated by Owen (1945) as X and Z. Plants with the Sxxx genotype are completely male sterile; Sxzz and Sxxzz are semi-male-sterile; and Sxzzz are also semi-male-sterile, but may be visually indistinguishable from normal male-fertile plants. Bliss and Gabelman (1965) described the dominant allele, gene X, as the restorer of complete male fertility and the dominant allele, gene Z, as a restorer of partial fertility to plants having S cytoplasm. Gene Z also was reported to be independent of and hypostatic to X, and plants containing N cytoplasm were male-fertile, regardless of the particular alleles of the X and Z genes. Crosses between plants with genotypes Nxxxz (normal cytoplasm and pollen fertile) and Sxzzz produce all male-sterile progeny. Maintainer lines of the Nxxxz genotype were designated O type by Owen. In other crops, such as maize, the O type is referred to as a B line. Introduction of CMS into an O-type breeding line is accomplished by crossing a CMS plant with an O-type male. Several backcrosses to the O-type parent will produce a male-sterile equivalent of the original O type, referred to as an A line in other crops.

When hybridizing sugar beet without the use of CMS, genetic markers have been identified which can be utilized to identify crosses. The most commonly used genetic markers in sugar beet are hypocotyl color, genetic male sterility, monogerm or multigerm seed, and annual growth habit.

The gene R consists of a multiple allelic series (R, R', R'', R^h, r) that governs hypocotyl, root, and foliage color. The R allele produces a pink to
reddish hypocotyl in the young beet seedling and usually red color in
crown buds of older plants. \( R^1 \) produces intense red stripes extending into
the petioles, and \( R^n \) produces pink petioles and hypocotyl and rose or
light pink tinted roots (Pedersen, 1944; Theurer, 1968b). \( R^b \) conditions
only red hypocotyl. The recessive genotype \( rr \) produces a plant with
green hypocotyl and crown buds. Crossing of a plant carrying \( rr \) to a plant
carrying \( RR \) produces \( Rr \) seed that will give red hypocotyl seedlings.
These plants will be easily identified from the \( rr \) plants with green hypocot-
yls that may have resulted from either selfing or sibbing. Associated with
the \( R \) gene is the \( Y \) gene. The \( Y \) gene controls yellow pigmentation, but its
expression is modified by the presence of the hypocotyl color \( R \) gene. The
\( rrY_\cdot \) combination results in plants with a yellow hypocotyl and root, with
the yellow color extending to the petioles and larger leaf veins. The \( R_\cdot Yy \)
combination produces plants with a red hypocotyl and white roots. How-
ever, the \( R_\cdot Y_\cdot \) combination produces plants with a red hypocotyl and root.
The presence of modifier genes along with the \( R_\cdot Y_\cdot \) combination may
produce entirely red foliage and intense red root epidermis. Linde-Laur-
sen (1972) identified a recessive gene, \( p \), which when present causes the
absence of red and yellow color, regardless of the \( R \) and \( Y \) genes. The
dominant allele \( P \), which is indispensable for red and yellow color forma-
tion, is carried by the majority of beet strains.

Genetic male sterility is useful for emasculation of individual plants in
breeding programs and in genetic investigations (Owen, 1952). Genetic
male sterility is especially useful for hybridization between self-fertile
lines. It also has been used with cytoplasmic-genetic male sterility to
produce double-cross hybrids (Owen, 1954). The dominant gene \( A_1 \)
conditions male fertility, whereas the homozygous recessive condition \( a_1a_1 \)
conditions male sterility.

Annual growth habit, designated \( B \), can be used to speed generation
cycles (Munerati, 1931; Abegg, 1936). Plants with the dominant \( B \) allele
produce seedstalks (bolt) in an environment of 18- to 24-hour photoperiod
and 24 to 27°C. Homozygous \( bb \) plants remain vegetative under the same
conditions. Additional modifying factors sometimes influence bolting and
make it difficult to classify plants for annual habit. These genetic markers
and cytoplasmic male sterility are used in artificial greenhouse hybridiza-
tion, as well as in the field.

**Mutagenesis**

Mutation breeding has not been used widely in sugar beet breeding. This
is probably because this natural cross-pollinator mates readily with other
species within the genus, and when genes for specific traits were needed, a
natural source usually has existed.
BREEDING PROCEDURES

Until 1856, mass selection was the only method used for improvement, and during this period, specific gravity was used to determine sugar content. In 1856, Louis de Vilmorin introduced family selection and progeny testing, as well as the polarimeter for determination of sugar content. Progeny testing and polarimetric sucrose determination were used for sugar beet improvement in the last half of the nineteenth century. By the beginning of the twentieth century, sugar content of sugar beet was raised from 9% to about 17%.

The sugar beet breeder basically is concerned with developing lines that will either be used as females or males in hybrids. There probably is no single best way to obtain the male and female lines; however, the following description of procedures for obtaining improved parental lines is considered typical of sugar beet breeding programs.

Female Source Population and Line Development

Indexing for O-type maintainer plants and conversion to CMS lines are necessary procedures for development of female source populations and lines. O-type indexing is accomplished by crossing selected candidate plants to known CMS females (Fig. 15-3). Concurrently, bags are placed on branches of the candidate O-type plant, or meristems from terminal or axillary buds are collected. Seed is harvested from the CMS plant and the F₁ progeny is brought to flower. If all F₁ progeny from a candidate plant are sterile, this indicates that the pollen parent is carrying the $xzxz$ nuclear gene combination and is a good O type. The selfed seed from such plants is pooled to form the O-type maintainer line. If meristems are used, as with highly self-incompatible O types, the meristems are used to produce seedlings for intercrossing and synthesis of the O-type population. Conversion of selected O types to CMS lines is accomplished by crossing the O type to a CMS line (Fig. 15-4). Subsequent backcrossing using the O type as the recurrent male parent results in a CMS equivalent after five or six backcrosses.

