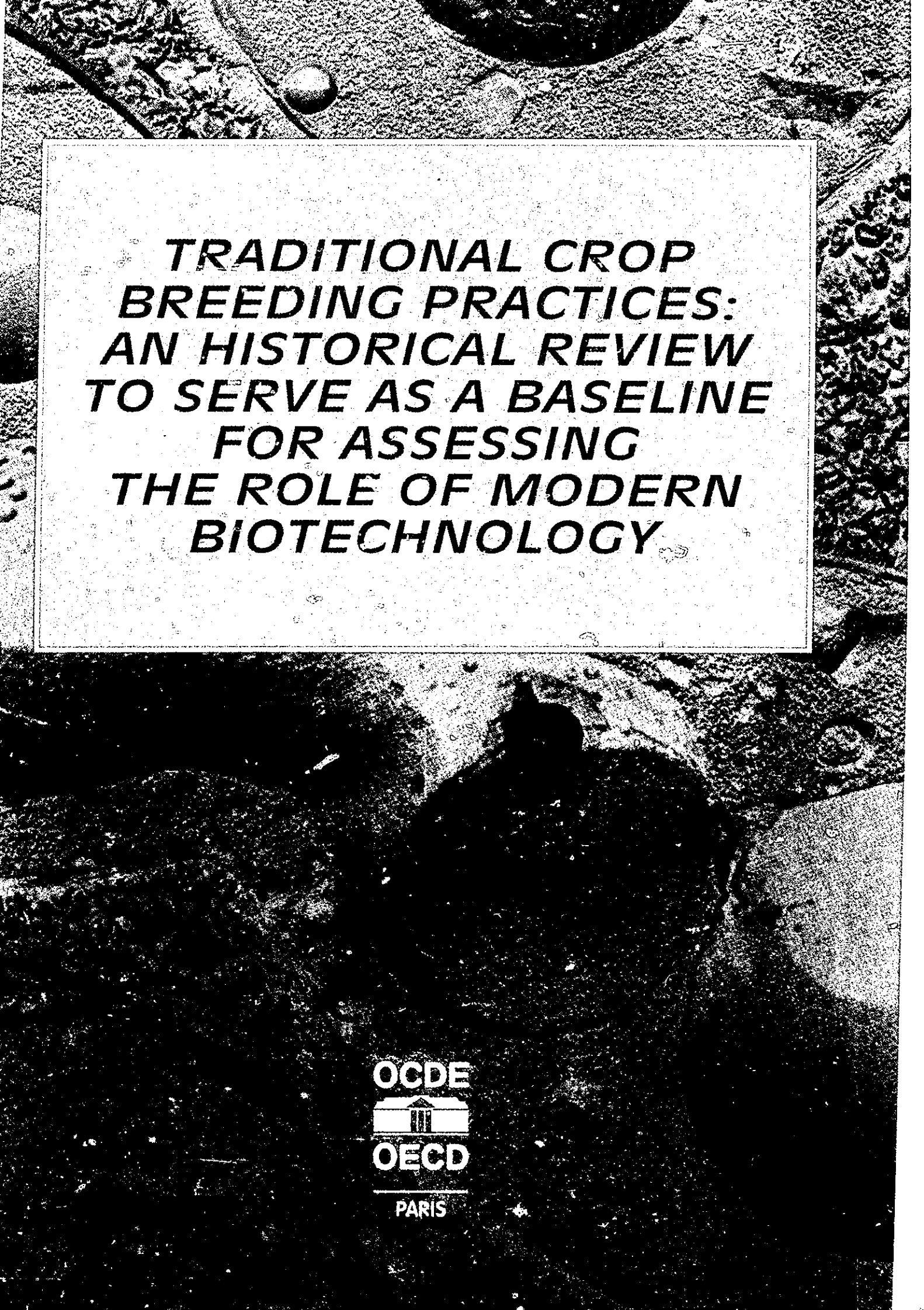

TRADITIONAL CROP BREEDING PRACTICES: AN HISTORICAL REVIEW TO SERVE AS A BASELINE FOR ASSESSING THE ROLE OF MODERN BIOTECHNOLOGY

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ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

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Publié en français sous le titre :

MÉTHODES TRADITIONNELLES DE SÉLECTION DES PLANTES :
UN APERÇU HISTORIQUE DESTINÉ À SERVIR DE RÉFÉRENCE POUR
L'ÉVALUATION DU RÔLE DE LA BIOTECHNOLOGIE MODERNE

Foreword

The OECD's Group of National Experts on Safety in Biotechnology (GNE) has, for many years, developed concepts and principles relevant to risk and safety assessment in modern biotechnology. As the success and safe record of new techniques lead to their widespread application in various sectors, including agricultural crop plants, the GNE considered that a "baseline" review of traditional crop breeding practices against which to appraise the biosafety aspects of the new techniques would be valuable.

In 1991, it was agreed to commission the present work based on a proposal and guidelines prepared by the GNE Chairman, Mr. W. de Greef, of Belgium. A panel of experts from Member countries prepared contributions on different crops, according to these guidelines.

The document has been reviewed by the GNE and its parent body, the Committee for Scientific and Technological Policy, and approved by the Secretary-General as an expert scientific and educational publication. It is not a statement of OECD policy nor of its Member governments, and the opinions expressed engage only the authors.

The OECD work on safety aspects of biotechnology, of which the present publication forms a part, has been made possible in particular by the generous financial support received from the Commission of the European Communities and from the Government of Japan.

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Preface

by

J.E. Veldhuyzen van Zanten

As long as life has existed it has been at risk. Living organisms face hazards at every step of their existence. In the struggle for life, some survive, some perish. The survival of the fittest is decided by natural selection.

Breeding crop plants is a way to enhance natural selection. Man composes a cultural environment that ranges from extensive agriculture to the growth chamber. He rearranges the genes of crop plants and, by doing so, gains the knowledge and experience that enable him to adapt living organisms to human use and to help them survive more easily.

It is to this historically built-up wealth of knowledge and experience that the Subgroup on Crop Plants of the Group of National Experts on Safety in Biotechnology of OECD decided to devote their attention. As a result, 17 major crops of world-wide importance and their parent species, considered to be prime targets for genetic modification projects, have been studied for their important characteristics, physiology, toxicology and environmental behaviour. A group of eminent authors from all over the world, each an authority in his field of plant breeding, contributed to this overview.

The *Historical Review of Crop Breeding Practices* published apart is by no means a scientific textbook on plant breeding. It is written for the layman. It provides insight into how the crop was derived from its wild origins and what goals have been sought in order to make use of the special features of each plant species. Nutritional quality and crop safety received special attention. "Common practice" in exposing plants to the environment is made clear, and observations are made on field release during the different stages of selection, crossing and testing.

Breeding material eventually becomes varietal seed, which is planted in quantity in agricultural systems. A variety is a superior combination of genes. It carries disease and pest resistance and strong factors for sustainable growth.

It can be argued that a limited number of varieties, used over a wide area, might reduce the genetic variation of a crop. This study shows how plant breeders cope with this potential problem by replacing the germplasm periodically, in reply to the needs of society.

The crop reports have been ordered according to their inherent mode of reproduction. The first group is strictly or predominantly self-fertilised. It includes soybean, wheat, rice, tomato, cotton, and tobacco. Cucumber and melon also show a high degree of

self-fertilisation. The self-fertilising principle tends to provide uniform varieties at high inbreeding levels. However, in most of these crops, the advantage of F_1-hybrids is recognised and employed.

The second group has a natural tendency for outcrossing and provides offspring of a more heterogeneous nature. In seed production, safe isolation distances must be observed and are well established for each crop. These crops include maize and sugar beet, whose pollen grains are dispersed by the wind over long distances, and sunflower, alfalfa, *Brassica* crops and onion, as well as squash and watermelon, all insect-dependent cross-fertilising crops. Examples of distances observed in some countries are given in the review. The advantage of uniform F_1-hybrid varieties in this second group is very obvious. Breeders have made great efforts to engineer reproduction mechanisms that allow the controlled production of F_1-seeds.

The third group consists of crops whose varieties are propagated by cuttings, stecklings, roots, tubers, or even by tissue culture. Under this heading fall cassava, potato and *Prunus*. In the hands of the plant breeder, the flowers of these crops may be crossed in order to create new variability, but in normal practice these crops are multiplied as uniform clones.

For every crop, the topics treated appear in a fixed order. This makes it possible to read the historical review either for individual crops or for specific subjects. Hence, it is possible to search the document for "dispersal and survival mechanisms of propagules" or "toxicological effects".

The historical review was written by experienced plant breeders, whose task was merely to describe "common practice", including existing seed regulations. The document is therefore not exhaustive. The authors were discouraged from discussing regulatory issues in connection with modern biotechnology. Their only instruction was to make their texts accessible to policy-makers, regulators etc., who are not experts in the field, in order to provide a historical baseline on environmental safety of crops based on "common practice".

In his *Origin of Species,* Charles Darwin pointed to the fact that familiarity may make us overlook new perspectives. In his case, familiarity with the facts obscured the law of natural selection. Today, familiarity could lead to ignoring the possibilities offered by gene modification, until and unless the value of these historical data are better understood.

Plant breeders are very familiar with the genetics of their crops, and they are starting to seek ways to make safe and meaningful use of the new technologies in their science. The historical review provides many examples.

The 17 crops in this study originated in the Middle East, central Asia, China, India, the Mediterranean basin, Australia and the Americas. Yet, all have spread around the globe.

All domesticated species are seen to be related to wild species, although in very different frequencies. Over 60 related species exist in the case of tobacco, sunflower, alfalfa, cassava, potato and *Prunus*. Less than ten closely related species are found in soybean, wheat, maize and sugarbeet. Maize, in fact, has but one related wild species, teosinte, which grows only in Mexico and Guatemala, where it can outcross to maize in seed production fields.

Natural outcrossing is reported. The number of cases, however, is low. Apart from maize, there are: cross-compatible red rice in Japan, India and the United States; wild cotton from Hawaii, where cotton is not usually grown; wild beets in the Mediterranean area, likely to contaminate seed multiplication plots; and the wild ceara rubber that crosses freely with cassava. Adequate crop management can deal with these problems. Seed production of population varieties of sugarbeet in the presence of wild beet offers one example. Provided the fields are large, the varieties produce high amounts of pollen, and the clouds of pollen grains prohibit wild beet pollen from contaminating the seed crop. However, hybrid seed crops cannot be grown near wild *Beta*. Seed producers now avoid such areas.

The toxicology issue is less serious than one would expect at first glance. Soybeans contain trypsin inhibitors that are undesirable in cattle feed. Wheat gluten in food sometimes causes health problems, and wheat pollen may cause inhalation allergy. Lignin in over-mature alfalfa hay lowers digestibility in livestock, so crop management of alfalfa must include early harvesting of forage. Some compounds never reach levels of toxicity that prohibit the use of the product. Examples are bitterness in cucumber, tomatin in some wild green tomatoes, solanin in potato, oxalic acid in fodder beet. Cassava, which is an important tropical staple food for human consumption, contains an enzyme, linamarase, that produces poisonous glucosides once the root cells are ruptured and exposed to the air. Tobacco is purposely grown for nicotine and contains dangerous levels of tar. It can be argued, however, that these poisons are familiar traits. The cyanamide of cassava is managed by proper processing and cooking. This is everyday practice in areas where cassava is a common food crop. Nicotine and tar in tobacco are legalised drugs, and managed accordingly. These points may need further attention.

Plants, by their very nature, are cultivated in open, unprotected environments, as are modified plants, regardless of the cause of the modification. Greenhouse isolation appears to be more the exception than the rule. Isolation or containment is not related to stages of development. For example, elite plants for basic seed production of an established sugarbeet variety enter the greenhouse, but an inbred of a cross for cold-resistant maize enters into field tests. New cotton candidate varieties must pass 20 trials, expressed in number of years times number of locations. After approval of the best candidate, there are four more stages of seed multiplication, some of them with contract seed growers, before the variety is allowed to reach the marketplace.

The crop study reveals that the era of plant biotechnology has already begun. Plant breeders use the diagnostic tool of RFLP (restriction fragment length polymorphism), the so-called "finger-printing" technique, to locate more precisely the genes they handle in tomato, maize and sugarbeet, among others. Regeneration of haploids and somatic hybrids is mentioned for tobacco and sugarbeet; it is also practised in cotton, tomato, alfalfa, *Brassicae* and potato. Transgenesis is now applied in many places and in many crops, including maize and rice. It is generally considered by plant breeders as an additional tool, complementing their conventional breeding schemes.

In conclusion, biosafety measures in crop plants require a thorough knowledge of the nature of crop plant breeding. This "historical review" provides such a baseline, thanks to the wealth of knowledge and experience offered by competent authors familiar with conventional plant breeding.

Introduction

by

Wally D. Beversdorf

This study, prepared by a panel of national experts following discussions in Paris on 9-10 November 1991, presents current and traditional mechanisms for genetically modifying plants in order to develop new varieties. It also offers a brief discussion of the paths by which the products of plant breeding programmes enter production systems and current commercialisation channels in OECD countries.

Plants have a unique ability to capture light energy from the sun and to draw physical elements from the earth and convert them into biological energy and many other biological products. Through photosynthesis and other metabolic systems, plants satisfy directly or indirectly the nutritional requirements of nearly all the other life forms on earth. In addition, plants provide oxygen, fibres, flowers, aromas, therapeutic medicines, etc., that are essential or aesthetically desirable to human beings. Plant cultivation makes use of various technologies – in tillage, planting techniques, plant protection, plant product harvesting, and storage and processing of plant products – on a relatively small number of plant species (and associated microbes) in order to sustain or enhance human and domestic animal populations. As society has evolved, cultivated plant species have undergone genetic modification (domestication), and cultivation technologies have changed.

Following the introduction, more than 150 years ago, of the Vilmorin selection technique in sugarbeet, many cultivated plant species have been systematically improved through plant breeding processes. These processes of genetic manipulation, coupled with improved techniques for cultivation and plant protection, are in large measure responsible for improved nutritional and physical well-being in OECD countries and elsewhere, despite the dramatic population growth (400 per cent) that these societies have sustained during the past century. Improvements in the biology of cultivated plants have been made by genetic modification of plant species through systematic breeding. Plant breeding programmes involve a series of activities:

i) acquisition or development of genetically variable plant populations;
ii) selection for desirable characteristics in order to increase their frequency in breeding populations;
iii) use of techniques to stabilise improved genetic composition from generation to generation or year to year;

iv) careful evaluation of genetically modified populations for adaptation, productivity, and other processing or end-user requirements to ensure that the genetically modified populations will meet producer, processor and end-user expectations; and

v) maintenance, purification and multiplication of the genetically modified population to provide stable starting propagules for commercial plant production systems.

While plant breeding generally aims to enhance cultivated plant species, specific goals reflect the needs of producers, processors, and end-users of these species. Some of these are:

- improved tolerance or resistance to organisms that consume or contaminate cultivated plants;
- improved tolerance to abiotic stresses (drought, heat/cold, atmospheric pollutants, and other physical stresses encountered in plant ecosystems);
- improved productivity (conversion of carbon dioxide and soil nutrients into useful biological products);
- improved processing and nutritional characteristics (improved human or livestock nutritional composition, reduced content of natural toxins, improved storage characteristics, etc.); and
- improved adaptation (to increase the geographic region or environmental niches within which a crop can be cultivated).

Although there are common steps associated with most plant breeding programmes, specific procedures employed by plant breeders are affected both by biological characteristics (*e.g.* method of reproduction, mating behaviour, genetic resource availability, etc.) and by market requirements defined by producers, processors and end-users. The biological characteristics of cultivated species differ tremendously. Some species (*e.g.* potato and numerous fruit trees) are propagated asexually. Many others are reproduced sexually through formation of seeds (embryos that result from sexual fertilisation). Among those propagated by seed (including all of the world's major grain crops), some are propagated by self-pollination (*e.g.* wheat, soybean, barley and common beans), while others reproduce by cross-pollination (*e.g.* maize, sunflower, sugarbeet, cucumbers, rye, alfalfa, etc.). Among species that reproduce by cross-pollination, cultivated crop varieties may be synthetics (random mating populations of genetically distinct individuals (*e.g.* rye and alfalfa) or hybrids produced by controlled matings between different populations (*e.g.* hybrid maize, hybrid sugarbeet, hybrid sunflower, etc.). Still other species fall somewhere between cross-pollinated and self-pollinated (*e.g.* oilseed rape) and result in varieties that may reflect pure lines, synthetics or hybrids, depending on the breeding strategies and biological resources available.

Pure-line varieties (typical of highly self-pollinated species like soybean) are populations of genetically identical individuals in which parental generations and progeny generations are identical. A synthetic variety typical of some cross-pollinated species (like alfalfa) is formed of a population of genetically distinct individuals, each reflecting the unique combinations of alleles received by either parent. While a synthetic population consists of many genetically distinct individuals within any generation, the specific type and frequency of genetic individuals (genotypes) vary only slightly from generation to generation of random mating. A hybrid variety (typical of maize or sunflower) is com-

posed of identical or very similar individuals, but unlike pure lines, they are genetically very different from both their parents and their progeny. Thus, reproductive characteristics, mating behaviour (self- or cross-pollination) and the capacity to control matings play a major role in determining the plant breeding processes employed in variety development.