Table 15-3 presents a time-sequence outline using recurrent selection for developing source populations and female parental lines. In year 1, lines or populations of plants known to have disease resistance, good combining ability, monogerm seed, a high frequency of the $xzxz$ genotype for good O type, and which have been allowed to intercross for several generations are planted as stecklings (mother roots). These roots are space planted in a polycross isolation plot. $s_0$ plants are selected for good seed production and good monogermness. These selected plants are self-pollinated by bagging one or more branches, and the remainder of the
Figure 15-3  Indexing for O type plants. Candidate $S_0$ plants are indexed by crossing each plant to a cytoplasmic male-sterile (CMS) tester. $S_1$ seed is produced by bagging flowering branches on the candidate plant. $F_1$ seed from the CMS tester is grown (usually in the greenhouse) and plants are checked for pollen production. If the $F_1$ progeny are partially or completely fertile, the $S_1$ seed from the candidate plant is discarded. If all $F_1$ progeny are male sterile, the candidate plant is assumed to have the homozygous recessive $x$ and $z$ genes for O type. The $S_1$ seed from the selected O-type candidate plants is retained and either increased by intercrossing the $S_{o1}$ lines or used in population improvement programs. Axillary or terminal meristems also can be used to increase O-type plants.

plant is allowed to open-pollinate. Pollen also is collected in bags and used to pollinate CMS plants for O-type indexing. In year 2, the open-pollinated seed is used to evaluate each $S_0$ plant in replicated field tests for the characters of interest, which usually include yield and disease resistance. Based on the results of the replicated tests, the $S_1$ seeds from the best performing $S_0$ plants are planted in the greenhouse, induced to flower by photothermal induction, and intercrossed. The intercrossed seed is the first synthetic generation (Syn 1). Plants within $S_{o1}$ lines also are sib-mated for maintenance and seed increase of the line, and crossed to a CMS line to provide females for crossing to a tester in year 4. The Syn 1 and CMS $\times$ $S_{o1}$ stocklings are grown in year 3. In year 4, top cross seed is obtained from the CMS $\times$ $S_{o1}$ females for replicated tests in year 5. Also in year 4, Syn 2 seed is obtained by intercrossing Syn 1 plants. In year 5, Syn 2 seed is planted to produce stocklings, and the top crosses made in year 4 are evaluated for yield and disease resistance. In year 6, Syn 2 plants are intercrossed to obtain Syn 3 seed and seed of the $S_{o1}$ lines with the best
performing top crosses in year 5 is increased by sib-matings. In year 7, good O-type, \( S_{m1} \) lines which performed well in disease evaluation tests and topcrosses are available for advanced combining ability testing and conversion to CMS. Also, Syn 3 populations can be used to begin another cycle of population improvement.

The time required to produce improved female populations can be reduced considerably by growing stocklings in the winter in favorable environments and shipping the stocklings to another location for crossing in the field or greenhouse. A method favored in some European breeding programs is referred to as the winter stockling method. Plants are established in pots in the greenhouse between 10 October and 20

**Figure 15-4** Conversion of an O-type maintainer line to a CMS equivalent by repeated backcrossing. Parent B is the recurrent male parent, and has normal (N) cytoplasm and the nuclear genes \( x x x z z \) for O type. Parent A is the donor for the male-sterile (S) cytoplasm. Progeny from each generation are backcrossed to parent B. After about six backcross generations, most of the nuclear genes from parent B will be transferred into a line with the sterile cytoplasm donated by parent A.
Table 15-3 Sequential Outline for Typical Development of Source Populations and Female Parental Lines

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Breeding Procedure</th>
</tr>
</thead>
</table>
| 1    | Summer field      | a. Establish polycross plot with genetically diverse stocklings (mother roots) that are self-fertile, monogerm, and have a high frequency of \( xz \) genes for O type.  
       |                   | b. Phenotypically select \( S_0 \) plants for good seed production and monogermness.  
       |                   | c. Open-pollinate (OP), self-pollinate, and cross \( S_0 \) plants to a CMS line. | Index CMS × \( S_0 \) progenies for O type (see Fig. 15-2). |
| 2    | Winter greenhouse |                                                                                                         |
| 2    | Summer field      |                                                                                                         |
| 3*   | Summer field      |                                                                                                         |
| 4    | Summer field      | a. Syn 1 is advanced to Syn 2 via interpollination of Syn 1 plants; selection is practiced for good seed production.  
       |                   | b. Plants from the CMS × \( S_0 \)-derived line mating are indexed for O type, and topcrossed to a tester.  
       |                   | a. Syn 2 stecklings are produced.  
       |                   | b. Topcrosses are evaluated for yield and disease resistance in replicated tests, and the best \( S_0 \)-derived lines are selected. | Syn 1 stocklings are grown; stocklings from the CMS × \( S_0 \)-derived line mating are grown. |
| 5*   | Summer field      |                                                                                                         |
| 6    | Summer field      | a. Syn 2 is advanced to Syn 3 via interpollination of Syn 2 plants; selection for seed production is continued.  
       |                   | b. The best \( S_0 \)-derived lines are increased.  
       |                   | a. Syn 3 populations are used to begin another cycle of selection by repeating years 1 to 6.  
       |                   | b. \( S_0 \)-derived lines are available for combining ability testing and conversion to CMS. |                                                                                          |

*Production of stocklings can be accomplished in winter, if seed is planted in a favorable environment. Stocklings also can be winter grown in a favorable location and shipped to another location for hybridization.
November. The plants are grown under enough light and temperature (about 20°C) to maintain growth. On about 25 December, light is reduced and temperature is reduced and maintained at 5 to 9°C. Temperature is regulated by opening and closing greenhouse windows. Plants are transplanted to the field at the normal stockling planting time and begin flowering in about 60 days.