In spite of these differences, similar steps are followed in the development of a variety. Genetically variable breeding populations are produced through controlled matings between genetically different parents (a technique commonly employed since the late 19th century), mutagenesis (employed since the early 20th century), or through acquisition of land races which have undergone random matings or natural mutations. Modern parasexual hybridisation (non-sexual combining of genetic material through fusion of genetically distinct plant cells) differs from controlled matings (hybridisation) in that it permits hybridisation between sexually incompatible individuals. Hybridisation as a result of controlled matings may be very simple (a one-time cross between two individuals), very complex (successive generations of controlled matings involving many distinct individuals), or very specialised, as in backcrossing (a sequence of matings initially involving two genetically dissimilar parents with subsequent generations mated to only one of the parents), a technique employed to transfer one or more specific characteristics from one population (donor) to another (recurrent parent). Modern technologies used to transform plants are conceptually identical to backcross mating schemes, but they make it possible to use donor populations that are sexually incompatible with the recurrent parent (recipient) population.

Selection procedures used to change the frequency of individuals with specific characteristics within genetically variable breeding populations simply involve measuring the characteristics of individuals within populations and eliminating those with undesirable characteristics. They are normally followed during consecutive generations and may be conducted on individual plants or on plant families. Selective retention or elimination of individuals within populations is commonly referred to as mass selection technique. Selective retention or elimination of families is commonly referred as to as pedigree breeding in self-pollinated species, or half-sib (individuals with one common parent) and full-sib (two common parents) in cross-pollinated species. There are many variations of these basic selection procedures, depending on the specific objectives of the breeding programme and the species involved.

For all selection procedures, measurements undertaken by plant breeders to include or eliminate individuals or families from subsequent generations in a breeding programme depend on the requirements of producer, processor or end-user. Such measurements may involve simple procedures, such as measuring plant height, lodging, or attractiveness. Others may be very complex, such as measuring the response of individuals following artificial inoculations with a plant pathogen, or chemical analysis of the oil, protein and toxicant content of grain). Selection decisions are usually made by considering many measured characteristics sequentially (independent culling) or collectively (using weighted selection indices), although the former is generally economically more efficient.

Genetic stabilisation of populations is required for sexually propagated species. It is accomplished by inbreeding to genetic uniformity among individuals within families for self-pollinating species or through random matings of individuals within families for cross-pollinating species. Genetic stabilisation may precede selection (in self-pollinated species), occur concurrently with selection processes (self- and cross-pollinating species),

or follow selection (typical of cross-pollinated species). Stabilisation of breeding populations is required to ensure that subsequent generations of families retain the attributes selected by the breeder. Asexually propagated species are stabilised by the multiplication procedure.

Genetically stable selected families (potential varieties) within plant breeding programmes are normally carefully evaluated for:

- geographic and production system adaptation (to determine if, where and how the selected family can be produced);
- performance characteristics (to determine the relative value of the selected family in comparison to varieties already in the commercial production system for productivity, pest and stress tolerance, harvestability, etc.);
- processing characteristics (*e.g.* milling characteristics of wheat, extraction and sugar yield characteristics of sugarbeet, storage characteristics of fruits and vegetables, etc.); and
- end-user characteristics (protein content of soybeans, bread-making or pastry-making characteristics of wheat, flavour characteristics of many fruits and vegetables, appearance and appearance retention characteristics for many ornamentals, etc.).

These evaluations are normally conducted across the range of potential geographic adaptation for an improved family and are usually carried out over several years to ensure that measurements reflect a variety of weather patterns in the potential areas of adaptation. Normally, evaluation trials begin at one or a few locations and subsequently spread to cover the potential range of adaptations and market interest. Expansion of evaluation trials, to which a family (potential variety) is subjected, is governed initially by seed (propagule) availability and subsequently by consideration of information from previous evaluations regarding: the range of potential adaptation; the sensitivity of the species to environmental variables; the intensity of production within zones of potential adaptation; and, in many OECD countries, the regulatory requirements for registration of new varieties. Evaluation trials are commonly a co-operative effort among plant breeders, plant variety regulators, agronomists and plant protection scientists with expertise in the species, plant processors capable of evaluating processing and end-user attributes of the families, and extension personnel familiar with producers' recommendations for the species or regions of adaptation.

At the time of scale-up evaluation of potential varieties, plant breeders normally initiate scale-up multiplication and purification of basic seed stocks or asexual propagules of the selected families. These activities normally continue throughout a breeding programme to ensure a sufficient supply of seed or propagule stocks for commercial multiplication, and to ensure that basic seed or propagule stocks do not change genetically through drift, mutation or contamination for as long as there is commercial potential for the variety.

The systematic plant breeding process described above has displaced the gradual domestication process of the past. A century of systematic plant breeding has refined the basic genetic resources (genetic variability) of cultivated species into superior cultivars capable of fulfilling the demands of modern society and has played a significant role in the remarkably improved nutritional well-being of modern societies. Superior cultivars coupled with superior production processing and distribution systems in most OECD countries have resulted in a marked decline in the portion of human activity devoted to

agricultural activities. Those active in plant breeding are generally aware that there is no room for complacency. Ever increasing market demands, declining genetic resources within many cultivated species, declining environmental resources for plant cultivation (arable land, plant nutrients, etc.) and uncertainty about both changing climatic conditions (*e.g.* the greenhouse effect) and global population stabilisation will undoubtedly tax plant cultivation systems over the next few decades.

The following chapters present a number of important cultivated plant species along with a brief description of their history, the roles they play, and the means employed to develop superior varieties. Collectively, they show how improved plant varieties are developed and enter modern plant cultivation systems.

1. Soybean

by

Wally D. Beversdorf

A. Characteristics of the crop

a) Geographic origins

Soybean, *Glycine max* L. Merrill, is a cultivated species of the legume family. The origin and early history of soybean production is not well known. According to Hymowitz (1970), soybean was probably domesticated around the 11th century BC in what is now northeastern China. Cultivated soybean is closely related to wild soybean, *G. soja* Sieb. et Zucc., with which it is highly cross-compatible. *G. soja* (previously known as *G. ussuriensis*) is an annual herb naturally distributed through much of China, Taiwan, Korea and eastern Russia. Collectively, *G. max* and *G. soja* make up the natural crossing range of cultivated soybean.

b) Geographic distribution of use; main production areas

Outside of Asia, soybean remained more a curiosity than a crop until the present century. Today, annual world soybean cultivation exceeds 50 million ha. Major soybean producing nations include the United States, Brazil, China, Argentina, and India.

c) Taxonomic status

In addition to the sub-genus *Soja,* in which both cultivated soybean and *G. soja* reside, two other sub-genera contain relatives of soybean. The sub-genus *Glycine* includes six species that are distributed naturally in Australia, southern China, Taiwan, the Philippines and several South Pacific islands. The sub-genus *Bracteata* includes a number of sub-species of *G. wightii,* a climbing, vine-like perennial that is distributed in Africa and Southeast Asia. This species includes "perennial soybean" which has some agricultural usefulness as a tropical forage crop.

d) Genetic and cytogenetic characteristics

Members of the sub-genus *Soja* (wild and cultivated soybean) have 40 chromosomes. Members of the sub-genus *Glycine* contain either 40 or 80 chromosomes. Some interspecific hybrids between species within sub-genus *Glycine* have been achieved through hand pollination. Within the sub-genus *Bracteata,* sub-species of *G. wightii*

appear to have either 22 or 44 chromosomes. *Glycine wightii* appears to have much larger chromosomes than members of the sub-genera *Glycine* and *Soja.*

No naturally occurring hybrids have been observed between sub-genera of *Glycine.* Cultivated soybean appears naturally cross-compatible only with wild soybean (*G. soja*). *G. soja* and *G. max* could be taxonomically defined as a single species (Hadley and Hymowitz, 1973).

e) Current phytosanitary considerations in movement of germplasm

Like many other major agronomic crops, soybean is susceptible to a number of economically important diseases. Pathogens include an array of fungal, bacterial and viral organisms (many of which can be seed-borne), nematodes, and other soil-borne organisms (which can move with seed in soil pedes). Brown spot (*Septoria glycines*), downy mildew (*Peronospora manshurica*), and stem canker (*Diaporthe phaseolorum*) are among the fungal pathogens that can disseminate through infected soybean seeds (Athow, 1973). Bacterial blight (*Pseudomonas glycinea*) is probably the most common seed-transmitted soybean disease (Kennedy and Tachibana, 1973). Soybean mosaic virus, which commonly causes a seed coat discoloration around the soybean hilum (placenta scar), is probably the most common seed-borne virus (Dunleavy, 1973). Soybean mosaic virus is widely distributed in soybean production areas. Like the seed-borne diseases already mentioned, *Phytophthora* root rot (*Phytophthora megasperma*), a fungal root pathogen, and soybean cyst nematode (*Heterodera glycines*), a major pathogen (Good, 1973), can probably move with soil pedes in shipments of seed or grain.

Many soybean pathogens are well established in older production areas of North America and Asia. Phytosanitary considerations in the movement of seeds, soil and inoculants may reduce the spread of diseases into new or uninfected soybean production areas.

f) Current end uses

Soybeans have evolved as a major food, feed and industrial crop. Soybean seed is approximately 40 per cent protein, 21 per cent fat, 34 per cent carbohydrate and 5 per cent ash (Orthoefer, 1978). Prior to 1900, soybean was used in the Orient for its medicinal and food value. Foods prepared from soybean included beverages, pastes, fermented flavourings (*e.g.* soya sauce), and a variety of curds.

Although soybean oil and meal were introduced into Europe in the 1700s, there was little interest in soybean production or processing outside the Orient until the 19th century. Japan used soybean meal as a fertiliser in the late 1800s, at about the time that soybean production took hold in the eastern United States. In the United States between 1930 and 1945, soybean gradually became used for oil and defatted soybean meal rather than for fodder.

Soybean oil remains a common edible vegetable oil used in many refined oil/fat products. Soybean phospholipids (by-product of soy oil processing) are marketed as lecithins, with food applications in emulsifiers, wetting agents, antioxidants, dispersing agents and anti-splattering agents (Smith, 1989). Numerous other oil-related by-products have industrial and personal health care applications.

The nutritional value of defatted soybean meal in swine and poultry rations was recognised soon after the Second World War. Today, most soybean meal is used as a protein source for livestock nutrition. Full-fat cracked soybeans also find applications as livestock feed, particularly where transportation costs to crushing facilities justify "on-farm" consumption. Roasted full-fat soybeans provide both protein and highly digestible energy in poultry and swine rations. Soybean protein (isolated from soybean meal) is also used for a growing number of food and medical applications. Soybean also continues to be a source of several traditional "oriental" foods (tofu, miso, etc.) whose popularity is growing worldwide.

Reproductive mechanisms

a) Mode of reproduction and pollination

The soybean and its close wild relative (potential parent) *G. soja* are self-compatible annuals. Fehr (1980) estimated that soybean outcrossing frequency is between 0.5-1.0 per cent. Natural cross-pollination between soybeans requires insect pollinators. Erickson (1976) reported that honey-bees are attracted to the flowers of some soybean varieties. Male-sterile soybean plants (not grown commercially) can approach normal seed set in the presence of male-fertile plants (pollen source) if large numbers of honey-bees or other insect vectors are available and climatic conditions are suitable (Davis, personal comment).

b) Dispersal and survival mechanisms of propagules

Both soybean and *G. soja* are propagated solely by seed. Both cultivated and wild soybean disperse seed through pod shattering. The cultivated form shatters seed under some climatic conditions if harvest is delayed. The wild soybean shatters seed rapidly as pods mature. Seeds of cultivated soybean survive poorly in soil (normally less than one year). The smaller seeds of wild soybean may survive longer, although this is not known.

c) Ability to cross with related species

As mentioned above, soybean and *G. soja* are fully cross-compatible and may be considered cultivated and wild representatives of a single species within the sub-genus. No natural hybrids between soybean or *G. soja* and other species of the genus *Glycine* have been observed. Attempts to hybridise soybean with *Glycine* species other than *G. soja* suggest there is a high degree of interspecific cross incompatibility; when they are successful, hybrids are sterile (Kenworthy, 1989).

Toxicology

Like all oilseed proteins, soybeans contain natural toxins (Smith, 1989), and raw soybean can inhibit growth, reduce fat absorption, cause enlargement of the pancreas and stimulate hypersecretion of pancreatic enzymes in monogastric organisms (poultry, swine, rats, etc). Trypsin inhibitors (proteinase inhibitors) are the most active; they are inactivated by heating to 100°C for 15 minutes or by atmospheric steaming at 25 per cent moisture for 20 minutes. Toasted soybean meal normally does not exhibit the adverse nutritional properties associated with active trypsin inhibitors. Although genetic variation

exists for some soybean trypsin inhibitors, breeders have generally not exploited it because of the effectiveness of soy processing (heat treatments).

Other active agents include phenolic constituents, saponins, phytic acid and hemagglutinins (Orthoefer, 1978). Hemagglutinins can cause clumping of red blood cells *in vitro,* although there is no evidence of red cell agglutination following ingestion of soy hemagglutinins. Soybean saponins (glycosides of triterprenoid alcohols) are not absorbed upon digestion. Phenolic compounds include genistin and daidzin, which can exhibit low levels of estrogenic activity. The significance of phenolic compounds in livestock rations or the human diet is as yet unknown.

Environmental requirements for life cycles

a) Climatic restrictions to extension of the crop

An annual, soybean is adapted to agricultural regions from equatorial to temperate zones. Soybeans grow most rapidly when air temperatures are between 25 and 30°C. Photosynthesis declines markedly when temperatures within the soybean canopy approach 40°C. Soybeans are very susceptible to frost damage during the crop growing period and somewhat susceptible to excessive drought and extended flooding.

b) Biological restrictions to extension of the crop

As soybeans are legumes, they can fix atmospheric nitrogen as a source of nitrogen for growth and development in a symbiotic relationship with *Bradyrhizobium japonicum.* When they are grown in new production areas, seeds are normally inoculated with *B. japonicum* prior to planting.

Soybean seeds deteriorate rapidly in storage as temperatures approach 40°C. Soybeans have difficulty germinating when soil temperatures exceed 42°C (Whigham and Minor, 1978). They are also susceptible to a number of nutrient deficiencies; normally, these can be offset by adjustment of soil pH and/or fertiliser applications.

Soybeans are photoperiod sensitive and typically an annual "short day" species. Most cultivars have delayed flowering when exposed to longer photoperiods and accelerated flowering when exposed to shorter photoperiods. Soybean cultivars are now commonly classified into 13 maturity groups (MG) from MG 000 to MG X. Soybeans of MG 000 are adapted to the longest photoperiods (highest latitudes) while MG X are adapted to shorter photoperiods (equatorial zone). Delayed flowering and generally unsuccessful reproduction (seed maturation) result when a high MG soybean cultivar is grown at a high latitude. Conversely, accelerated flowering and early maturity with low seed yields occur when a low MG cultivar is grown in a tropical or subtropical zone.

For higher latitudes, plant breeders continue to develop soybeans with earlier maturity, less photoperiod sensitivity and greater cold tolerance. Over the next decade, an MG 0000 soybean may emerge for maritime zones at high latitudes.

B. Current breeding practices and variety development research

a) Main breeding techniques

i) Germplasm maintenance

Breeding systems for soybean are similar to those for other self-pollinated grain crops (such as wheat, barley, and peanuts). Soybean breeders depend upon the germplasm available in current cultivars and the soybean collections which are maintained at institutions in many countries. In China, collections include more than 15 000 accessions of *G. max* and 1 000 accessions of *G. soja*; the United States maintains more than 11 000 accessions of *G. max* and nearly 700 of *G. soja*.

As both wild and domesticated soybeans were distributed primarily in China, Taiwan, Japan, Korea and Russia prior to the present century, collection and cataloguing of soybean germplasm have been prerequisites for soybean breeding programmes elsewhere. The USDA maintains two well-catalogued collections, one for early maturing soybeans (MG 000-MG IV) at Champagne-Urbana, Illinois, the other for later maturing soybeans at Stoneville, Mississippi.

ii) Basic breeding

Most soybean breeding activities are directed at development of improved varieties. Basic breeding programmes include development of populations (commonly, recurrent selection populations) for gradual modification of important but complex characteristics (*e.g.* protein content and oil content). Other activities in basic breeding programmes include measurement of inheritance, creation and evaluation of new genetic variability, and production of specific genotypes for research or germplasm maintenance purposes.

iii) Variety development

Soybean variety development programmes follow the basic breeding steps for self-pollinated species. Genetically variable populations are produced through artificial hybridisation followed by inbreeding. Genetically variable (segregating) populations are subjected to selection pressure for desired agronomic and quality characteristics as the populations are inbred through self-pollination. As breeding populations approach homozygosity, superior lines are evaluated for agronomic performance in replicated performance trials and subsequently in multiple location performance trials. Lines with superior agronomic performance or quality characteristics are multiplied under controlled conditions to increase seed stocks for commercial use and to maintain genetic purity.