Table 15-4 outlines an alternative developmental sequence for female line identification when self-incompatibility precludes selfing. Self-incompatible, monogerm, O-type populations are used to isolate good-combining O-types by a paired-plant crossing system. In year 1, pairs of random or selected roots from a source population are mated and crossed to a CMS tester. These crosses are usually made under small tent isolators, which allow air movement without pollen contamination. The isolators usually are constructed of metal frames and covered with a white muslin type material. They can be used in a relatively small field or greenhouse area. Within these tents, random or selected roots from source populations are mated in pairs and simultaneously crossed to a CMS tester with good general combining ability. Stocklings from the CMS × (So + So) seed can be grown in a favorable environment, such as Arizona in the United States, during the winter of year 1. Also during the winter of year 1, seed from the CMS tester is used for an O-type index of the So × So pairs.

In the summer of year 2, CMS × (So + So) plants are crossed to a topcross pollinator to provide seed for general (GCA) and/or specific (SCA) combining ability evaluation. In the summer of year 3, GCA and SCA tests for yield of CMS × (So + So) lines are conducted in the field. In the winter of year 3, stocklings are grown from the best So × So crosses as determined by the combining ability tests. In the summer of year 4, superior So × So pairs are crossed with CMS testers for SCA evaluation. The So × So crosses also may be backcrossed to obtain their first backcross generation increase; [CMS × (So + So)] × (So × So). So × So pairs evaluated for combining ability can be returned to year 1 as a new source for population improvement. In the winter of year 4, So × So pairs and CMS BC1 stocklings are grown in a favorable environment for further backcrossing. In year 5, SCA tests are conducted with the seed from CMS tester × (So × So) crosses, and the backcrossing program for synthesis of the CMS version of the So × So cross is continued.

Population Development for Identification of Male Pollinator Parents

Recurrent selection with progeny testing has proven to be an effective method of population improvement for the identification of good pollinator parents. This method is used most often when plants are at least par-
Table 15-4  Identification of Superior Female Lines Via Paired Crossing of O-Type Monogerm from an Improved Source Population

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Breeding Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Summer field or greenhouse</td>
<td>Pairs of plants from a source population are crossed to each other ($S_0 \times S_0$) and to a cytoplasmic male sterile (CMS) line; CMS × ($S_0 + S_0$).</td>
</tr>
</tbody>
</table>
| 1*   | Winter field or greenhouse| a. Stocklings (mother roots) of the CMS × ($S_0 + S_0$) crosses are grown.  
b. The CMS × ($S_0 + S_0$) crosses are indexed for O type of the pairs of $S_0$ plants. |
| 2    | Summer field              | The CMS × ($S_0 + S_0$) plants are crossed to a topcross pollinator to obtain seed for general (GCA) and specific (SCA) combining ability tests. |
| 3    | Summer field              | Replicated topcross tests of CMS × ($S_0 + S_0$) crosses are grown for GCA and SCA evaluation.                                                   |
| 3*   | Winter field or greenhouse| Based on the topcross test, stocklings of selected O-type $S_0 \times S_0$ pairs are grown.                                                      |
| 4    | Summer field              | Selected $S_0 \times S_0$ pairs are (a) crossed with CMS testers,(b) backcrossed as [CMS × ($S_0 + S_0$)] × ($S_0 \times S_0$) = BC$_1$, and/or (c) returned to year 1 as $S_0 \times S_0$ crosses. |
| 4*   | Winter field              | $S_0 \times S_0$ and CMS BC$_1$ stocklings are grown for further backcrossing.                                                                   |
| 5    | Summer field              | a. Replicated yield tests of CMS × ($S_0 \times S_0$) crosses are grown.  
b. Selected $S_0 \times S_0$ pairs and CMS BC$_1$ crosses are utilized in hybrid program as male or female parents.  
c. CMS BC$_1$ plants are backcrossed to develop CMS equivalents of the selected ($S_0 \times S_0$) pairs. |

*Stocklings can be produced in the winter in an area with a favorable climate, such as southern Arizona, or in the greenhouse by a winter stockling method where induced seedlings are transplanted to the field at normal stockling planting time.

...tially self-fertile. The method is about the same as that used for female source population development with two exceptions. First, source populations are almost exclusively multigerm, and lines derived from any source population are multigerm. Second, there is no need to select for an O-type maintainer line in the final pollen parent. Multigerms are better pollen producers and provide better pollen dispersal and seed production...
in hybrid matings with CMS lines than monogerm lines. Because the majority of sugar beet cultivars are hybrids synthesized by the use of CMS, the multigerm trait in the final pollen parent of sugar beet hybrids does not interfere with the production of monogerm seed for commercial planting.

As outlined in Table 15-5, development of source populations for pollinator parents begins with planting of partially self-fertile stocklings.
which are genetically broad based. Recombination among these source plants is achieved in the first year by open-pollination in the space-planted nursery. In the same nursery, S₀ plants that flower normally have flowers that are spaced close together on the branches, and that produce adequate amounts of pollen are self-pollinated by placing bags on several branches of each plant. In year 2, the open-pollinated progeny from each S₀ plant are evaluated in replicated yield and disease tests. In the winter of year 2, S₁ progeny from S₀ individuals with the best performance in the replicated tests are grown, induced to flower, and intercrossed to form the Syn-1 generation. Plants within a S₀₁ line also may be sib-mated for advancement as pollinator lines in the cultivar breeding program. In years 3 through 6, synthetic populations (Syn 2, Syn 3, etc.) are produced by intercrossing selected plants from the previous generation. Also during these years, hybrid matings and evaluations are made of commercial CMS lines with Syn populations or with specific S₀₁ lines from the Syn populations.

In breeding programs where self-incompatibility precludes adequate seed set from selfing, a paired-plant crossing system can be used effectively. Table 15-6 outlines the developmental sequence of such a system for pollinator line development. These paired-plant matings and crosses to CMS testers usually are accomplished using small tent isolators. Seeds from the CMS testcrosses are used in yield trials to identify superior pairs of S₀ plants. Selected S₀ × S₀ pairs are sib-mated as a family and recycled for further paired-plant selection. The S₀ × S₀ families or reselected paired-plant crosses from them can be tested for specific combining ability at any time, and increased for use as a pollinator in a commercial hybrid. Progenies from paired-plant crosses can be used to form synthetics for further population improvement.

**Methods of Hybrid Synthesis**

Sugar beet cultivars currently being produced in the Western world are nearly all true hybrids. It is important to note that for all methods of hybrid synthesis, the fertility of the commercial hybrid is inconsequential because they are not harvested for seed. The development of a hybrid involves three distinct components:

1. CMS lines
2. O-type maintainer lines
3. diploid or tetraploid pollinators

Use of these three components enable the production of the following types of hybrids.
Table 15-6 Developmental Sequence for Male Pollinator Line Development Using a Paired-plant Mating System

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Breeding Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Summer field</td>
<td>Selected stocklings (mother roots) from an improved population are mated in pairs (S₀ × S₀) and crossed to a cytoplasmic male sterile (CMS) line, CMS × (S₀ + S₀).</td>
</tr>
</tbody>
</table>
| 2    | Summer field   | a. Progeny of CMS × (S₀ + S₀) crosses are evaluated in replicated yield and quality trials.  
           b. S₀ × S₀ seeds are planted and selected for disease resistance.  
           c. S₀ × S₀ pairs are selected based on disease evaluation and yield and quality tests. |
| 3    | Summer field   | a. Selected S₀ × S₀ pairs are interpollinated to form the first synthetic generation (Syn 1).  
           b. Selected S₀ × S₀ pairs are mated to CMS topcross testers.  
           c. Selected S₀ × S₀ pairs are advanced to evaluations as potential pollinators in commercial hybrids. |
| 4    | Summer field   | a. Replicated topcross tests are conducted for yield quality and disease resistance.  
           b. Superior S₀ × S₀ pairs are identified in the topcross tests for potential use in commercial hybrids.  
           c. Syn 1 population is open-pollinated to obtain the Syn 2 generation. |
| 5    | Summer field   | a. Paired plant matings are made within the progeny of selected S₀ × S₀ pairs. Evaluation of the new pairs is conducted with the procedure described for years 1 to 4.  
           b. Syn 2 is open-pollinated to obtain the Syn 3. The Syn 3 population is used to begin another cycle of selection with the procedure described for years 1 to 4. |

Source: After Hecker and Helmerick (1985).

Single-cross Hybrids. A single-cross hybrid is produced by crossing parent A with parent B. The principal requirement in this type of cross is that the female parent be a CMS monogerm line. Inbred lines are the most common type of parent, especially for the female parent, and maximum homozygosity of both parents may provide the maximum expression of heterosis. The single-cross hybrid may be triploid when either parent is a tetraploid. Triploid hybrids usually are synthesized with tetraploid pollen parents. Although heterosis and uniformity may be maximized in single crosses, their use is limited by the reduced seed production on inbred parents. In the United States, single crosses have been used
when disease resistance, especially for curly top, was needed in both parents.

*Three-way Hybrids.* The three-way or three-way topcross is the predominant type of hybrid in North America and Europe. Figure 15-5 outlines the synthesis of a three-way topcross hybrid. In this hybrid, female parent A is always CMS and monogerm. The maintainer line for parent A is an O-type equivalent. Parent B is a monogerm O-type pollinator. The F₁ of the A × B cross is sterile and monogerm. Both parent A and B are typically inbred or partially inbred. The pollinator used as parent C is typically a broad-based multigerm line. The origin of this pollinator is variable and may include progeny from crosses between inbred lines, paired-plant crosses, synthetic populations, or open-pollinated heterogeneous populations. The three-way cross hybrid seed harvested from the F₁ plants of the single-cross parent (A × B) is phenotypically monogerm and genetically multigerm. The three-way cross hybrid is triploid when pollinator parent C is tetraploid. Triploid hybrids produced from tetraploid male-sterile monogerm parents have been synthesized in France and possibly other countries as well, but purported advantages may be offset by problems with maintaining the tetraploid male-sterile parents.

*Double-cross Hybrids.* Double-cross hybrids may be produced by three systems, all of which use cytoplasmic male sterility and one of which uses

---

**Figure 15-5** Synthesis of three-way topcross sugar beet hybrid. Parent A, which is CMS and monogerm (mm) is crossed to the O-type, monogerm parent B. The progeny (AB) which is CMS and monogerm is crossed to the multigerm (M__), non-O-type parent C. The hybrid seed harvested from the AB female parent will be monogerm.
Figure 15-6 Synthesis of double-cross sugar beet hybrid using a self-incompatible (SI) male pollinator and a CMS female parent. The CMS, monogerm (mm) line A is crossed to the O-type, mm, line B to produce the CMS single-cross female parent. Lines C and D, which are self-incompatible and multigerm (M), are crossed to give the single-cross male parent. The hybrid seed harvested from the single-cross female parent will be monogerm and will include some three-way cross seed due to the self-pollination in the SI parent.

both cytoplasmic and genetic male sterility. In the first system (Fig. 15-6), inbred A is CMS (Sxxzz) and monogerm and inbred B is O type (Nxxzz) and monogerm, so that the single-cross plants from the inbred A × inbred B mating will be male sterile. Parents C and D are self-incompatible, and their interpollination produces seed of the single-cross pollinator parent used for synthesis of the double-cross hybrid. Because a small amount of selfing occurs in self-incompatible lines, the seed harvested from interpollination of parents C and D will be primarily hybrid, but with mixtures of some sib or self-pollinated seed of parents C and D. As a consequence, the hybrid seed used commercially will be primarily the double-cross AB × CD, but with some seeds of the three-way crosses AB × C and AB × D.