In public and commercial soybean breeding programmes, the specific procedures employed in cultivar development vary widely. Simple inherited traits such as disease resistance are commonly bred into existing cultivars through backcrossing. When several traits are combined from two or more parents, hybrids from single, three-way, double or other complex crosses may be advanced through any of several procedures to provide homozygous lines. Commonly used methods include bulk population advance, the pedigree method, the pedigree method with early generation testing, single seed descent and modified single seed descent. All methods produce an array of homozygous (true breeding) lines, but vary in the time frames during which selection pressures are applied.

iv) Techniques used

As soybeans are primarily self-pollinated, hybridisation between parents usually involves emasculation (removal of anthers) from flowers of one parent (female or seed parent) and artificial transfer of pollen from the alternate (male) parent. Soybean hybridisations are commonly undertaken in controlled environment rooms or glass house facilities, but they can be accomplished in field nurseries (if the parents involved are of the same or adjacent maturity groups). If parents are from considerably different maturity groups, the planting dates have to be staggered to synchronise flowering periods. Alternatively, the photoperiod of the later maturing parents can be artificially reduced to accelerate flowering.

A hybrid plant reproduces to form a segregating population (segregation and recombination of genes). Development of a new variety usually involves inbreeding a segregating soybean population for three to seven generations, during which selection is applied and individuals in the population become increasingly homozygous (true breeding). Narrow crosses (similar parents) normally require fewer generations of inbreeding than wide crosses (very different parents) to become true breeding.

Winter nursery facilities are used for rapid inbreeding of soybean breeding populations. Because soybean is photoperiod sensitive, breeding programmes in temperate zones can use a tropical winter nursery for rapid winter generation advance. For example, one programme grows F_1 hybrids during the summer in Canada followed by two generations of inbreeding (F_2 and F_3) between October and May in Central America. Although selection is less effective in tropical winter nurseries, inbreeding accelerates the move towards homozygosity, thereby improving selection efficiency in the F_4 population in Canada (June-September). In this programme, populations are inbred and individuals are selected in the F_4 and F_5 generations. Progeny of selected F_5 plants (from narrow crosses) are maintained and evaluated as F_5-derived lines (potential varieties).

b) Main breeding objectives

The objectives of individual soybean breeding programmes vary widely. Because soybeans are very photoperiod sensitive, individual breeding lines will normally be adapted to only a few degrees of latitude. Breeding objectives normally include performance, quality, and stability characteristics required for the zone of adaptation. These may include necessary or desirable resistance to diseases associated with a particular production area, resistance to physical environmental stress associated with the region, improved harvestability (resistance to lodging or higher pod height on the stem), improved yield characteristics, and any of several desired market characteristics (*e.g.* characteristics for special food use in tofu, natto or specialty oil products).

Herbicide tolerance in soybean has received considerable attention in recent years. Weed control problems may reduce yields, particularly in narrow row production systems that restrict inter-row cultivation. Natural variability in soybean for tolerance to some herbicides (*e.g.* metribuzin) is well known. Mutations and plant transformations have provided new sources of genetic herbicide tolerance, including resistance to sulfonylurea (Sebastian *et al.*, 1989) and glyphosate herbicides. New genetic herbicide resistance may provide producers with opportunities for more cost-effective weed control with fewer environmental and toxicological risks.

Composition and quality characteristics have also recently received increased attention. Breeders have identified, created and/or used genetic variability for a number of these characteristics, including oil and protein content, fatty acid components of oil (Rennie and Tanner, 1989), anti-nutritional factors (Orf, 1989) and flavour characteristics for human food (Wilson, 1989).

c) Assessment of general performance

Selection (during and after inbreeding) and evaluation of potential varieties represent major aspects of soybean breeding. Once selected, lines undergo evaluations for composition and performance at an increasing number of replicated trials within and adjacent to areas of potential adaptation. Superior lines are normally released for commercial multiplication following two to five years of evaluation trials. Evaluations are usually conducted through co-operative trials among or within breeding institutions or enterprises. Because many important soybean characteristics are influenced by environment, evaluations of candidate varieties usually involve performance and quality comparisons with established cultivars within individual evaluation trials.

National policies governing the release of commercial soybean varieties vary. Several nations have well-defined performance and quality requirements which must be met by candidate varieties prior to registration and commercial release (*e.g.* Canada, Italy, France), while others have few or no specific requirements (*e.g.* the United States).

C. Seed multiplication for commercial use

a) Stages in seed production

A new soybean variety is normally commercially available when breeder (pre-basic) seed is released by the breeding institution or associated seed multiplication organisation. It may also be made available by the seed production division of a commercial soybean breeding enterprise. Breeder seed of a potential soybean variety is formulated, maintained and multiplied to preserve the genetic characteristics of the variety over generations. Multiplication of breeder seed in commercial quantities usually involves multiplication through three or four classes of seed (breeder > select > foundation > registered > certified). If the variety is adapted to a small market, one or two classes of seed (*e.g.* registered) may be eliminated.

b) Isolation practices

Isolation distance and cropping history requirements for soybean seed multiplication (commonly a few metres and one to two years, respectively) vary with class of seed and jurisdiction, but are usually less stringent than for crops with higher outcrossing frequencies and/or greater seed dormancy.

c) Surveillance of variety behaviour; lifespan

During multiplication, varieties continue to undergo evaluation for performance and quality characteristics. Following commercial release, evaluations continue, usually for the commercial life of a cultivar (5 to 20 years), through co-operative public or private cultivar evaluation trials.

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2. Wheat

by

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A. Characteristics of the crop

a) Geographic origins; taxonomic status

Wheat is a member of the angiosperm class, the monocot sub-class, and the grass family. Within the grass family, wheat is a member of the tribe Hordeae and the genus *Triticum.* Names of the different species of wheats are given in the Table 2.1 at the end of the chapter.

The geographic centre of origin of wheat is considered to be western Iran, eastern Iraq, and adjacent southern and eastern Turkey. Some of the first domesticated (primitive) wheats grown in this region were diploid *Triticum monococcum,* known as einkorn; a tetraploid *Triticum turgidum* var. *dicoccum,* known as emmer; and a hexaploid known as spelt (*T. aestivum* var. *spelta*).

Records of einkorn wheat date back to the 7th millennium BC in Iraqi Kurdistan, south-eastern Turkey and the southern Balkans. This wheat was also grown by the earliest civilisation in the Mesopotamian plains, and it is thought to have spread into Europe through the Danube and Rhine valleys. Emmer was the most prominent cereal grown by the early farmers of the Near East. It was probably transferred from the mountain areas of the Fertile Crescent to the lowlands of Mesopotamia in the 6th millennium BC and taken to Egypt, Europe, central Asia, and India in the 5th and 4th millennia. Archaeological information suggests that spelts were cultivated in the Upper Rhine region in about the 2nd millennium BC.

Today, while farmer selections (landraces) of diploid wheat species are probably grown along with landraces of tetraploid and hexaploid wheat species near or in the geographic centre of origin of wheat, all commercially grown wheat species are hexaploid or tetraploid. The grain of many primitive cultivated wheat species, including einkorn, emmer and spelt, is hulled, whereas the grain of all modern wheat species, including durum, club and common wheats, is free-threshing.

b) Geographic distribution of use: main production areas

Wheat production worldwide is dependent on the performance of hundreds of cultivars specifically bred and selected for high yielding ability and desirable end-use characteristics (quality) under constraints of local climate, weather, pests, diseases, and

soil conditions. These wheat cultivars are continually replaced or updated by a worldwide network of public and private breeding programmes in response to changing markets and the stresses imposed by changing disease and pest complexes and cultural practices. A new wheat cultivar may be released when it has been shown in performance evaluation trials to be superior to the nearest counterpart already available and grown in the area. Wheat cultivars tend to be environment-specific; even the most widely adapted cultivars are limited to specific geographic areas or environments and are totally dependent on cultural practices to perform to their full potential.

Durum wheat (*T. turgidum* var. *durum*) is a free-threshing tetraploid that began to replace hulled emmer in the Near East in about the first millennium BC. Durum wheat is grown today as a source of semolina flour in North America, Europe, North Africa, and Asia as well as the Near East.

Club wheats (*Triticum aestivum* var. *compactum*), so named because of the shape of the spike (head or ear), are hexaploid wheats. In contrast to common wheats (*T. aestivum* var. *aestivum*), characterised by spikes with spikelets arranged alternately and uniformly on a long rachis, the spikes of club wheats have spikelets densely arranged on a short rachis. Thus, club wheats are distinguished from common wheats by their shorter, denser, laterally compressed spikes. Club wheats are thought to have been common in the Neolithic and Bronze Ages.

c) Genetic and cytogenetic characteristics

The basic number of chromosomes in wheat species is seven. Thus, diploid wheat species have 14 chromosomes, the tetraploid emmer and modern durum wheat species have 28 chromosomes, and the common hexaploid wheat species have 42 chromosomes.

Tetraploid wheat species arose as the consequence of rare but natural crosses between two diploid wheat species. Through natural hybridisation, one diploid species combined its set of chromosomes with a different set of chromosomes of another diploid species by a process known as amphidiploidy. The genomes of the different wild diploid species have been labelled by cytologists for scientific purposes as AA, BB, CC, DD, etc. A diploid species with genome AA in a normal out-cross with a diploid species of genome BB, for example, would produce a diploid hybrid of genome AB, which would be sterile. In rare instances, spontaneous doubling of the chromosomes occurs to produce the tetraploid hybrid AABB, which is fertile.

Hexaploid wheat species arose by the same process: a diploid of genome DD combined with a tetraploid of genome AABB to produce a hexaploid hybrid of genome AABBDD. This process can be duplicated experimentally in the laboratory. In addition, chromosomes from the diploid rye (*Secale cerealis*) can be cytologically substituted for chromosomes in the genome of hexaploid wheat, particularly for chromosomes IA and IB in hexaploid wheat, as a source of genes for disease or pest resistance or other traits. Other substitutions are cytologically possible, but the progeny are typically sterile, or the plant types are considered too remote or different from agronomically desirable wheat to be useful in a breeding programme. However, one man-made species combination, triticale, showed adaptability to agriculture. Cultivated triticale is a fertile hexaploid hybrid between wheat and rye. Fertile wheat/rye hybrids that are octaploids have also been produced.

d) Current end uses

It has been estimated that two-thirds of the world's people rely upon wheat and rice for sustenance. Wheat grain is processed into flour, the principal constituent of an array of leavened and unleavened baked products, pasta, noodles and cereals. Each of the wheat foods imposes its own quality requirements on the flour from which it is made. Processing quality, therefore, is a major component of wheat breeding programmes. Throughout the industrialised western world wheat cultivars are bred and selected for specific quality traits according to end-use. Commercial acceptance of new wheat cultivars depends closely on the processing quality of the grain as well as on grain yield and agronomic traits.

Most countries use market classes that are based on the protein content of the grain. In the United States and Canada, for example, wheat cultivars are divided into hard red spring wheat, hard red winter wheat, white wheat (spring, winter, club and hard or soft types), soft red winter wheat, and durum wheat. Wheats high in protein content (13-16 per cent) are used for bread baking, and wheats relatively low in protein content (8-11 per cent) usually are used for pastries, cookies, crackers, flat breads, and oriental noodles. Durum wheat is used for semolina. Both protein content and protein quality of wheat grain are complexly inherited traits. Both are strongly influenced by the production environment. These quality differences reflect on world trade in wheat. Heavy importation of high protein Canadian and US spring wheat by western European countries is due to the high protein content as well as the protein quality of the Canadian and US wheat.

Reproductive mechanisms

a) Mode of reproduction

Wheat is monoecious with perfect flowers. It reproduces sexually as a self-fertilising (self-pollinating) crop. Some cross-pollination occurs, but usually this is less than 3 per cent.

Wheat is an annual plant. Some distant relatives of wheat are perennial, and perennial wheats have been developed through cytological manipulations involving chromosome substitutions with wild relatives. The perennial characteristic is genetically complex. No contemporary wheat breeding programme has seriously considered the development of perennial wheat, because pest and disease control by crop rotation and changing cultivars would no longer be an option.

Wheat may exhibit either a winter or spring growth habit. "Winter wheats" are planted in the autumn and produce grain the following spring or summer. They require a vernalisation period of temperatures near or slightly below freezing as well as minimum accumulation of growing degree days and/or length of daylight to convert from vegetative to reproductive growth. Accumulated growing degree days are the total number of days average temperature above 0°C. "Spring wheats" are planted in the spring and produce grain the following summer. They require a minimum number of accumulated growing degree days and/or length of daylight but not a vernalisation period to convert from vegetative to reproductive growth.

b) Dispersal and survival mechanisms

The growing point for wheat in each stem (mainstem and tillers) does not extend above ground until after the plant has converted from vegetative to reproductive growth. The vernalisation requirement is therefore a survival mechanism for wheats that establish as seedlings in the autumn and produce their spikes with grain the following spring or summer (winter wheats); it prevents the frost- and winter-sensitive growing points of the plants from extending above ground until after the danger of winter kill or frost is past. Today, winter wheats are grown in temperate areas with mild winters, while spring wheats are grown in areas where winters are too severe for wheat to survive (*e.g.* northern Great Plains of North America) or too warm for wheat to vernalise (*e.g.* tropics and sub-tropics).

Wheat is propagated from sexually produced seed. The spikes (heads or ears) of wild wheats and some primitive wheats are prone to dispersing their seeds on the ground by "shattering" which is a means of self-propagation. The tendency for shattering has been eliminated from most modern cultivated wheats so that the heads remain intact for harvesting. Cultivars that spread their seeds on the ground are not acceptable. Wheat seeds can be moved by birds and animals but not by wind.

As a survival mechanism, the seeds of wild and primitive wheats form germination inhibitors during seed maturation to prevent both sprouting in the heads and precocious germination in the soil. Expression of this dormancy factor is greatest when temperatures are high (above 25°C). It confers a survival advantage to wheats in their natural habitat in areas dominated by types with a winter growth habit. This dormancy factor is gradually dissipated and/or ceases to be produced or expressed as the soil cools and growing conditions for the seedling become more favourable. Modern cultivated wheats have been selected for expression of seed dormancy in areas where sprouting in the head is a potential problem, and for little or no seed dormancy in areas where the crop is planted into a warm seedbed.

c) Ability to cross with related species

Wheat cultivars are subject to a low frequency of cross-fertilisation by pollen from the weedy relative, *Aegilops cylindrica,* provided it is within range of movement of pollen. Commonly known as jointed goat grass, *Aegilops cylindrica* is a diploid relative of wheat that occurs widely in association with cultivated wheat west of the Mississippi River in the United States and in adjacent Canada. Some wheat cultivars are more prone than others to male sterility and hence to outcrossing. Hybrids of wheat × *A. cylindrica* are sterile, but some are female-fertile and therefore can be further cross-fertilised. With a sufficient number of generations and repeated outcrossing, a self-fertile individual with traits both of wheat and jointed goat grass may be possible.

Toxicology

Some people are allergic to wheat gluten as a food, and some people are allergic to wheat pollen in the air. Except for these two common and relatively well-understood kinds of allergies, wheat has no known toxic effects on people or animals.

Environmental requirements for life cycles

a) Climatic restrictions to extension of the crop

Modern cultivated hexaploid wheat is possibly the most widely adapted of all crop plants. In addition to its normal range within temperate climates, it is grown as a crop plant well into the northern and southern latitudes and at the higher elevations in the tropics. It is well adapted to dryland conditions and is produced in some areas on fallow with as little as 150-200 mm of precipitation per year. It cannot survive temperatures below –10°C and above 40°C. Respiration outpaces photosynthesis at temperatures above 31-32°C. It responds well to irrigation but is unsuited to waterlogged soils.

While wheat is widely adapted climatically, genetically different cultivars have been developed to take optimal advantage of the growing conditions unique to each climatic area. For example, wheats developed for the higher elevations in the tropics have been selected for insensitivity to length of daylight, whereas those developed for spring seeding in the northern Great Plains have been selected for sensitivity to length of daylight as well as ability to produce mature spikes with fewer accumulated growing degree days.

b) Biological restrictions to extension of the crop

Some wheat cultivars have carried unforeseen vulnerabilities, such as susceptibility to previously unimportant disease agents or biotypes/pest agents. Each new cultivar introduced into agricultural practice carries this risk, but it is often detected in field performance trials. In that case, the cultivar is not approved for introduction into agricultural practice. There is also the risk that controversial cultural practices may be made economically more attractive by some genetic modification in the cultivar. Thus, the introduction of a gene for semi-dwarf stature in wheat also made wheat less prone to lodging, and this paved the way for heavy use of nitrogen fertiliser. In the course of these changes, however, the average yield of wheat increased in some areas by three and fourfold.

B. Current breeding practices and variety development

a) Main breeding techniques

i) Main breeding schemes

Three types of wheat cultivars are currently being developed. These are pure lines, multilines, and hybrids. Pure lines are produced by cross-breeding followed by selection until the line is genetically uniform (usually eight to ten generations). Multilines are mixtures of pure lines. Hybrids are produced by either the cytoplasmic male-sterile method or by the chemical hybridisation agent method. Not all countries with wheat breeding programmes have approved the use of chemical hybridisation agents for wheat. Pure-line crossbred cultivars are by far the most common of these three kinds of cultivars.

The initial selection of candidate pure-line wheat cultivars is based on performance of segregating progeny, usually fourth and fifth generation lines (also referred to as F_4 or

F_5 lines) in small-scale field tests on the local experimental station (usually the "home" station of the breeder). The best genetic lines are then evaluated for performance for one to three years in more advanced nurseries, still at a local experimental station. A very small number of advanced generation (F_6, F_7, and F_8) wheat lines are moved each year into performance evaluation trials carried out at other locations and sometimes in co-operation with growers. Each year, the very best performers from among the very best advanced-generation wheat lines, if they appear to have potential as new cultivars, are submitted for performance evaluation. Regional, national or international tests are carried out by co-operating wheat breeders, the extension service (as in the United States), or a national testing service (as in the United Kingdom and other countries). Many OECD countries carry out and require national tests of each new wheat cultivar prior to its introduction into agricultural practice.

The disease-resistant Russian wheat varieties Kavkaz and Aurora have been used extensively by wheat breeders. The disease resistance is derived from a translocation of a segment of rye chromosome IR with a segment of the wheat IA or IB chromosomes. The translocation also imparts inferior bread-making and dough-handling properties.

Performance evaluations typical of local, regional, national or international nurseries include appropriate check cultivars, *i.e.*, the current cultivars grown in the area and subject to replacement if outperformed by a candidate new cultivar. Breeding programmes typically produce thousands of genetically unique lines initially; these are then reduced by selection in performance evaluation trials to hundreds, then to less than ten and finally to the one introduced into agricultural practice. The process is ongoing in the sense that new early generation material resulting from hybridisations enters the programme each year. Private and public breeding programmes follow similar protocols.

ii) Use of exotic germplasm

The most serious implication of requirements of high grain quality in modern wheat breeding may be the impact on breeders' choices of germplasm for cultivar development/ improvement. Much of the world's wheat germplasm possesses traits that are unsuitable for modern processing of wheat grain for various food products. Wheat breeders are reluctant to rely on exotic germplasm for cultivar improvement because they are unlikely to identify progeny of acceptable quality from crosses involving such parents, unless they have many generations of backcrosses and selection. Normally, they rely instead on relatively adapted parental stocks that have acceptable quality traits, and they tend to relegate poor quality exotic materials to long-range germplasm enhancement. Clearly, their preference for narrow crosses also reduces opportunities for significant advances in yield and other complexly inherited traits.

iii) Growth characteristics limiting the range of breeding methods

Many of the necessary or desirable characteristics of wheat needed for solving specific production problems or meeting specific market or nutritional needs cannot be achieved by traditional breeding technologies. Nor is adequate resistance to many important diseases and insect pests available in the existing bank of wheat germplasm. Where breeding has failed, environmentally unacceptable, undesirable, or controversial cultural practices – such as the use of pesticides, more intensive tillage, open-field burning of the stubble – have often been introduced to overcome the problems and improve the performance of an otherwise vulnerable cultivar. Without exception, environmental problems

associated with current agricultural practices relate not to genetic modifications of the crop, but rather to the cultural practices introduced or encouraged to maximise the performance of the crop cultivars.

Some heretofore intractable problems associated with current agricultural practice might now be solved by using recombinant DNA technology to introduce new and useful genetic information from a wide array of sources into wheat. For example, the barley yellow dwarf virus (BYDV), which resides in many weed and alternate crop hosts and is vectored to wheat by aphids, has not been controlled by conventional breeding. However, it may someday be controlled by the development of transgenic wheats that express the coat-protein or other genes from BYDV. The new "breeding" tools will broaden the genetic base but are not likely to reduce the number of site-years necessary to evaluate the performance of these new lines or cultivars. Transgenic wheat cultivars, like wheat cultivars developed by traditional breeding, will be required to perform at least as well as their nearest counterparts when the targeted stress factor which it was designed to overcome is absent. Such information can only be obtained by comparative performance evaluations in co-operative regional, national and international tests like those carried out for wheats developed by traditional technologies.

b) Main breeding objectives

New cultivars are usually developed and selected for their ability to overcome specific production constraints or meet some need in the market place, while performing at least as well as the nearest counterparts over a wide range of production criteria. In a few cases, cultivars are developed for use only in a specific area to overcome a production constraint unique to that area, *e.g.*, to control snowmould on wheat in the areas of North America where winter wheat must survive under snow for up to four months of the growing season.

Wheat breeders recognise that quality requirements may negatively affect yield improvement. Unfavourable genetic linkages between genes for specific quality traits and genes affecting performance undoubtedly exist, but relatively few have been reliably identified. Many studies have demonstrated a negative correlation between grain yield and grain protein content. The negative correlation does not necessarily reflect genetic linkage. Yield enhancement usually involves starch accumulation and an accompanying protein dilution in the wheat grain. Production environments that promote high grain yields (US Pacific Northwest and western Europe) usually result in low protein grain as well. For bread wheats in which high grain protein content is essential for satisfactory leavened bread production, the negative correlation of yield and protein content may have serious consequences.

c) Testing the crop for important breeding goals

End-use characteristics, including milling and baking qualities, are identified in some programmes by a micromilling and baking test as early as the initial stages of performance evaluation and in standard tests for grain quality before submission to regional or national performance evaluations.

Candidate wheat cultivars grown in advanced performance trials are exposed to typical if not best cultural practices for the local area, and to the natural or artificial stresses necessary to reveal superior performers. An example of an "artificial" stress is

the introduction of inoculum of a local pathogen into the experimental plot area in order to create an epidemic of sufficient severity to experimentally distinguish degrees of resistance in a segregating population. Performance evaluations using introduced inoculum of pathogens are carried out almost exclusively on the experimental station and not in growers' fields. Performance evaluations under conditions of natural stress are carried out both on and off the experimental station. Natural stresses include exposure to winter injury, saline soil, high soil temperature during stand establishment, high air temperature during grain fill, and natural disease epidemics or insect infestations. The experiments are designed to permit evaluation of the data by standard statistical methods.

d) Assessment of general performance

Most new cultivars are selected on the basis of data from 50 to 60 site-years of testing and some after 75 or even 100 site-years of testing. In spite of the many years and plant generations required, many new wheat cultivars are made available every year, because of the importance of wheat and the large number of private and public breeding programmes.

Wheat breeders have a long history of close national and international co-operation, including the sharing and exchange of useful wheat germplasm. As an example, the *RHt-1* and *RHt-2* genes for semi-dwarf growth habit, originally inherited in the Norin line from Japan, were successfully transferred by standard hybridisation in the United States into Bevor to produce the line Norin 10/Bevor 14. This plant line, in turn, was shared freely with breeding programmes worldwide, including the programme of Norman Borlaug in Mexico. Within about 20 years, these two genes for semi-dwarf habit were used, singly or in combination, in roughly 50 per cent of the wheat cultivars worldwide. As another example, the *Pch* gene for resistance to *Pseudocercosporella* (eyespot) foot rot was first transferred to hexaploid wheat in France by a wide cross from the wild tetraploid *Aegilops ventricosa* (Doussinault *et al.*, 1983). The transfer involved many years of research and testing. This germplasm was shared with wheat breeding programmes in the United States and the United Kingdom, and the gene was transferred by standard hybridisation into cultivars adapted to the local conditions in these countries where eyespot was a problem.

C. Seed multiplication for commercial use

a) Stages in seed production

Most, if not all, countries with wheat breeding programmes follow carefully established guidelines for the release, multiplication, distribution and maintenance of seed of new wheat cultivars. Typically, the release of a new cultivar is approved by a formal procedure carried out within a ministry of agriculture, as in Europe, or a state agricultural experiment station, as in the United States. Seed of the approved cultivar is made available by the breeder (''breeder seed''), to be multiplied through two or more stages to produce the seed grown for food. In the United States, breeder seed is used to produce ''foundation seed'', which is used to produce ''registered seed'', and this is used to produce the ''certified seed'' grown for domestic use and for export.

Many, if not most, countries with wheat breeding programmes have laws to ensure the identity, purity, and germinability of seeds, including wheat seeds. In the United States, each of the three stages of seed production – foundation, registered, and certified – is carried out by professional seed growers. Based on established phytosanitary standards, each seed field is inspected, as is the harvested seed, for the presence of certain weeds, diseases, and insects. Wheat seed production is overseen by state certification agencies in the United States and by federal agencies in Canada and most European countries.

b) Isolation practices and surveillance

The international movement of wheat seeds as germplasm for breeding programmes is subject to the phytosanitary laws of the countries importing the seed. Some countries have placed quarantines against seed from certain other countries because of diseases present in those countries. Some quarantines have also been extended to wheat grain imported for food. Seeds must either be certified as free of the particular disease, in the case of zero tolerance, or meet a minimum tolerance level set by the importing country.

Most or all countries with wheat breeding programmes also carry out surveillance of cultivar behaviour in the seed-producing fields, even, in some countries, after the cultivar is in commercial use. If, during seed increase or in commercial use, an approved cultivar exhibits an unexpected vulnerability to disease or environmental stress or is discovered to exhibit other agronomically undesirable tendencies not previously recognised during performance evaluations, approval may be withdrawn.

In areas where wheat is grown in close proximity to wild relatives, traits may be transferred by cross-pollination either from the relative to cultivated wheat or from cultivated wheat to the relative. In the United States, all seed wheat fields grown in areas infested by jointed goat grass (*A. cylindrica*), the diploid weedy relative of wheat, are inspected for the presence of readily recognisable hybrids. The US seed law does not

Table 2.1. **Names of wild, primitive cultivated, and modern cultivated wheats**

Wild wheats	Primitive cultivated wheats	Modern cultivated wheats
T. monococcum var. *boeoticum* diploid (AA)	*T. monococcum* var. *monococcum*, einkorn (hulled) diploid (AA)	*T. turgidum* var. *durum*, durum (hull-less) tetraploid (AABB)
T. tauschii diploid (DD)	*T. turgidum* var. *dicoccum*, emmer (hulled) var. *durum* (hull-less) tetraploid (AABB)	*T. aestivum* var. *spelta*, spelt (hulled) var. *compactum*, club wheat (hull-less) var. *aestivum*, common wheat (hull-less) hexaploid (AABBDD)
T. turgidum var. *dicoccoides* tetraploid (AABB)		
T. timopheevii tetraploid (AADD)	*T. aestivum* var. *spelta* (hulled) var. *compactum* (hull-less) var. *aestivum* (hull-less) hexaploid (AABBDD)	
T. aestivum hexaploid (AABBDD)		

Source: Adapted from Feldman (1976).

allow certification of seed from fields with even a single hybrid plant of wheat × *A. cylindrica.*

Gene flow can also occur, potentially, from cultivated wheat to a wild relative such as *A. cylindrica.* For example, the *Pch* gene for eyespot resistance, transferred to hexaploid wheat by a wide cross from *Aegilops ventricosa,* could conceivably move by outcrossing to *A. cylindrica.* This trait is not known to occur in *A. cylindrica,* a host for the fungus that causes eyespot on cultivated wheat. While no formalised surveillance is carried on within populations of *A. cylindrica* to monitor for gene transfer, easily recognised wheat traits such as red coleoptile and pubescent leaves have never been observed in populations of *A. cylindrica.* Moreover, if the *Pch* gene were to move into *A. cylindrica* by outcrossing, there is no evidence that the transfer would make it a more important weed, and it might become a less hospitable host for the eyespot fungus.

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3. Rice

by

Chukichi Kaneda

A. Characteristics of the crop

a) Geographic origins; centre of diversity

Two rice species are cultivated: Asian rice, *Oryza sativa* and African rice, *O. glaberrima.*

Ancestral forms of Asian cultivated rice are considered to have appeared in the Neothermal age (10 000 to 15 000 BC) in the southern borders of the Himalayas and in south and south-west China; annual forms gradually developed in north-eastern and eastern India, northern Southeast Asia and southern China. They dispersed and diversified to form three ecogeographic subspecies – *indica, japonica* and *javanica.*

African cultivated rice originated in the Niger River delta. The primary centre of diversity is the swampy basin of the upper Niger River; two secondary centres are to the north-west, near the Guinean coast (Chang, 1985).

b) Geographic distribution of main production areas

Cultivation of rice spans a latitude from 53°N to 40°S, under various physiographic, hydrologic and edaphic conditions. More than 90 per cent of the production comes from Asia, with 36.3 per cent from China, 21.1 per cent from India, 8.4 per cent from Indonesia, 5.7 per cent from Bangladesh, 3.9 per cent from Thailand and 3.7 per cent from Vietnam, about 5 per cent from the Americas, and 2 per cent from Africa (IRRI, 1991*b*, p. 320).

c) Taxonomic status

In addition to the cultivated species, the genus *Oryza* includes 21 wild species. Most are diploid (2n = 24) and seven are tetraploid. *O. perennis* (presently *O. rufipogon*) is considered to be the progenitor of the Asian cultigen, while *O. breviligulata* (*O. barthii*) is that of the African cultigen. *O. nivara,* named in India, is also considered to be an annual form of *O. rufipogon.*

The 23 species comprising the genus *Oryza* are classified into five sections, as shown in Table 3.1.

Table 3.1. **Taxa in the genus *Oryza*: the species complexes and genome groups**[1]

Species complex	Taxa	Genome group	Distribution
	O. schlechteri	Unknown	Papua, New Guinea
	O. brachyantha	FF	Africa
O. ridleyi complex			
	O. longiglumis	Tetraploid	Irian Jaya, Indonesia
	O. ridleyi	Tetraploid	SE Asia
O. meyeriana complex			
	O. granulata	Diploid	S and SE Asia
	O. meyeriana	Diploid	SE Asia
O. officinalis complex			
	O. officinalis	CC	Tropical and subtropical Asia
	O. minuta	BBCC	Philippines
	O. eichingeri	CC	Sri Lanka, Africa
	O. rhizomatis	CC	Sri Lanka
	O. punctata	BB CC BB	Africa
	O. latifolia	CCDD	Latin America
	O. alta	CCDD	Latin America
	O. grandiglumis	CCDD	South America
	O. australiensis	EE	Australia
O. sativa complex			
	O. glaberrima	A^gA^g	Africa (mainly West)
	O. barthii	A^gA^g	Africa
	O. longistaminata	A^lA^l	Africa
	O. sativa	AA	Worldwide
	O. nivara	AA	Tropical and subtropical Asia
	O. rufipogon	AA	Tropical and subtropical Asia
	O. meridionalis	A^mA^m	Tropical Australia
	O. glumaepatula	$A^{gl}A^{gl}$	South America

1. Genome groups are defined by the ability of chromosomes to pair at meiosis.
Source: Chang and Vaughan (1991).

d) Genetic and cytogenetic characteristics

Species in the *O. sativa* complex can be hybridised, although there are various levels of cross-incompatibility. The first great success of interspecies hybridisation was the development in 1974 of the variety IR28 through the incorporation of a resistance gene to grassy stunt virus from *O. nivara.* It is also well known that cytoplasm of *O. rufipogon* provided the key genetic material for male sterility in the production of hybrid rice varieties.

In order to overcome the problems of breakdown of resistance to important diseases and insect pests in tropical rice, IRRI is working with intersection hybridisation. *O. australiensis, O. latifolia, O. minuta, O. brachyantha, O. eichingeri, O. alta,* and *O. rhizomatis* are used as donors of resistance to brown planthopper, blast, bacterial blight, or sheath blight, or as a way to widen the rice gene pool (IRRI, 1991*a*, p. 317).

e) Current phytosanitary considerations in movement of germplasm

In most countries, the phytosanitary restrictions designed to prevent introduction of rice diseases are extremely strict. In Japan, for example, rice seeds with hulls can be imported only with special permission of the Minister of Agriculture, Forestry and Fisheries for specified experiments inside a designated facility, and on the condition that they will be autoclaved or incinerated after the experiment. When germplasm is introduced, rice plants are grown in a specially designed greenhouse that prevents the air from flowing out freely. The seeds are harvested only after examination by the quarantine officer. The plant quarantine system in the United States is quite similar (Cooper, 1988, pp. 57-60).

f) Current end uses

Rice, polished or brown, is used for food in various forms. In limited amounts, rice is also used to manufacture starch. As the size of rice starch granules is very small, it has been used as an important material for the photosensitive membrane of films, for "oshiroi" (a cosmetic), etc. The germ is used for nutritious foods, vitamins, cakes and animal feed. The bran is an important source of oil for food and manufacturing; husks are used for fertilisers and animal feed and straw for making various materials for wrapping, mats, feed, horticulture, etc. Rice plants are now also considered good material for producing ceramics due to their high silicate content.