In the second method (Fig. 15-7), inbred A is CMS and monogerm and inbred B is O type and monogerm. The single-cross plants from the inbred A × inbred B mating will be monogerm and male-sterile. For the single-cross pollinator parent, inbred C is genetically male-sterile (a_A) and inbred D is male-fertile (A_A). The genetic male-sterile line C must be rogued to remove pollen-producing plants and retain only the recessive
a1a1 male-steriles. The single cross from the inbred C × D mating will be heterozygous (A1a1) and will produce pollen. The double-cross seed harvested from the AB × CD mating is phenotypically monogerm, regardless of the seed type of parents C or D. This method is not used extensively because of the labor required to rogue the pollen-fertile plants from inbred C.

The third method for producing the double-cross hybrid utilizes a fertility-restoring gene (Fig. 15-8). The single cross of parent A with parent B is produced with CMS in parent A (A line) and the O-type condition in parent B. Both parents, A and B are monogerm. Parent C is CMS (A line), and parent D carries a gene for fertility restoration Rf (R line). The male-fertile CD single cross is used to pollinate the AB male-sterile single cross to produce the double-cross hybrid. The double-cross seed will be monogerm, regardless of the seed type of parents C and D. The third method, referred to as the fertility-restorer method, has been used very little because of incomplete fertility restoration and inadequate pollen production by the C × D single-cross pollinator parent in some environments.
Example of the Developmental Sequence of a New Sugar Beet Cultivar.

**Perspective and Rationale for Development.** ‘USC-1’ is a commercial three-way cross hybrid whose component lines were developed and tested by the USDA at Salinas, California. The Union Sugar Company produced and marketed the hybrid seed. ‘USC-1’ is the result of nearly 40 years of breeding effort to combine multiple disease resistance and productivity in a monogerm hybrid cultivar. It involves two distinct classes of germplasm with unique requirements and developmental histories. One class of germplasm was used to develop the female parent of the hybrid, and was required to be monogerm and CMS. The other germplasm was used to develop the pollinator parent and required extensive breeding effort to improve its combining ability for disease resistance.

In the early 1970s, the predominant sugar beet hybrids grown in California and Arizona were ‘US H9’ and ‘US H10’ or similar types produced by commercial companies. ‘US H10’ has the pedigree

![Figure 15-8](Image)

Synthesis of double-cross sugar beet hybrids using pollen restoration genes and cytoplasmic male sterility. Parent A, which is CMS and monogerm (*mm*), is crossed to parent B, which is O type and monogerm, to produce the AB single-cross female (CMS) parent. Both A and B are homozygous for the nonrestoring allele *r* so that the AB cross is male sterile. Parent C, which is CMS, *mm*, and *rr*, is crossed to the multigerm parent D. The *R*r allele in parent D will restore pollen fertility in the single-cross male parent, CD. The hybrid seed ABCD harvested from the female parent will be monogerm.
(C562CMS × C546) × C17. 'US H10' was moderately resistant to prevalent diseases including curly top (CT) virus, virus yellows (VY) incited by beet yellows and beet western yellows viruses, and downy mildew (DM) incited by Peronospora farinosa Fr. It was a very nonbolting type, which was required because of fall, winter, and early spring plantings and overwintered productions.

In the early to mid-1970s, two new important diseases were identified in sugar beet. Erwinia root rot (ERR) incited by Erwinia carotovora betavascularorum caused a soft rot of the storage root that reduced yields and greatly impeded processing efficiency. Powdery mildew (PM) incited by Erysiphe polygoni D.C. was introduced into North America in 1973 or 1974 and rapidly spread to all production regions causing losses of up to 35% in US H10. In the breeding program of R. T. Lewellan at Salinas, California, breeding strategies were formulated to combine the favorable traits of US H10 with resistance to ERR and PM and to continue the improvement in productivity (extractable sucrose yield per hectare).

Development of Parental Lines

Female Parent. The CMS parent C546H3 developed by McFarlane and Skoyen (1965) has been widely used in California since about 1966. It is a single cross of C562CMS × C546, which has high seed yield and quality, and contributes better than average general combining ability when used in three-way hybrids. The inbreds C562CMS and C546 have the following traits: (a) good monogerm with few doubles and freedom from polyembryony; (b) high resistance to CT virus; (c) bolting resistance; (d) wide adaptation; (e) DM resistance; and (f) adequate sucrose concentration and juice purity. In addition, C546 provides partial resistance for ERR. These qualities, plus the fact that these inbreds had been widely tested and used, made C546H3 the best candidate as the female parent for 'USC-1.'

Male Parent. Because the development of pollinators is easier than that of females due to wider availability of multigerm and non-O-type germplasm, and fewer technical requirements for breeding, the objective of developing a hybrid with improved resistance to ERR and PM was achieved by improvement of the pollinator.

C17, the pollinator of the hybrid 'US H10,' was chosen as the best available source of resistance to bolting, CT, VY, and DM, and had adequate general combining ability for extractable sucrose. However, tests had shown that it was highly susceptible to ERR and PM. C64, the multigerm pollinator used for the hybrid 'US H7' had genetic variability for resistance to ERR (Lewellan et al., 1978), PM, and, in the absence of VY,
had higher sucrose concentration. C64 was highly susceptible to VY. The resistance of C64 to bolting, CT, and DM was nearly equal to that of C17. All of these traits are quantitatively inherited with low to medium heritabilities.

In 1973, C64 and C17 were crossed. In 1974, the F₁ was backcrossed to C17 to produce a BC₁ source population. The backcross to C17 was made to enhance the recovery of resistance to VY.