Reproductive mechanisms

a) Mode of reproduction; ability to cross with related species

Cultivated rice is basically propagated by seeds produced by self-pollination, of which the degree varies considerably among varieties and especially among sub-species. In general, *indica* rice shows a lower rate of self-pollination than *japonica*. Japanese varieties have been made more homozygous by reducing outcrossing. This is done by changing morphological characteristics of the flower, such as the length and position of the stigma, the size and extension of anthers, or the timing of pollen shedding.

A very high rate of outcrossing is usually observed in wild rices, and the outcrossing mechanism (contrary to that in cultivated Japanese rice) is being incorporated into cultivated rice so that, under controlled conditions, F_1 seeds can be produced more efficiently.

b) Perennial vs annual habits

Cultivated rice is an annual. This habit came from *O. nivara*, the annual form of *O. rufipogon* which has both annual and perennial forms. However, rice scientists often propagate experimental rice materials vegetatively, or keep ratoon (tillers from basal nodes produced after harvest) plants in order to continue their trials. Ratoon plants can resume the vegetative phase after treatment under long day conditions. In Japan, ratoon plants of some varieties can survive winter in the warmer regions (*e.g.* southern Kyushu).

c) Dispersal and survival mechanisms of rice seeds

Seed of wild relative species of cultivated rice shatters easily. Many traditional *indica* rice varieties express this trait strongly, but most *javanica* and *japonica* varieties do not. A similar tendency is seen in length of dormancy of seeds. Therefore, degradation of seed quality occurs much faster in *indica* rice. Red rice is a problem because the seed shatters easily, and it has a longer dormancy period.

Toxicology

Rice has no toxic effects in the ordinary sense. However, probably because of changes in dietary habits in Japan – from foods high in carbohydrates to foods with greater fat and protein content – an allergic reaction to rice (atopic dermatitis) has been identified and is increasing, especially among small children. The major allergenic factor is a globulin with a molecular weight of 16 kD. Several mutants from irradiated rice lack this 16-kD globulin, and breeding for an allergen-free rice for commercial use has begun.

Environmental requirements for life cycles

a) Climatic restrictions for extension of the growth range of rice varieties

The maturation period of a rice variety is determined by the length of its basic vegetative growth phase, and its sensitivity to day length and temperature. Under Japanese climatic conditions, the maturation period is a critical factor, whence the number of varieties in different regions. Temperature is also important, and causes various kinds of cold damage in northern Japan and higher elevations, and lower grain: straw ratios in southern Japan.

b) Biological restrictions to wider distribution

At present, the most important biological factors in Japan are diseases such as blast and stripe virus, and insect pests such as planthoppers and leafhoppers. Introducing resistance genes to these biotic problems is essential for wider distribution of a variety, and in general, this has been achieved by using *indica* rice as a donor. A problem that arises for these resistant varieties is how to achieve taste quality comparable to that of traditional Japanese varieties.

B. Current breeding practices and variety development research

a) Main breeding schemes/techniques

i) Germplasm maintenance

In Japan, rice germplasm is maintained in the gene bank system of the central bank of the National Institute of Agrobiological Resources and in sub-banks in national agriculture experiment stations in different regions of Japan. Sub-banks assist in the multiplication of newly introduced germplasm and/or germplasm which needs reproduction of seeds. In the central bank, the base collections are kept at –10°C, and active collections at –1°C and 30 per cent relative humidity.

ii) Basic breeding

Major basic breeding research areas can be classified into those that use orthodox and those that use biotechnological techniques. The former use mainly backcross breeding, for introducing and/or accumulating resistance/tolerance genes to biotic and abiotic stresses, and mutation breeding, for novel characteristics. The latter, practised in prefectural experiment stations and the private sector, include haploid breeding through anther culture and somatic mutation breeding through protoplast culture. In both national institutions and the private sector, recombinant DNA techniques have also been adopted. Rice with coat protein DNA incorporated into the genome from stripe virus is scheduled to be tested in an isolated field.

iii) Variety development

For variety development, orthodox cross-breeding coupled with bulk selection (usually up to F_3-F_4) dominates. In general, as parents in *japonica/japonica* crosses are rather closely related, it takes eight to ten years before a commercial variety of genetically high homogeneity is released.

Breeding for variety development in Japan can be defined as those activities which result in sending annual selections to prefectural experiment stations for testing local adaptability and other principal traits, including growth habit and yield. In the country as a whole, about ten new varieties are listed for recommendation each year.

Products from basic breeding research are not distributed in this way. However, when they become very promising and should be tested for practical use, they are sent to the varietal development laboratory. In Japan, hybrid rice breeding is at present considered as basic research.

iv) Techniques used

Due to plant characteristics, one of the limitations to the range of breeding methods may be the limited number of life cycles that can be achieved in one year. Because basic vegetative growth prevents panicle initiation at the very young seedling stage (Vergara *et al.*, 1969, p. 31), only three generations can usually be grown, even using the photoperiod control system in greenhouses, except for thermosensitive rice for very cool areas.

b) Main breeding objectives

Primary objectives are, as for other crops, high yield and good quality, the definition of which may be different for millers and final consumers. Yield should be accompanied by stability of production, through tolerance and resistance to biotic and abiotic environmental factors. Attaining good quality often means sacrificing yield in subsequent crosses, so that plant breeders have difficulty in realising both objectives.

The most remarkable progress in yield has been attained by the use of semi-dwarf genes both in Japan and the United States. The genes were identified in some traditional varieties and also in artificially induced mutants. Dwarfness is not the only component for obtaining high yield. Stiffness of culms is another important vegetative factor, but it seems to affect palatability negatively. Generally speaking, the best table-quality rice varieties have taller and weaker stems.

With regard to tolerance and resistance to different environmental factors, much emphasis has been placed in Japan on diseases such as blast, bacterial blight and virus diseases, on insect pests such as planthoppers and leafhoppers, and on cold tolerance. Gene sources for combatting problems may often be found in areas exempt from those problems. For example, a resistance gene for *hoja blanca* virus in Latin America was found in *japonica* rice, and high levels of tolerance to low temperatures were found in tropical *indica/javanica* rice.

Broadening the genetic base is an emerging strategy and is essential in rice breeding in tropical countries, especially for resistance to diseases and insect pests, due to the continual breakdown of resistance. For this reason, IRRI is tackling the problem of hybridisation using distant wild rice species other than the *Sativa* complex. However, in temperate countries such as Japan and the United States, breakdown can be avoided by good management of technological factors including the choice of varieties to be grown. Under appropriate management, the gene sources within *O. sativa* almost suffice for controlling these pests. In terms of biotechnology, tests for introducing single genes for insect tolerance (BT endotoxin) and storage protein modification have been carried out in both countries.

c) Testing for major breeding goals

i) Yield and stability

These characteristics are quantitative and controlled by many unidentified genes; therefore, they can be tested only after the breeding materials become, to some extent, genetically homogenous. Otherwise, heterosis hinders proper evaluation, especially in the early generations. For this reason, plant selection usually starts in the third to fourth generation in ordinary crosses, and in the sixth to eighth generation in distant crosses. In the latter case, the size of the populations grown is also several times larger, *e.g.* 10 000 to 20 000 in *japonica/indica* crosses.

Testing needs replicated trials under different conditions to simulate various growing practices, such as levels of fertiliser application, planting density, and planting time. When a selection is promising, it is advanced to local adaptability testing and replication trials in prefectural experiment stations. At least three years of such testings (plot size: 5-10 m^2) plus at least two years of demonstration plantings (plot size: 0.05-0.1 ha) in several farmers' fields are necessary before the selection is adopted as a recommended variety.

ii) Testing resistance to diseases and insect pests

Laboratory or greenhouse tests are conducted to identify reactions to specific pathotypes or biotypes inoculated by different methods, and field tests are commonly conducted for several years to determine the level of resistance and the extent of yearly fluctuations. It should be noted that, in some cases, the result of laboratory testing does not reflect the genetic characteristics. For example, in the case of resistance to the brown planthopper, the level of resistance of rice was much lower under laboratory conditions of low light intensity than in the field. This may be attributable to inferior physiological conditions inside the laboratory, leading to relatively degraded resistance or tolerance (Kaneda *et al.*, 1982).

iii) Testing for qualities

Milling recovery can be tested by small test mills in the laboratory. The table quality or palatability could be roughly estimated by testing several parameters such as amylose content, gelatinisation temperature, and some physico-chemical traits. But in Japan the final judgement is made by taste tests. So far, no other testing procedure can evaluate palatability better than a team of experienced panelists.

d) Assessment of general performance of breeding material

Besides the various tests stated above, plant type, maturation period, and performance of different plant characteristics are observed under different field conditions for several years. Observation and note-taking continues from germination in seedbeds to maturity, and overall evaluation of the breeding material is made after all the yield and quality checks have been carried out. The ranking system is different according to the characteristics of the variety, but the system of IRRI can serve as an example (IRRI, 1980, p. 40).

Box 3.1. **Exploiting heterosis in self-pollinating rice**

Hybrid rice varieties are now grown in more than half of the total rice land in China and yield 15-25 per cent more than ordinary varieties. The yield gap between hybrid and ordinary varieties arises from heterosis, *i.e.* plant vigour in hybrids between two remotely related parents. In grain crops, heterosis should not be so great in traits such as maturation period and vegetative growth, except the sink of photosynthesis, *i.e.* the total volume of spikelets.

The degree of remoteness of the two parents is also important: parents too distantly related produce partially sterile hybrids, and parents that are too closely related produce no effective heterosis. Chinese *indica* and tropical *indica* were good enough to produce fertile plants with sufficient heterosis, but Japanese *japonica* and *indica* produced hybrids with such high sterility as to make no use of heterosis in the sink size. Sterility in *japonica-indica* hybrids can be reduced by introducing cross-compatibility genes found in specific groups of rice, such as *javanica* or *boro*.

Hybrid rice varieties are usable in practice only as F_1 because of segregation in the F_2 generation. Therefore, seeds of hybrid varieties have to be produced every year. As cultivated rice has been improved so that it self-pollinates, the efficiency of hybrid seed production was very low at first, *e.g.* 100 kg per ha. Conversion of parental lines for hybridisation was achieved by introducing traits of traditional *indica* rice or wild rice – such as the stigma exserted outside the hulls or bigger pollen sacs which dehisce after complete exsertion of filaments – is expected to improve seed production efficiency. However, this will also cause a rapid degradation of varietal purity.

The mechanism causing male sterility in female parent lines is another important development. Male sterility should be stable and maintained without much labour. This problem should be solved because of the recent discovery of genetic (recessive) male sterility caused by different day length or temperature levels.

Box 3.2. **Problems with red rice**

Red pericarp rice is especially esteemed in some areas, *e.g.* the Kerala state of India and Sri Lanka. In Japan, at the very primitive age of rice cultivation, red rice varieties were commonly grown, and until the late 19th century, they were grown by poor farmers for several reasons. Transplanting rice by regular spacing, coupled with strong guidance from the authorities, effectively eradicated red rice from paddy fields in Japan. At present, red rice is sporadically found in some areas of upland rice cultivation where direct seeding is practised. Eradication of red rice was urgently needed in Japan because many red rice varieties were *indica* type and produced, by natural pollination to ordinary varieties, sterile hybrid plants, thus inducing lower yields and poorer grain quality.

In the United States, red rice causes significant commercial losses. It is very hard to eradicate by chemical and cultural control measures, but California has succeeded in obtaining fields free of red rice by the practice of water seeding, which effectively suppresses red rice emergence. A grain width separator fitted with a screen with 2.88 mm diameter perforations was found effective in removing 98-99 per cent of red rice contaminants in a long grain variety (Delouche, 1988).

In India, there was a trial of breeding rice with purple leaf colour to differentiate it from red rice, so that farmers could rogue green-leafed red rice from their fields. The first commercial variety, Shyamla, was released in Madya Pradesh State. Its yield is 10-15 per cent lower, due to the lower photosynthetic ability of the leaves.

C. Seed multiplication for commercial use

a) Stages in seed production

Rice seeds of a recommended variety are supplied to farmers after three steps of seed production. In Japan, the breeder seed from about 50 individual plants of the line selected as the genuine pedigree is sent to the agricultural experiment station of the prefecture where the variety is or will be recommended. It is grown in the pedigree nursery in order to observe whether genetic uniformity exists within and among pedigree lines. Only the lines showing no segregating offtypes are harvested to make up the foundation seed. A portion of this seed is used to produce registered seed, and the rest is placed in cold storage for use in subsequent years. The final step, production of the certified seed, is usually entrusted to a few leading farmers recommended by the experiment station. The seed production fields are supervised at several critical stages (from heading to maturity) to ascertain the genetic purity of the stands. Offtype plants are rogued, and the seeds harvested are examined for purity.

b) Isolation practices

Both spatial and temporal isolation of the field is usually practised for pure seed production. Adjacent fields are planted with varieties of different flowering time, and seeds from several border rows are excluded from the harvested seed lot. The necessary distance between two fields to prevent natural outcrossing is at least 2.5 m. Isolation is

especially important for waxy rice which exhibits *xenia* in the endosperm (appearance of character belonging to the male parent) when contaminated by non-waxy rice.

c) Surveillance of variety behaviour; lifespan and market spread

Degradation of seeds in *japonica* rice is much slower than in *indica* rice, because of less shattering, less seed dormancy, and other factors relating to field management. In Japan, the rate of replanting of farmers' seeds has recently risen (nearly 50 per cent compared to less than 20 per cent in the mid-1960s) due to the massive seedling supply system of the co-operative for mechanised transplanting, which covers around 95 per cent of total rice acreage.

In recent years in Japan, the lifespan of a variety after commercial release has been determined by consumers, on the basis of its palatability. A highly resistant variety that successfully subdued very severe outbreaks of a virus disease, rice stripe, in the early 1980s was replaced by a susceptible older variety of better eating quality as soon as the damage became economically bearable.

Koshihikari, first released in 1956, is now planted in more than 28 per cent of the rice area because of its table quality. This is an exceptional case, and each prefecture makes efforts to have its own rice variety win the rice production competition.

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4. Cucurbits

by

Henry M. Munger, Molly M. Kyle and Richard W. Robinson

The cucurbits include a number of species in three different genera of the family Cucurbitaceae. The genus *Cucumis* includes both cucumber and many forms of melon; watermelon is in the genus *Citrullus*, and a wide range of squashes and pumpkins are found in four cultivated species of *Cucurbita*. Breeding practices and objectives for cucurbits have many elements in common, but a general discussion is not feasible because of the differences among the seven species. Cucumber will therefore be used as a model species because it has been the most studied genetically and its varieties most improved. The other cucurbits will be covered more briefly, emphasising the ways in which they differ from cucumber, specific breeding objectives for each, and sources of germplasm.

I. CUCUMBER

The cucumber (*Cucumis sativus* L.) has several distinctions among the cucurbits: it is the most widely grown throughout the world, the least nutritious, the most studied genetically, and its varieties have been improved the most in recent years, especially in terms of disease resistance. It has the shortest life cycle and requires the least space per plant.

A. Characteristics of the crop

a) Geographic origins; main production areas

This species originated in Asia, probably in India, and is now cultivated in all parts of the world.

b) Genetic and cytogenetic characteristics

Cucumber has seven pairs of chromosomes. Tetraploids have been produced but not found to have any advantage. There are no related species crossable with cucumber. The weedy *C. hardwickii* found in India was originally described as a separate species, but is completely fertile in crosses with cultivated cucumber and now considered a group within that species. The great genetic variability in *C. sativus* has not been fully utilised to provide superior varieties, especially for tropical countries.

c) Phytosanitary considerations in movement of germplasm

There are so few seed-transmitted diseases of cucurbits that there are few, if any, phytosanitary considerations in the movement of germplasm. The primary concern in squash and melons is seed transmission of squash mosaic virus; this is especially serious where cucumber beetles are prevalent since they also transmit this virus. The United States has no restrictions on importation of cucurbit seeds, although some countries do.

d) Current end uses

The immature fruits are eaten fresh wherever the cucumber is grown, and in temperate climates it is preserved extensively in the form of pickles.

Toxicology

Cucurbitacins are a group of related bitter compounds found in many cucurbits. The forms that occur in cucumber are less toxic to humans and less concentrated than those in the *Cucurbita* species. They are seldom found in the fruit. We know of no instance where consumption of bitter fruit has resulted in illness. Most varieties grown in the United States have bitter seedlings, but bitterness is hard to detect in older plants. A single recessive gene *bi* makes the plant free of bitterness, and this not only eliminates the possibility of bitter fruit but makes the plants less susceptible to injury by cucumber beetles. At the same time, however, the non-bitter plants are more susceptible to spider mites. A number of non-bitter varieties have been developed in the United States but are not widely used. Non-bitter varieties have become more important in glasshouse cucumber production in Europe.

A gene for bitter fruit is present in some wild or primitive cucumbers in India. These can pose a health hazard if eaten.