In 1975, the BC₁ population (cycle 0) was grown in the field and 12-week-old plants were wound-inoculated with *E. carotovora betavascularorum*. A low percentage of the population seemed to be resistant. Roots of the resistant plants were harvested in October. The selected roots were induced to flower in a photothermal induction room (4 months’ exposure time) and increased by open-pollination in the greenhouse in 1976 to produce the cycle 1 population.

In 1977, the cycle 1 population was planted in the field and inoculated with ERR, VY, and PM. In November, roots free of soft rot were selected and harvested on the basis of size, sucrose concentration, and shape. A selection index was used that gave greater weight to sucrose concentration than root weight. The selection index also was used as an indirect procedure to select for resistance to VY and PM. About 50 selected roots were grown in the greenhouse and, following 4 months of photothermal induction, cycle 2 seed was obtained in June 1978 by open pollination of about 50 roots. In November 1978, seed of the cycle 2 population was planted in the field and subjected to natural cold induction. In mid-June, plants were inoculated with ERR, VY, and PM. In November 1979, nonbolted plants were selected and harvested on the basis of freedom from soft rot, root shape, and a high index for yield. During the winter, selected roots were used to obtain cycle 3 seed by open-pollination.

In June 1980, the cycle 3 population was sown and plants were inoculated with VY, ERR, and PM. In 1981, selected individuals were intercrossed to obtain Syn 1 seed of the cycle 4 population. Syn 2 seed was obtained by open-pollination of the Syn 1 generation.

In 1982, Syn 2 seed of the cycle 4 population was released as germplasm line C46. It was increased by commercial companies in 1982 and registered in 1985 (Lewellan et al., 1985). In 1982 and 1983, C546H3 was crossed with C46 to produce semicommercial seed quantities for wide scale testing. In 1984, small-scale production of ‘USC-1’ was conducted by the Union Sugar Company for marketing in 1985.

During the development of C46, the population developed after each cycle of selection was increased and crossed to a number of CMS parents for replicated tests of general combining ability for seed yield and other traits. Concurrently, the populations and hybrids were tested and moni-
tored for changes and improvements in reaction to diseases by evaluations in appropriate disease nurseries. The selection program to combine moderate levels of resistance to CT, VY, bolting, DM, PM, and ERR was successful.

FIELD- PLOT TECHNIQUES FOR GENOTYPE EVALUATION

Plant Density, Plot Types, and Replication

Plant density for commercial sugar beet production is designed to maximize sucrose yield, not total plant yield. In Europe and North America, for practical reasons including irrigation and cultivation, seed is planted in rows spaced 50 to 80 cm apart, with final plant densities between about 50,000 and 80,000 plants per hectare. Beds are used to facilitate furrow irrigation. Frequently, especially in North America, wide beds spaced 100 to 112 cm apart are used with two rows planted per bed. With this pattern, row spacing on the beds can range from 30 to 40 cm. Within-row spacing of beets is accomplished by precision planting or, more commonly, by thinning. Plant spacing following thinning is about 10 to 25 cm. Thinning of commercial fields is done manually or with mechanical or electronic thinners. For experimental test plots, manual thinning is most common.

Testing programs for experimental hybrids strive to emulate commercial conditions as closely as possible. The initial yield evaluation of hybrid cultivars typically is conducted with limited amounts of seed. Consequently, the first yield trials are conducted with fewer and shorter rows per plot than more advanced tests. Single- or double-row plots about 8 to 15 m in length are frequently used during the earlier testing phases.

As the better hybrids are identified, seed increases are made to permit the use of multiple-row plots with four or more replications per location. Plots are commonly 2, 4, or 6 rows wide, and 6 to 15 m in length. Hybrids that reach the more advanced testing stages, often referred to as semicommercial tests, are typically the most vigorous and not as subject to intergenotypic competition. However, in many advanced yield tests, only the innermost rows of multiple-row plots are harvested.

Test Environment and Location

The number of locations and the nature of the test environments primarily depends on the intended market area for the hybrid. In North America, almost all hybrids are developed for rather limited areas, each of which
has a unique set of conditions. A similar situation exists in Europe, where the largest differences are between the northern and the southern areas. For example, *Cercospora* leaf spot, a consistent problem in southern European countries, is not a problem in the more northern beet-growing areas of Europe. In North America, intermediate and advanced hybrid evaluation tests are limited to two or three locations each year. Within a growing region, such as the Red River Valley of Minnesota and North Dakota, replicated tests are conducted at different sites within the region each year to ensure that data are from a diverse range of soil types common to the region. From the time that a promising hybrid is first identified until is approved for commercial sale, 3 to 6 years of testing are required.

**Experimental Designs and Data Analysis**

The most common design for testing sugar beet hybrids is the randomized complete-block. It is less limiting than other designs for the number of hybrids that can be tested. It is especially useful in early testing stages, when the number of hybrid combinations being tested is usually large. Other frequently used designs are latin squares and lattice designs, both rectangular and square. The latin square and, to some extent, the lattice designs can be limited by the area required to test large numbers of entries with adequate replications.

Data collected from test plots may include seedling vigor, disease reaction, root yield, percentage sucrose, and juice purity. Juice purity may be determined by the use of a purity index, which depends on laboratory determination of such soluble nonsucrose compounds as sodium, potassium, nitrate, and amino nitrogen. The data are used to make decisions about which hybrids should be advanced for further tests and at which locations they should next be tested. Critical evaluation of data from multiple locations and years facilitates making the decision to increase seed of a new hybrid to commercial quantities. When a decision is made to market a hybrid, the parental lines must be grown in seed-producing areas on a scale large enough to meet anticipated demands.