Environmental requirements

The cucumber is probably the most widely adapted of the cucurbits, being grown from the extremes of the temperate zones to the tropics. It is almost insensitive to photoperiod and less affected by extremes of temperature than most other cucurbit species. However, low soil temperatures can cause problems with greenhouse cucumbers in winter.

Susceptibility to diseases is probably the greatest restriction to its wider distribution. The variety Poinsett, bred at the Clemson University Experiment Station in Charleston, SC, USA, is widely grown in tropical countries in part because of resistance to four diseases: anthracnose (*Colletotrichum obiculae*); angular leafspot (*Pseudomonas syrin-*

gae); downy mildew (*Pseudoperonospora cuberisis*); and powdery mildew (*Sphaerotheca fulingenea*). Susceptibility to target leafspot (*Corynespora cassiicola*) and several virus diseases (CMV, PRV-W = WMV1, WMV, and ZYMV) have limited its usefulness to some extent, but this will be changed with the imminent release from Cornell University of Poinsett with resistance to 11 diseases, and similar improvements from various seed companies.

B. Current breeding practices

a) Description of main breeding techniques

i) Basic breeding

Most cucumbers are monoecious in flowering habit, *i.e.* with separate male and female flowers on the same plant. Since this leads to about 75 per cent natural cross-pollination of unprotected flowers, controlled pollination is necessary in breeding this species. Self- and cross-pollinations are easy to make but do require considerable labour.

Cucumber varieties fall into two main categories: F_1 hybrids and open-pollinated. Modern open-pollinated varieties are essentially inbred lines since little or no vigour is lost through inbreeding, and it is not unusual for the same line to be used directly as a variety and also as a parent for a hybrid variety. The first hybrids in the United States in the 1940s were produced by hand pollination of individual female flowers, using male flowers from a second parent. Application of Ethephon to inhibit male flower production on the female parent was suggested as an alternate procedure but not used extensively because the gynoecious (all female) flowering type became available and drastically reduced the cost of producing F_1 seed.

Gibberellic acid or silver nitrate applied to seedlings that are genetically female induces male flowers and permits the maintenance of seed lots that are true breeding for the *E* gene. When interplanted with a second parent that is monoecious (*ff*), seed harvested from the gynoecious parent (*FF*) is gynoecious (*Ff*) because that trait is completely dominant under most conditions. Consequently, a variety that produces male flowers must be blended (plus or minus 10 per cent) with the gynoecious F_1 in order to insure pollination. Gynoecious hybrids are somewhat earlier and more concentrated in production than open-pollinated varieties, but they often produce a higher *proportion* of unmarketable fruit in spite of a high *total* marketable yield. Unlike crops where there is more true hybrid vigour, there is still an important place for open-pollinated varieties when earliness and concentration of maturity are not important and cost of seed must be considered.

ii) Variety development

In cucumber, the variety designations "open-pollinated", "inbred line", "true-breeding", and "non-hybrid" are essentially interchangeable. These are developed by the pedigree method, the backcross method, or some combination of the two. In the former, crosses are made between parents each of which has some good features lacking in the others. In the segregating generations that follow, plants that recombine the good traits of the parents are selected. Self-pollination of desirable plants is essential, and since some traits are not selectable at flowering time, many more plants must be hand-

pollinated than are eventually saved. This procedure is followed until the new combination is sufficiently stabilised for testing, usually from four to six generations after the cross.

When one is attempting to improve a variety with many good features by adding one or a few traits from a less desirable parent, the backcross method is useful, especially if the trait to be added is simply inherited. After the initial cross, the subsequent generations are crossed to the variety to be improved (the recurrent parent), selecting in each generation the trait to be added from the donor parent. After four to six backcrosses, the population will have 95-99 per cent of the genes of the more desirable parent while retaining the gene(s) selected from the donor parent. Self-pollination is then carried out for two to three generations to make the introduced trait true-breeding. This is the most predictable breeding method, requires relatively few plants and pollinations, and can be carried out quickly if locations are available for growing more than one generation per year. Less testing of a potential new variety is required if it is bred by the backcross method because most of the characteristics are already known.

b) Main breeding objectives

Earliness, high yield, good appearance, and disease resistance are common objectives. For pickles, additional objectives may include processing quality, crispness, locule size, and adaptability to machine harvesting. Cucumbers are not grown for their nutritional value. Quality differences in taste and texture are ill-defined and almost impossible to evaluate in segregating material. A variety known to be acceptable in quality or adaption is best improved by using it repeatedly in a backcross programme while adding improvements that are more readily selectable.

It is possible to improve earliness by selecting plants which produce early female flowers or by breeding gynoecious hybrids. Yield is difficult to improve if factors such as disease limit the expression of a plant's yield potential. With this crop, the greatest yield improvements in many areas have come from disease resistance.

With the backcross method one can easily and quickly transfer genes such as gynoecious flowering (one dominant), non-bitter leaves (one recessive), resistance to scab caused by *Cladosporium cucumerinum* (one dominant), resistance to powdery mildew (two or three recessive), resistance to *Cladosporium* and *Ulocladium* (one dominant for both), resistance to ZYMV and WMV (two linked dominant genes), and resistance to PRV (one dominant). Somewhat more difficult to transfer is resistance to CMV (two or three partially dominant genes needed for high resistance), to downy mildew (difficult to evaluate), anthracnose (difficult to evaluate and complicated because there is more than one race) and angular leafspot. There are an unusual number of associated resistances in cucumber. Selection for powdery mildew resistance usually results in some downy mildew resistance and vice versa. Resistance to *Corynespora* and *Ulocladium* seems to be controlled by the same gene, which in most US varieties has been linked with susceptibility to powdery mildew. The gene for scab resistance is believed to confer *Fusarium* resistance as well.

Except for resistance to scab, found in an old US variety, most resistance has come from introductions from India, China, and Japan. These are frequently not uniformly resistant and may have resistance combined with undesirable features. In looking for the best sources of resistance one should evaluate commercial varieties and advanced germplasm available from seed companies and public plant breeders who are actively breeding

cucumbers. These breeders can also provide information on the best current methods for testing for resistance.

There is still a need for better sources of resistance to some diseases, including higher levels of resistance to downy mildew and gummy stem blight (*Didymella bryoniae*). Resistance to nematodes is another need for some areas.

c) Testing methods

It is not possible in limited space to describe the testing methods used for the diverse breeding goals in the several cucurbit species. Most would be carried out in greenhouses and fields and relatively few in laboratories. Testing methods for individual objectives are described in some of the general references but many are found in various scientific journals or in less formal publications. In the latter category, the *Cucurbit Genetics Cooperative Reports,* issued yearly since 1976, are particularly useful. In addition to research notes, each report gives names, addresses, and telephone numbers of the members along with the research interests of many. The best information on current testing methods can be obtained by contacts with appropriate members.

After progenies have become relatively uniform for a desired combination of characteristics, it is important to evaluate them by harvesting them as would be done for market. With cucumbers, number of marketable fruits is a better criterion of yield than weight because smaller sizes are frequently worth more than larger ones. Fruit type can be evaluated better in repeated harvests than by observing unpicked plants. A single harvested replicate of four to six plants will give considerable information, especially when results are combined with similar results from additional years or locations.

For other species, mature fruits are harvested, counted, weighed, and recorded by market grade or as marketable yield. Single plots of four to ten plants repeated in at least two locations or for two years give considerable information when results are combined. The better progenies can then be advanced to replicated tests. Quality evaluation can be made by testing and/or by determining soluble solids with a hand refractometer. Appearance as well as yield and quality will also enter into decisions on release and naming of a progeny advanced to yield tests.

II. MELON

A. Characteristics of the crop

a) Geographic origin; centre of diversity

The melon, *Cucumis melo* L., originated in Africa and tremendous diversity is found within the species. This has led to the naming of many botanical varieties, now called groups, some having only trivial differences from each other and some being given different names in different countries.

b) Taxonomy; genetic and cytogenetic characteristics

The following is an attempt to simplify and at the same time make the groups in *C. melo* more inclusive than most previous treatments of the subject.

1. *C. melo cantaloupensis* Naud. Cantaloupe or muskmelon. Medium size fruits with netted, warty, or scaly surface, flesh usually orange but sometimes green, flavour aromatic or musky. Fruit dehiscent at maturity. Usually andromonoecious, *i.e.* each plant has both male and perfect flowers, the latter having male parts as well as female parts. Resistance to *Fusarium* wilt is found occasionally in this group but in general it is susceptible to diseases.
2. *C. melo inodorus* Naud. Winter melons. Smooth or wrinkled surface with flesh usually white or green and lacking musky odour. Usually larger, later in maturity, and longer-keeping than *cantaloupensis*, and not dehiscent at maturity. Usually andromonoecious. Highly susceptible to virus diseases but a source of resistance to *Fusarium* wilt.
3. *C. melo flexuosus* Naud. Snake melon. Synonym of snake cucumber, a common name causing confusion and therefore to be avoided. Fruit long and slender, used when immature as an alternative to cucumber. Monoecious. Probably more tolerant to heat and cold than most other groups.
4. *C. melo conomon* Mak. Pickling melon, sweet melon. Small fruit with smooth skin, white flesh, early maturity, and usually with little sweetness or odour. However, some melons in this group have high sugar content when mature and are eaten like apples, rind included. Vines of both types have similar appearance and have in common resistance to cucumber mosaic. Andromonoecious.
5. *C. melo chito* and *C. melo dudaim* Naud. Mango melon, vine peach and other similar names for the former; pomegranate melon, Queen Anne's Pocket melon for the latter. Distinction between these two groups is not clear from published descriptions. Long vines with small leaves, small fruit, and monoecious flowering. Resistance to gummy stem blight, watermelon mosaic, and possibly to other virus diseases has been found in this group.
6. *C. melo momordica.* "Phut" or snap melon. Grown in India and other Asian countries and distinct from any other group. Flesh is white or pale orange, low in sugar, and mealy. The smooth surface of the fruit cracks as maturity approaches and the fruit disintegrates when barely ripe. PI 371795 and 414723 belong to this group and have provided important resistance, such as to aphids, zucchini yellow mosaic, and watermelon mosaic, but they are also very susceptible to cucumber mosaic. Monoecious. Many in the group are resistant to powdery mildew.
7. *C. melo agrestis* Naud. Wild types with slender vines and small, inedible fruit. Probably synonymous with *C. melo callosus* and *C. melo trigonus*. Resistance to watermelon mosaic has been found in this group, and it deserves study as a possible source of other resistance.

C. melo has 12 pairs of chromosomes and there have been attempts to cross it with other *Cucumis* species with the same number. Crosses are difficult to make, and there has been little success in transferring anything useful to melons. Even more than with cucumber, there is great variability within the species that has not yet been used for varietal improvement.

Toxicology

Cucurbitacins are present in the young leaves of many melon varieties but occur in such small amounts in fruit that it is not toxic or bitter. We know of no instance in which anyone has been made ill by a toxic compound in fruits of cultivated melon. The wild melon *C. melo agrestis* (= *callosus*) has bitter toxic fruit.

Environmental requirements

Melons are considerably more exacting than cucumbers in needing sun and heat but they are less in need of a steady supply of moisture. Low temperatures, even several degrees above freezing, slow development greatly, and low soil temperatures slow water uptake. This may result in wilting and even death if cool, cloudy weather is followed quickly by sunny weather that causes rapid transpiration.

Melons are grown in many arid regions where soil salinity may be a problem, and there have been a number of studies on salinity tolerance. Varietal differences have been found that give some guidance on choices under saline conditions. The leading US cantaloupe "Topmark" is one of the more tolerant.

B. Current breeding practices and variety development research

a) Main breeding techniques

Until recently most breeding has involved crossing within groups in spite of the complete fertility of crosses between groups. The groups that are less commonly cultivated have some important and distinctive feature that deserve much more attention. These are not well represented in most germplasm collections, but any search for traits such as disease resistance or salinity tolerance should include all of the diverse groups.

Most commercial melons are andromonoecious in flowering. Crossing between adjacent plants is only about 10 per cent. Controlled self-pollination is not essential when selecting in populations with a low proportion of desirable plants. When crosses are desired, the perfect flowers, identifiable by having ovaries, should be emasculated in the bud stage on the day prior to anthesis. There are several alternatives for cross-pollination. First, if many crosses are to be made with the same male parent, male buds may be picked in the afternoon, kept at room temperature in a plastic bag or other container to avoid drying, and their pollen brushed on the emasculated flowers after the anthers dehisce the next morning. Second, if only a few crosses are to be made with a given male parent, male buds may be fastened shut or enclosed in place and picked after anther dehiscence the next day for immediate use. Third, male flowers may be prepared as above, refrigerated from time of dehiscence the next morning until buds are emasculated in the afternoon and then pollinated immediately, the perfect flowers being receptive the afternoon prior to their opening.

For self-pollinating when all outcrossing is to be avoided, a male and a perfect bud on the same plant are fastened shut or enclosed on the afternoon before anthesis. In many varieties the perfect flower may require some emasculation to expose the stigma sufficiently. The pollinated flower should be protected from insect visits for the rest of the day by bagging or covering with half of a gelatin capsule.

A single dominant gene conditions monoecious flowering and simplifies both controlled self- and cross-pollination. Unfortunately this gene often leads to elongated fruit, reduced netting, and poor "slip". Considerable effort is needed to combine it with the modifier genes that overcome the undesirable effects, but acceptable monoecious forms are now in use for producing seed of hybrid varieties.

In melons there is the same choice of open-pollinated or hybrid varieties as in cucumbers, and the same breeding practices apply. Seed of hybrids has been much more expensive in the past because of the hand-emasculation required, and it has mainly been used where the earlier maturity of certain hybrids is important. There appears to be a decided increase in the use of hybrid varieties in recent years but the extent of and reasons for this trend are not clear. The availability of good monoecious parents is probably important.

Gynoecious (all-female) melons are known and genes for male sterility are available, but neither has been used to a significant extent in hybrid seed production to date.

b) Main breeding objectives

Common objectives include earliness, high sugar content, appropriate aromatic flavours, appearance that matches market preference for shape, colour, size, and netting, and resistance to the diseases important in the target area. Many of these are superficial characteristics that vary with locality and are subject to rapid change. High sugar content and high marketable yield are always desirable, and in many areas diseases limit both. Consequently, disease resistance has become a major objective of many melon breeders.

Several dominant genes confer resistance to powdery mildew and some are associated with autogenic necrosis, which can have worse effects on the vines than mildew itself. The gene for race 1 PMR in PMR45 seems safe to use and confers useful resistance in many locations. If race 2 PMR is a problem, the Texas varieties such as Oerlita and TAM Uvalde have resistance with little or no association with autogenic necrosis.

Two complementary dominant genes for resistance to downy mildew (*Pseudoperonospora cubensis*) come from PI 124111, and a single dominant gene for resistance to *Alternaria cucumerina* is available in the USDA line MR-1.

Dominant genes for various races of *Fusarium* wilt are known and readily transferrable by backcrossing. A single dominant gene for resistance to gummy stem blight has been reported in PI 140471, but the high resistance of that PI has not been transferred to commercial varieties in spite of considerable effort, suggesting more complicated inheritance.

Resistance to several aphid-transmitted viruses has been found in melons from India. The single dominant gene for papaya ringspot virus, formerly watermelon mosaic virus 1, can now be obtained in the well-netted, orange flesh melon WMR29 of the US Department of Agriculture. Resistance to this virus can also be found along with a dominant gene for resistance to zucchini yellow mosaic virus in some plants of PI 414723. Resistance to watermelon mosaic virus (formerly WMV2) is also present in this PI, probably as a single dominant gene. Resistance to CMV has been found in the *conomon* group and is partially dominant; at least three genes are needed for a high level of resistance.

Resistance to individual aphid species has been found in PI 414723 but to date has not appeared in a successful commercial variety, probably because diseases are a greater

limitation to melon production. Multiple disease resistance is needed urgently because it is difficult to predict which single resistance may be most important in a given production area.

III. SQUASH AND PUMPKINS (*CUCURBITA* SPECIES)

A. Characteristics of the crop

a) Geographic origin and distribution of use

The genus *Cucurbita,* with several cultivated species, originated in the Americas and is now grown throughout the world.

b) Taxonomy

Squash and pumpkin are common names applied to some types within each of the four principal species – *C. pepo, C. moschata, C. mixta* (now considered to be *O. argyrosperma*), and *C. maxima.* Common names are confusing because what is called squash in one area may be called pumpkin in another. "Summer squash" usually refers to fruits of *C. pepo,* while "winter squash" refers to the mature fruits of all four species. "Pumpkin" in the United States generally means fruits of any species used for decorative purposes, for pies, or, less commonly, as feed for livestock. Some representative varieties or varietal groups within each of the species follow:

Cucurbita pepo	Pie and Jack O'Lantern pumpkins, summer squash, *i.e.* fruits eaten when very young (yellow straightneck, cocozelle, zucchini, patty pan), winter squash (table queen or acorn), ornamental gourds;
Cucurbita moschata	Butternut squash, golden cushaw, Kentucky field pumpkin;
Cucurbita mixta	Japanese pie, green-striped cushaw;
Cucurbita maxima	Hubbards, delicious, Boston marrow (squash), Queensland blue pumpkin (Australia), atlantic giant pumpkin;
Cucurbita ficifolia	Fig-leaf gourd (the only cultivated perennial, very restricted usage).

c) Genetic and cytogenetic characteristics

The four main cultivated species all have 20 pairs of chromosomes but do not cross readily with each other. Large numbers of crosses yield only a few seeds which give either partially fertile or self-sterile plants. The most successful crosses have been *C. pepo* × *C. moschata.* From such crosses bush habit has been transferred to *C. moschata* and disease resistance to *C. pepo.* Some varieties of *C. pepo* produce several seeds

per fruit when pollinated with *C. moschata* but others yield no seeds at all. Therefore, several varieties should be used if this cross is attempted.

Some inedible species of *Cucurbita* cross more readily with certain cultivated ones than the latter cross with each other. *C. andreana* and *C. ecuadorensis* give fertile hybrids in crosses with *C. maxima*, and *C. martinezii* crosses readily with *C. moschata*.

d) Current end uses

Seeds as well as the flesh of fruits are eaten in some places and are very nutritious.

Reproductive mechanisms

All of the *Cucurbita* species are monoecious in flowering and a given plant has at least 75 per cent natural crossing with those around it. Controlled self-pollination is therefore required along with selection in most breeding efforts. No appreciable vigour is lost in inbreeding and very uniform inbred lines can serve as varieties in themselves or as parents for hybrid varieties. Large-scale production of hybrid seed can be accomplished by removal of male flower buds from parent rows or by spraying with Ethephon to prevent male bud development. Hand pollination for self- or cross-pollination is similar to that described for melons except that there is less need to consider pollinating female buds because no emasculation is needed for individual squash flowers.

A gene for male sterility has been found in an Egyptian *C. pepo* and transferred to other types in this species. Its use in hybrid seed production has been limited because half the plants in female parent rows are fertile and must be removed. Male sterility has also been found in *C. maxima* and used to a limited extent in producing hybrid seed.

Toxicology

The cucurbitacins found in certain *Cucurbita* are more toxic than those in other cultivated cucurbits and have occasionally caused severe illness. This has been a problem in some *C. pepo* summer squashes, because there are bitter ornamental gourds in the same species which can initiate introgression of bitterness if not completely isolated in seed production. After several generations of seed production, natural backcrossing can result in a very small proportion of bitter plants that look exactly like the rest of the population.

Some of the wild species used as sources of disease resistance have a gene for bitter fruit (*Bt*) and this must be selected out in the early generations following crosses. Plants with bitter fruit cannot reliably be detected by tasting cotyledons.

Environmental requirements

Among the cultivated species, *C. maxima* is best adapted to low temperatures, *C. moschata* and *C. mixta* to high temperatures, while *C. pepo* is adapted to a wide range. However, some varieties of *C. pepo* respond to low temperatures early in their life cycle by producing only female flowers and early yields can be disappointing because of lack of pollination.

This genus has a tendency to be photoperiodically sensitive and some forms, particularly the wild ones, may be late in flowering if moved to higher latitudes. Time of flowering for these varies from year to year, suggesting that temperature interacts with photoperiod.

B. Current breeding practices and variety development

a) Main breeding techniques

As with other cucurbits the main choices are between pedigree selection with enforced inbreeding and the backcross method. The latter is almost essential for recovering commercial types from wide crosses. Either procedure leads to uniform true-breeding lines that can be used either as varieties or as parents for hybrid varieties. Hybrids are used most in summer squash (*C. pepo*) where earlier harvest is their main advantage and where the bush habit of growth facilitates hybrid seed production. Hybrid varieties are used to a lesser extent in winter squashes of the other species.

One breeding practice that is distinctive in *Cucurbita* is the use of mass selection in developing some recent varieties. Growers of pumpkins for display purposes have developed some preferred varieties by mass selection. This practice has undoubtedly been responsible for some of the land races found in developing countries. Even though changes are made slowly with this method, it should be encouraged because it is inexpensive, leads to varieties adapted to local growing conditions and markets, and permits local seed production.

b) Main breeding objectives

C. pepo is probably the most widely grown species of *Cucurbita* when one considers its varied forms and their popularity. It is also the most subject to loss from disease, and very little resistance has been found within it. Disease resistance is a high priority. Excellent powdery mildew resistance (PMR) is found in *C. martinezii* (synonymous with *C. okeechobeensis*) and has been transferred to *C. pepo* with *C. moschata* as a bridge species. It can now be backcrossed rapidly into any variety of *C. pepo*, a single largely dominant gene giving a satisfactory level of resistance under most field conditions. PMR gives better quality in *C. pepo* winter squash and decreases tendency to rot in the ornamental pumpkin. This same interspecific material also carries resistance to cucumber mosaic which is more complex in inheritance and more difficult to transfer. *C. martinezii* is also resistant to gummy stem blight.

Provvidenti *et al.* (1984) have found resistance to CMV, PRV, WMV, and ZYMV in *C. moschata* "Nigerian Local". The inheritance of these resistances is not well understood, but resistance to ZYMV has been transferred to *C. moschata* "Waltham Butternut" and seems to behave as a single dominant gene. Transferring ZYM resistance to *C. pepo* has been more difficult, partly because lack of fruit symptoms is sometimes independent of lack of foliar symptoms. There is little doubt that multiple virus resistance along with PMR will be incorporated into a range of *C. pepo* types within a few years.

C. moschata is much less susceptible to disease than *C. pepo*, but "Butternut", probably the most important *Cucurbita* in the United States, is sometimes damaged by mildew and ZYMV. The addition of the resistances is well underway.

C. maxima is also not as susceptible to disease as *C. pepo* but resistance would be useful, and R.W. Robinson has transferred resistance to several viruses from *C. ecuadorensis* to the "Golden Delicious" variety of *C. maxima*.

When *C. pepo* is grown in small plantings, as in home gardens, there are frequently large losses from the squash vine borer. It has long been known that "Butternut" is

hardly ever attacked by this insect. Attempts have been made to transfer this resistance through interspecific populations, but in the larger plantings needed for this purpose, there has not been sufficiently uniform infestation to permit effective selection. Attempts to raise the insect in culture to provide artificial infestation have not been successful so far.

Bitterness in the plant but not in the fruit occurs in *C. pepo* as in cucumber. Cucumber beetles damage the varieties with bitter plants much more than the non-bitter, and efforts are underway to convert the former to the latter, with the zucchini type as the prime candidate.

IV. WATERMELON

A. Characteristics of the crop

For many years watermelon was classified as *Citrullus vulgaris* but the correct name is now considered to be *Citrullus lanatus*. It originated in Africa where several related species occur. It is now an important crop throughout the world, especially in the tropics and subtropics. Seeds as well as flesh of the fruits are eaten in some localities. *C. lanatus* crosses readily with *C. colocynthis*, a perennial with bitter fruit found in northern Africa. It has been a source of disease resistance but its pollen can give rise to bitter hybrids if watermelon seed production is attempted too close to uncultivated land. Both species have 11 pairs of chromosomes.

Reproductive mechanisms

Most watermelons are monoecious (separate male and female flowers) but a few are andromonoecious (male and perfect flowers). They are naturally pollinated by insects and there is a high rate of cross-pollination. However, vigour is not lost when inbreeding is practised to achieve uniformity. Controlled self- or cross-pollinations are carried out in the same way as described for other cucurbits. Male sterility has been found but to date has not proven useful in large-scale production of hybrid seed.

Toxicology

Even though bitterness is present in fruits of the inedible species of *Citrullus* and their natural crosses with watermelons, no reports of illness from eating bitter fruit have been made. Apparently the cucurbitacins in watermelon are not sufficiently toxic to cause illness in the amounts that anyone is likely to ingest.

Environmental requirements

Watermelons are similar to other melons in needing relatively high temperatures for good production. However, early varieties are available that mature as early and are as well adapted as *C. melo* for the northernmost states of the United States. They are much less grown in those states, although they are there less subject to disease and more

dependable in producing fruit of good quality. This anomaly is probably explained by the fact that they are more reliable in southern states and shipped from them in large quantities for many weeks before local melons are available in the North, at which time consumers have become more interested in other fruits.

B. Current breeding practices

a) Main breeding techniques

Breeding practices are similar to those used for other cucurbits with one important exception: the use of triploid hybrids to produce seedless watermelons. Seed parents for these hybrids are tetraploid lines obtained by doubling the chromosomes of desirable diploids by colchicine treatment. Suitable diploid male parents are then identified by testing numerous experimental triploid hybrids. Diploid plants must be planted in commercial triploid fields, because fruit will not develop without the stimulus of diploid pollen. Some other factors have slowed the commercialisation of triploid hybrids. The seed is many times more expensive than that of diploid hybrids and is difficult to germinate unless high temperatures can be provided. Nevertheless, seedless watermelons are produced in substantial quantities in Taiwan and other Asian countries, and according to one reliable estimate, triploids now account for about 7 per cent of US production. Much effort is devoted to triploids in breeding programmes of US seed companies.

Diploid hybrid varieties increased considerably in importance in the 1980s and are now estimated to be used in nearly half the US production. Male sterility is not used. Hybrid seed is produced by hand-pollination or by removing male buds from female parent rows.

b) Main breeding objectives

Fusarium wilt resistance has been a major goal of watermelon breeding for most of this century. As a result of the first organised effort to breed a disease resistant variety in the United States, W.A. Orton of the US Department of Agriculture released the *Fusarium* resistant "Conqueror" watermelon in 1911. Its source of resistance was the inedible stock citron. Since then many *Fusarium* resistant varieties have come from numerous breeding programmes. Inheritance studies have been complicated by the existence of different races of the organism and because resistance is governed by several mostly recessive genes. However, one dominant gene for resistance to race 1 has been identified.

Resistance to anthracnose caused by *Glomerella cingulata* var. *obiculare* is fully as important as *Fusarium* resistance. "Charleston Gray" and "Crimson Sweet" have been dominant varieties in the United States for many years because of resistance to both diseases, combined with many desirable horticultural features.

On the other hand, "Sugar Baby", without any identified disease resistance, has been the main variety in some parts of Asia. It has smaller fruits than most other varieties, but the reasons for its success in Asia are not clear.

Other important objectives include resistance to gummy stem blight, durability for shipping, high sugar content, fruit of the preferred size, shape, and colour for the intended market, and dwarf habit of vine for ease of harvest.

C. Seed multiplication for commercial use

Once a desirable line has been identified as suitable for release either as a variety to be used directly or as a parent for hybrid varieties, cucurbit seed can be multiplied very rapidly. If plants are given wide spacing and good growing conditions, a single seed can be multiplied to a thousand or more. Like most vegetables, seed for commercial production of cucurbits is usually purchased from specialised seed companies. Public vegetable breeders usually provide small amounts of breeder seed to such companies, sometimes without charge, sometimes with a payment for the seed, and sometimes on the basis of royalties based on the amount of seed eventually sold. Certification is seldom used to ensure seed quality, this being done essentially by the known reliability of the seed companies involved.

One advantage of open-pollinated varieties of cucurbits as compared with hybrids is that the seed can be multiplied by farmers if seed from commercial seedsmen is not readily available.

Whatever the mechanism of seed multiplication, a public breeder or institution should maintain viable pure seed of its releases to renew the planting stock of seedsmen if necessary, and to have a pure source for comparison if deterioration in commercial sources is suspected.

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5. Cotton

by

Johnie N. Jenkins

A. Characteristics of the crop

a) Geographic origins; centres of diversity

Cotton, *Gossypium* ssp. has been cultivated for millennia in many parts of the world. All cultivated cottons are *G. hirsutum* L. (AD1) or *G. barbadense* L. (AD2), allotetraploids of the AD genome, except for small acreages of *Gossypium arboreum* L. (A1 diploid genome) in India and small acreages of *G. herbaceum* L. (A2 diploid genome) in drier regions of Africa and Asia. The geographic centre of origin for *G. hirsutum,* the predominant cotton of commerce, is North and Central America and Mexico; and that of *G. barbadense,* the extra-long staple (or fibre length) cotton of commerce, is South America. Records of cultivation of *G. arboreum* in India date back 3 000 years. Pre-Columbian cotton was important in Brazil, Peru, Mexico, and Central America. During the colonial period in North America, cotton was one of the first crops to be successfully cultivated in what is now the eastern part of the US Cotton Belt.

b) Geographic distribution of use; main production areas

About 90 per cent of the world production of cotton is *G. hirsutum.* The United States (4.7 million ha with a value of $5.1 billion), the former Soviet Union, and China (5.58 million ha) are the three largest cotton-producing nations. It is also grown in many other countries and makes a major contribution to the economy of each. Production figures for 1990 are: North and Central America (5.04 million ha), South America (3.5 million ha), Europe (3.1 million ha), Africa (3.4 million ha), and Asia, the Middle East and Oceania (17.7 million ha) for a total world production of 33.3 million ha (USDA, 1991). Most of the nations of Africa as well as the Middle East produce cotton. Cotton is primarily used worldwide for its lint, which is spun into yarn. Seed is a by-product in most countries. Only the United States and a few other countries have developed major commercial uses for the seed.

The San Joaquin Valley of California only allows commercial production of cultivars with fibre properties approved by the Acala Board. Therefore, all cotton produced in this valley of California has a high, uniform quality fibre, and it commands a premium on the market. In the United States, pima cotton (a type of extra long staple *G. barbadense* cotton) is primarily grown in Arizona, with limited acreage in New Mexico and

California. The Supima Association is the controlling co-operative for this extra-long staple high quality cotton. Egypt also grows an extra-long staple cotton that is *G. barbadense*. A fairly large acreage of *G. barbadense* is also grown in the Xinjiang province of western China.

c) Taxonomic status

There are 39 species of cotton but only four have spinnable fibres called lint. The remaining wild species tend to be perennial with a growth habit of small shrubs and only have short, non-spinnable fuzz on seed.

d) Genetic and cytogenetic characteristics

The allotetraploid cotton species *G. hirsutum* and *G. barbadense* are the result of an ancient natural cross between the old world diploid A genome and an unknown species of the new world diploid D genome, followed by spontaneous doubling of the chromosomes to form the allotetraploid. *G. arboreum* carries the A1 diploid genome and *G. herbaceum* the A2 diploid genome.

e) Current phytosanitary considerations in movement of germplasm

Insects are a major problem for cotton production. The most important are: worm insects in the order Lepidoptera; several insects in the Heteroptera Miridae; *Anthonomus grandis* Boheman, the boll weevil; *Aphis gossypii* Glover, the cotton aphid; *Bemisia tabaci* (Grennadius), the sweet potato whitefly; *Trialeurodes abutilonea* (Haldeman), the banded wing whitefly; and *Tetranychus* ssp., spider mites. They are generally controlled by pesticides, which add materially to production costs. Aphids and whiteflies secrete a substance called "honeydew"; it is high in sugar and causes sticky lint, a major problem in cotton spinning. Although the boll weevil is only a pest in parts of Mexico, North, Central and South America, many of the other insect pests occur worldwide.

The pink bollworm, *Pectinophera gossypiella* (Saunders), and certain diseases such as bacterial blight, *Xanthomonus malvacearum*, can be carried along with the seed. As a result, phytosanitary certificates and/or acid delinting (removal of fuzz from seed by treatment with sulphuric acid) are required for seed movement and germplasm exchange between many countries.

f) Current end uses

Fibre characteristics of importance to the spinning industry are length, strength, fineness, maturity, elongation, and uniformity of length. If fibre or lint do not meet certain criteria, they are not useful for commercial trade. Standard fibre measurements and methods are used worldwide. High volume instrument (HVI) testing is required in US trade and is used in many countries.

Reproductive mechanisms

a) Mode of reproduction and pollination

Cotton flowers are surrounded by three triangular bracts, and the buds with the bracts are called squares. Pollen is shed directly on the stigma when the anthers open, or it may be carried there by insects. Cotton pollen is heavy and thus not wind-borne. It is also spiny, and many insect pollinators, such as the honey-bee *Apis mellifera,* do not like it. Cotton is considered an "often crossed" crop species but essentially behaves like a self-pollinated one. The amount of cross-pollination varies with the insect pollinator population. Extensive use of insecticides for control of insect pests will essentially limit the extent of cross-pollination. Since only insects cross-pollinate, most cross-pollination involves plants situated within 30 m of each other.

b) Perennial vs annual

Cotton is a perennial in the wild; it has been forced, through selection, plant breeding, and management, to behave like an annual. In tropical zones and some temperate zones it will grow as a perennial unless forced through crop management, usually stalk destruction, to grow as an annual.

c) Dispersal of propagules and survival mechanisms

Wild species of cotton generally have a fairly high percentage of "hard seed", *i.e.*, seed that survives one or more seasons before germination. This is a positive survivability mechanism in wild cotton. Plant breeders have bred it out of modern cultivars. Cottonseed in commercial trade must be handled properly to preserve germination quality. In humid environments, seed left in the field will not usually survive until the next season.

d) Ability to cross with wild species

Cotton is not closely related to any other crop or genera, and crosses are usually only fertile within a species with some cross-species compatibility, such as *G. hirsutum* by *G. barbadense.* There are no important traits known to be inherited through the cytoplasm. A cytoplasmic-nuclear system has been reported that causes male sterility.