**Planting and Harvesting Equipment**

*Planting Equipment.* Establishing experimental plots for yield evaluation is accomplished by use of planting units that are two broad types: those designed specifically for planting sugar beet seed and those capable of planting seeds of a wide spectrum of crops, including sugar beet. Both types of planters are used for planting the commercial sugar beet crop,
and various modifications make them suitable for experimental plot planting. Planters that accommodate a wide spectrum of crops meter seed through horizontal seed plates with cells of correct size to accommodate the seed being planted. Furrow openers are generally of the double-disk type, similar to those of typical maize planters. The specialized sugar beet planters are designed for small seeds requiring precision planting. These planters use either belts, segmented cones, or vertical rotors for seed distribution. Some planters require that seed be graded precisely to fit a particular cell size. Planters most commonly sow four or six rows.

*Harvesting Equipment.* Sugar beet harvest is accomplished with machines that can harvest one to six rows. The particular harvesting machine available may dictate the plot size used for testing experimental hybrids. The harvesters remove the top from the beet, lift the beet from the soil, and convey it to a storage unit. Included in the operation is the mechanical separation of the roots from the soil. In some beet-producing areas, sugar beet plants to be harvested are first flailed to remove the tops and are removed from the soil with a lifter harvester. Harvested beets from experimental plots are taken to a laboratory where they are washed; then root weight, sucrose, and purity are determined.

**PROCEDURES FOR SEED PRODUCTION**

*Methods for Producing and Maintaining Seed Stocks*

In breeding programs, seed production in the field is most commonly achieved by planting stocklings or mother roots taken from storage. Alternate rows of CMS and male-fertile rows are planted 0.6 to 1.2 m apart. Within rows, plants are spaced far enough apart (0.6 to 1 m) to allow plants to be checked for male sterility or the multigerm seed character. Field crossing nurseries also may consist entirely of male-fertile plants, which are planted in alternate rows of red hypocotyl and green or yellow hypocotyl plants. In such arrangements, the non-red lines are considered the female parent and resulting hybrids are identified by red hypocotyl and root color. Field crossing plots may also consist of a male-fertile pollinator and one or more lines segregating for genetic male sterility \(a_1d_1\). Male-fertile plants in the segregating male-sterile lines are rogued before anthesis.

The primary concern in producing and maintaining sugar beet seed is prevention of contamination by foreign pollen. In North American breeding programs and commercial seed production, nurseries are separated by distances of 1.5 km or more. In European breeding programs, crossing
blocks are often close together, but are separated by rows of hemp (*Cannabis sativa L*.). The hemp provides a biological barrier which is considered adequate for minimizing contamination by foreign pollen in most cases.

In breeding programs, small quantities of seed are regularly obtained from greenhouse crosses. Greenhouse-produced seed is harvested from photothermally induced plants grown from seed or from mother roots that have undergone thermal induction by cold storage. In breeding programs with small plots for seed production, entire plants are hand-cut, placed in burlap bags, dried naturally or artificially, and threshed with a Vogel threshing machine or similar device.

**Commercial Seed Production and Marketing**

*Planting Methods.* Two methods of planting are used to produce commercial hybrid seed. One method is to plant a seed mixture of 85 to 90% of the monogerm male-sterile parent and 5 to 10% of the multigerm pollinator. Care must be exercised to ensure a uniform distribution of pollinator seed in the row. Seed is bulk harvested and the larger multigerm seed is mechanically separated from the smaller monogerm seed.

In the second method of commercial hybrid seed production, alternate strips of a male-sterile line and a pollinator line are planted. A distance of 1.8 m is left between the strips so that seed harvest can be accomplished without mixing the two parents. The ratio of pollinator to male-sterile rows is commonly 4 to 12 or 4 to 16, depending on the pollen-producing ability of the male parent. If seed of the pollinator does not need to be harvested, it is destroyed by tilling it into the soil about 3 weeks before harvest of the male-sterile line. The strip planting method is favored when the pollinator line is a weak pollen producer or when its growth habit will not permit effective competition in a mixed planting with more vigorous male-sterile lines.

*Seed Maturity and Harvest.* In commercial seed production areas, the first blooms appear 5 to 6 weeks from the time seedstalks begin to form. The full-bloom stage of development is reached approximately 2 weeks later. The crop begins to mature and is ready for harvest about 6 weeks after the full-bloom stage. Seed maturity is best determined by observing the stage of development of the endosperm. Fruits (seed balls) from representative branches are dissected and the endosperm is observed. Fruits may be very green and high in moisture content, but if the true seed is in a moderate to hard dough stage, the seed crop is ready to harvest. Definite changes in color of the seedstalks and foliage, and shattering of early ma-
turing seed are indicators of the proper time for harvesting the crop. As the seed approaches maturity, it usually takes on a straw color. The maturity of cultivars that are resistant to bolting, especially monogerm lines, can be difficult to determine because the seed stalks and foliage remain green when the seed is ready for harvest.

Commercial seed harvest is accomplished by windrowers with vertical sickle attachments, which permit cutting through tangled masses of branches. Threshing is accomplished with a combine after the plants are dried in windrows for 7 to 14 days. Threshed seed is cleaned by passing it through air separators and other equipment to remove trash and to size the seed to contract specifications. Seed is passed through equipment designed to polish the seed by grinding off a portion of the rough, cory material (pericarp) surrounding the true seed. This operation reduces the weight and bulk of the seed and makes the product more uniform in size.

Seed Storage. Processed seed is stored in bags under relatively cool temperatures of 13°C and low humidity of 15%. Under these conditions of low temperature and humidity, seed viability may be maintained for extended lengths of time. Seed lots in cold storage conditions have maintained good seed germination for more than 20 years.

Seed Marketing. Although seed is not the primary harvestable product in sugar beet, it nevertheless is a valuable commodity. Sugar beet seed in North America and Western Europe is marketed by either private companies that deal exclusively in seed or by companies that process beets for sugar and also develop cultivars of sugar beet for seed sale.

In the United States, the sugar beet seed market is regulated to some extent. Committees composed of processor and grower representatives establish minimal acceptable standards for growing areas or factory districts. These standards include minimal requirements for disease resistance, quality, and sucrose. Cultivar tests are established, which often include disease evaluation. These unbiased tests are conducted by state, federal, and private companies. A company may seek approval for a new or existing cultivar by paying a testing fee for subjecting the entry to about 3 years of testing. In general, only one or a few cultivars are grown in a production area. Specific environmental conditions, especially prevalent disease or diseases, dictate the cultivars grown.

FUTURE PROSPECTS FOR CULTIVAR DEVELOPMENT

Significant increases in the basic productivity of sugar beet cultivars will likely come from new methods that can accurately identify and isolate genotypes that have desired traits, such as disease resistance, pho-
tosynthetic efficiency, and drought tolerance. There is a need for improved screening methods, especially for quantitative traits with low heritability. Traditional plant breeding approaches, although usually successful, are predictably slow. Genetic engineering, protoplasmic hybridization, recombinant DNA techniques, and similar technology will not likely contribute to sugar beet cultivar development in the near future. However, there are several methods that seem to show more immediate promise as tools for use in cultivar development.

**Meristematic Cloning**

Techniques for obtaining multiple copies of selected genotypes utilizing axillary or terminal meristems recently have been described (Saunders, 1982). These in vitro procedures depend on exposing meristems from flowering plants or seedlings to nutrient media containing cytokinin for shoot multiplication or auxin for root initiation. Some of the uses for the in vitro cloning technique are for maintenance of O-type candidates, especially when the O type is self-incompatible; to provide genetically identical propagules for use in polycross nurseries to ensure equal and random pollination; for maintenance and increase of aneuploids for basic research studies; and for production of clones with specific selected traits, such as high taproot to leaf weight ratio (Snyder, 1978).

**In Vitro Sporophytic-Gametophytic Screening**

*Sporophytic Screening or Selection.* Seedlings, meristems or callus, when cultured in vitro, can be subjected to specific challenging agents added to nutrient media. Figure 15-9 outlines the procedure using a theoretical phytotoxin. Selection criteria for identifying tolerant genotypes may vary with the specific challenging agent. Susceptible genotypes may show severe stunting or irregular development, such as suppression of central bud development or complete tissue necrosis. The ultimate success of this system depends on the ability to regenerate plants following selection. Genotypes selected as tolerant after the first cycle may be recycled at the same or different concentrations of the challenging agent. Tolerant genotypes may be proliferated and rooted via subculturing and induced to flower for hybridization seed increase, or for screening in the gametophytic generation.

*Gametophytic Screening.* Pollen from sugar beet plants can be collected and placed in pollen germination media to which specific challenging agents have been added, such as herbicides or pathotoxins. Figures 15-10
and 15-11 present two methods by which this procedure might be accomplished. The direct method presented in Fig. 15-10 has not been tested, but is described because the techniques for its use are now available.

In brief, the method facilitates the crossing of self-incompatible clones selected simultaneously for tolerance to the same challenging agent. In the example presented, the challenging agent is a pathotoxin, such as that produced by a specific fungus. Pollen from each clonal source is collected and incubated for a short time in liquid media containing predetermined concentrations of the pathotoxin. Pollen that is not tolerant to the toxin would be killed, while tolerant pollen would remain viable. Recovery of the pollen from each incubation dish and subsequent pollination of alternate source plants completes the procedure. Plants or clonal lines used in this method need not have been screened previously at the sporophytic level.

The indirect method has been used by the author and seems to offer promise as a verification of selection accuracy for selections made in the sporophyte or for initial selection in the gametophyte (Fig. 15-11). With this method, plants or clones that are to be screened for tolerance are kept isolated until each of their pollen samples has been tested. Pollen samples
Figure 15-10 Direct method of gametophytic screening for phytotoxin resistance using pollen from self-incompatible lines or clones. Seedlings selected for phytotoxin resistance in the sporophytic generation or unselected plants are multiplied in vitro, and brought to flower. Two different, resistant, self-incompatible (SI) clones are chosen for gametophytic screening. The self-incompatibility within each of the clones prevents selfing and helps ensure that hybrid seed is produced. Pollen is collected from each of the two clones and cultured in medium containing phytotoxin. Pollen with resistant alleles should remain viable, while pollen with susceptible alleles will be inhibited or killed. The pollen is recovered and used to make reciprocal pollinations. In theory, pollen grains carrying alleles for phytotoxin resistance will result in seed containing a higher frequency of resistant alleles than those from unscreened pollen.

taken from each source are incubated in liquid media containing predetermined concentrations of a challenging agent, such as a toxin or herbicide. Tolerant plants whose pollen gives a positive germination in the media are intercrossed. All pollen germination tests include, as controls, pollen samples of each source tested on liquid incubation media without a challenging agent.

Using this indirect method, it has been found that a majority of clones selected in vitro for tolerance to the herbicide ethofumesate in the sporophytic generation were confirmed as tolerant in the gametophytic generation (Smith and Moser, 1985). In further tests of this indirect method, progeny from plants selected for ethofumesate tolerance by
gametophytic pollen tests were grown in soils containing various concentrations of ethofumesate. Plants identified as tolerant in the gametophytic test produced progeny that exhibited better germination, increased dry weight, and a higher percentage of seedlings with normal true leaf development after 4 weeks than the progeny of plants identified as less tolerant.
ACKNOWLEDGMENTS

The author extends special thanks to Dr. Robert T. Lewellan, Salinas, California, for supplying the developmental sequence for the new cultivar 'USC-1.' Appreciation also is extended to Dr. J. C. Theurer, East Lansing, Michigan, for supplying information for updating the gene symbol table and to Mr. Hal Moser, Fort Collins, Colorado, for his assistance in reviewing and assembling the manuscript.

REFERENCES