In the areas of the United States where cotton is grown, there are no wild species or relatives of cotton that will form fertile hybrids with commercial cotton. In Hawaii, there is a wild species (*G. tomentosum* Nutt.) that is cross-fertile with commercial cottons; however, no commercial cotton is produced in Hawaii. Some research plots of cotton are grown in Hawaii, and extra attention should perhaps be paid to field isolation. There, and in some parts of the world, accidental outcrossing to diploid cottons might occur. This is a legitimate concern for some traits, but should be considered on a case by case basis.

Toxicology

There are no toxicants of importance in the lint or fibre of cotton. Bissinosis, a disease of the lungs which is sometimes a problem in the spinning industry, may be related to biological or trash contaminants on cotton lint. Cottonseed contains phenolic-related components, typified by gossypol, which limits its direct use as an animal feed by

non-ruminants. Present levels of phenolics in seed do not generally cause problems when the seed is used as feed for ruminants. Gossypol in the seed limits its direct use as human food and discolours the oil. However, the oil can be clarified by commercial means and made fit for human consumption.

Environmental requirements for life cycle

a) Climatic restrictions to extension of the crop

Many wild species and relatives of commercial cotton are sensitive to day length and require short days and long nights to flower. Thus, they will not flower in summer in many cotton-producing regions, such as the US Cotton Belt. In general, commercial cottons are day neutral, *i.e.* they are not sensitive to day length. Cotton is generally cultivated in the temperate and tropical zones and requires about 125 to 160 days of growing season to produce a crop. It is grown as far north as 43°N in desert valleys in Russia and 45°N in China, and some efforts are being made to extend its production range northward in the United States.

B. Current breeding practices for variety and hybrid development

a) Main breeding schemes and techniques

i) Basic breeding

At one time public breeding programmes in the United States emphasised development of germplasm rather than of varieties. However, the research of most state agricultural experiment stations now covers both. The United States Department of Agriculture, Agricultural Research Service (ARS) research programmes emphasise high-risk studies and germplasm development. Pre-breeding research on pest resistance and fibre quality properties from wild relatives of cotton are emphasized by ARS. In addition, germplasm collection, maintenance and evaluation for *G. hirsutum* and *G. barbadense* are primarily the responsibility of ARS.

The Supima Association, a private body, develops pima varieties in the United States. There are several major commercial seed-breeding companies for *G. hirsutum* upland cottons in the United States. One firm emphasizes F_2 hybrids; they use a chemical male gametocide to make flowers male-sterile and pollinate for F_1 seed, using colonies of honey-bees in the fields. These F_1 seed are advanced one generation and sold as "F_2 from hybrids". Most US companies sell varieties; outside the United States, cotton seed breeding and variety development are generally the responsibility of various governmental agencies.

F_1 hybrids are produced by hand emasculation and pollination in China and India. Some hybrids in India are interspecific between *G. hirsutum* and *G. barbadense*. A satisfactory system for male-sterile, pollen restoration, field pollination for cotton does not exist at the present time. This limits the development of F_1 hybrids for commercial use; therefore, most cotton in world commerce comes from cultivars rather than hybrids.

ii) Techniques used

In a typical breeding programme, hand crosses are made between selected parents or F_1 hybrids. Seed are advanced by self-pollination to the F_2 generation, where individual plants are self-pollinated and planted in progeny rows. Selection is practised or the row is bulk harvested to F_5 or F_6, using either single or double progeny rows about 20 m long at one site. Beyond F_5 or F_6 generations (essentially true breeding), lines are evaluated at multiple locations, usually in two row plots. Selection for plant type, fibre properties and lint percentage is practised ir early generations, and progeny that do not meet certain minimal standards are discarded. Most varieties are the product of bulking several select sister lines in the F_6 or F_7 generation.

Single gene traits are often backcrossed into varieties through a straight backcross and selection programme. The wild relatives of upland cotton serve as a major reservoir of diversity for varietal improvement. The germplasm collection of wild races of *G. hirsutum* contains genes for improved pest resistance, fibre quality and other traits; however, these are usually found in unadapted lines and are relatively low in productivity. Genetic engineering and recombinant DNA technologies are providing additional methods to make use of sources of needed diversity for traits such as pest resistance and fibre quality. An example of a trait from a non-cotton source is the delta endotoxin produced by a gene in *Bacillus thuringiensis* and useful for control of caterpillar insects of cotton.

Genetically engineered cotton lines carrying genes from other organisms are currently being evaluated in the United States for resistance to lepidopterous insects (delta endotoxin gene from *Bacillus thuringiensis* var. *kurstaki*) and for tolerance to certain herbicides (glyphosate and bromoxynil). At present, the traits of interest are the product of single genes for each trait. The traits in these genetically engineered cotton lines should reduce use of pesticides and/or allow more selective use of those that are less damaging to the environment.

Genetic engineering is not likely to shorten markedly the time required to develop a variety. However, it offers the opportunity to use genes that are not available in *Gossypium* to improve varieties. Genetic engineering biotechnology provides an important means to broaden the germplasm base available for cotton improvement.

b) Important breeding objectives

Yield and fibre quality are important objectives in all commercial breeding programmes. Where genes for pest resistance are available, these are also worked into the programme. Fibre quality requirements are important worldwide.

c) Testing for the most important breeding goals

Instruments are used to measure fibre length, strength, uniformity, and micronaire (a combination of fineness and maturity). There is a negative correlation between lint yield and fibre strength; this presents problems in breeding programmes where increases in each are important.

d) Assessment of general performance

Yield is measured over several environments, and seed breeding companies usually have a testing combination of years by environments of 20 (N years by Y environments = 20) before they place a variety into commercial sale and production.

In breeding programmes in the United States, advanced lines, called superior strains, are entered in multi-location, state-supervised evaluation trials for two to three years before they are released as varieties or hybrids for commercial production. Nearly all advanced strains destined to become varieties are evaluated at least one year in the National Root-knot Nematode/*Fusarium* Wilt Nursery at Tallassee, Alabama. This nursery is operated as a co-operative project between ARS and the Alabama Agricultural Experiment Station. Seed breeding companies make the decision to turn a superior strain into a commercial variety.

C. Seed multiplication for commercial use

a) Stages in seed production

Most breeding agencies and commercial companies in the United States will store several tonnes of the original variety seed (called breeder seed) in environmentally controlled storage rooms set to maintain seed quality and germination over a period of years. Each year, a quantity of the breeder seed is removed from this reserve and provides the nucleus for a new seed increase called foundation seed. Foundation seed is grown (increased) for one year and becomes registered seed. Registered seed is increased one year and sold as certified seed to be planted by growers. Ten years usually elapse between the first cross and the sale of a new variety in commercial trade, but in certain circumstances hybrids could reduce the time span. During final testing and seed production the company breeder carefully studies the yield and fibre quality data from each location.

b) Isolation practices

Since cotton is generally self-pollinated and most certified seed production is in areas with few insect pollinators in the fields, only minimal (5 m) separation is required between different varieties unless there are obvious differences in morphology, such as flower color or leaf shape. In the latter case, 536 m between varieties are usually required.

c) Seed certification and registration; plant variety protection

During final seed production by certified seed growers, the fields and gins are regulated by state seed certification officials and seed breeding company officials.

In the United States, seed of varieties is usually sold as a class of certified seed. Seed certification is the responsibility of the various state regulatory agencies; however, the companies usually have contract growers, and all seed is sold by the developing company or under their variety and brand name by licensed growers or seed dealers. The purpose of plant variety protection under Public Law 91-577 is to promote and protect increased commercial investment to develop new, sexually reproduced non-hybridised varieties of crop plants, because of increasing concern for breeders' rights. A plant variety protection

office has been established in the Agricultural Marketing Service of USDA to administer this act.

According to the *Handbook of Seed Certification Regulations* of the Mississippi Seed Improvement Association, classes of certified seed are defined as follows:

Breeder: "Breeder seed is seed directly controlled by the originating or sponsoring plant breeding institution, or person, or designee thereof. As applied to certified seed, breeder seed is the source for the production of seed of the other classes of certified seed. If breeder seed is to be tagged, it must be tagged with a white tag labelled 'Breeder Seed'."

Foundation (White Tag): "Foundation seed is seed which is progeny of breeder or foundation seed produced under control of the originator or sponsoring plant breeding institution, or person, or designee thereof. As applied to certified seed, Foundation seed is a class of certified seed which is produced under procedures established by the certifying agency for the purpose of maintaining genetic purity and identity. Foundation seed shall be tagged with white tags issued by the official seed certifying agency."

Registered (Purple Tag): "Registered seed shall be the progeny of Breeder or Foundation seed handled under procedures acceptable to the certifying agency to maintain satisfactory genetic purity and identity. Registered seed shall be tagged with purple tags issued by the official seed certifying agency."

Certified (Blue Tag): "Certified blue tag seed shall be the progeny of Breeder, Foundation, or Registered seed so handled as to maintain satisfactory genetic purity and identity, and which has been acceptable to the certifying agency (see exception: *Limitation of Generations*)."

Limitation of Generations: "The number of generations through which a variety may be multiplied shall be limited to that specified by the originating breeder or owner of the variety and shall not exceed two generations beyond the Foundation seed class with the following exceptions. A. Recertification of the Certified Blue Tag class may be permitted for older varieties where Foundation seed is not being maintained. B. The production of an additional generation of the Certified Blue Tag class only may be permitted on a one-year basis, when an emergency is declared by the certifying agency stating that the Foundation and Registered seed supplies are not adequate to plant the needed Certified Blue Tag acreage of the variety. The permission of the originating or sponsoring plant breeder, institution, firm, or owner of the variety, if existent, must be obtained. The additional generation of Certified Blue Tag seed to meet the emergency need is ineligible for recertification."

d) Surveillance of varietal behaviour

All cotton-producing states in the United States conduct annual state variety trials in which all currently sold varieties are entered. These are multi-location trials. In addition, commercial seed breeding companies conduct their own variety trials. Results are published annually and are used by growers and extension personnel for variety choice or recommendations. The seed companies also use these data to determine the continued relative performance of their varieties. Cotton varieties are not subject to "run out", but new races or biotypes of pests can develop and affect cultivar performance. These variety yield trials allow detection of problems of this sort.

National variety evaluation trials are conducted in the United States only to measure progress in cotton breeding. The ARS, state experiment stations, and seed breeding companies co-operate in carrying them out. ARS assembles the yield and fibre data and publishes the data annually. In these trials, gossypol and oil content of seed are also measured. Published data include yield, boll size, lint percentage, seed size, seed gossypol and oil content, fibre length, strength, micronaire, elongation, and uniformity. All fibre measurements are made at the same facility each year (Starlab, Knoxville, Tennessee). ARS pays for the fibre measurements and for publication and distribution of the data.

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6. Tobacco

by

René Delon

A. Characteristics of the crop

a) Geographic origins

The *Nicotiana* genus, to which all cultivated tobaccos belong, forms part of the family Solanaceae. There are 66 known species (Table 6.1), most of them natives of South America and Australia. In Africa (Namibia) a single species, *N. africana,* was discovered by Merckmüller and Butler in 1975.

Most tobaccos commercially cultivated today belong to the polymorphous species *N. tabacum* which has 24 pairs of chromosomes. It is now generally considered that the species descends by amphidiploidy from the crossing of *N. sylvestris* (n = 12) with *N. tomentosiformis* (n = 12).

b) Geographic distribution of use; main production areas

Although originally a warm-region plant, tobacco can flourish in a variety of soils and climates, and its area of cultivation is very extensive. World production of the various varieties – Virginia, Burley, Aromatic, Dark, etc. – is expanding slowly but steadily (Figure 6.1) and currently stands at about seven million tonnes. Asia and America together account for over three-quarters of the world's output. The major producing countries include China, the United States, India, Brazil, Turkey, the former USSR, Zimbabwe and Malawi. The leading producers in the EEC are Italy and Greece (Figure 6.2).

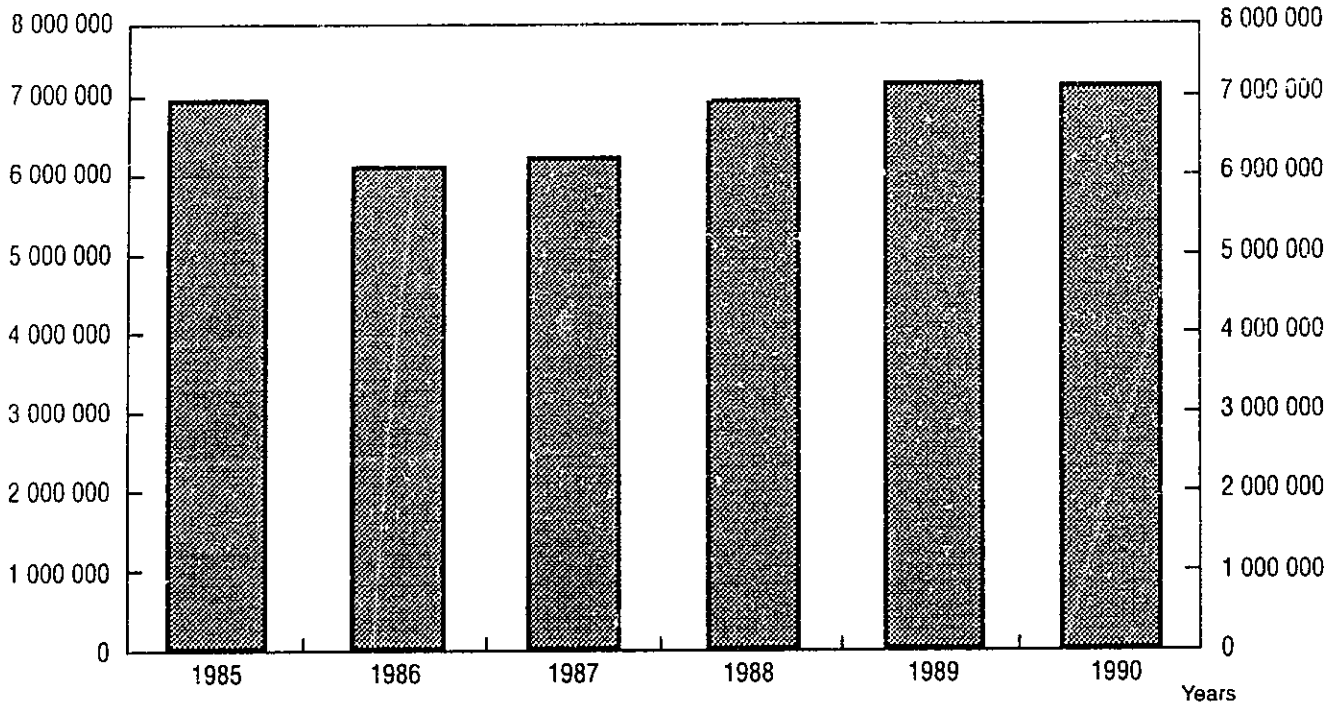
c) Taxonomic status

T.H. Goodspeed (1954) carried out the most recent classification of the *Nicotiana* genus, subdividing it into three sub-genera, *Rustica, Tabacum* and *Petunioides,* 14 sections and 60 species. Since then, revisions and discoveries by Burbidge (1960), Merckmüller and Butler (1975) and Ohashi (1976) have raised the number of species to 66 (Table 6.1).

Table 6.1. **Nomenclature of the genus *Nicotiana***

Sub-genus	Section	Species	Author	No. chromosomes (2n)
Rustica	Paniculatae	*glauca*	Graham	24
		paniculata	Linnaeus	24
		knightiana	Goodspeed	24
		solanifolia	Walpers	24
		benavidesii	Goodspeed	24
		cordifolia	Philippe	24
		raimondii	Macbride	24
	Thyrsiflorae	*thyrsiflora*	Bitter ex Goodspeed	24
	Rusticae	*rustica*	Linneaus	48
Tabacum	Tomentosae	*tomentosa*	Ruiz and Pavon	24
		tomentosiformis	Goodspeed	24
		otophora	Grisebach	24
		kawakamii	Ohashi	24
		setchellii	Goodspeed	24
		glutinosa	Linnaeus	24
	Genuinae	*tabacum*	Linnaeus	48
Petunioides	Undulatae	*undulata*	Ruiz and Pavon	24
		arentsii	Goodspeed	48
		wigandioides	Koch and Fintelman	24
	Trigonophyllae	*trigonophylla*	Donal	24
	Alatae	*sylvestris*	Spegazzini and Comes	24
		langsdorffii	Weinmann	18
		alata	Link and Otto	18
		forgetiana	Hort ex Hemsley	18
		bonariensis	Lehmann	18
		longiflora	Cavanilles	20
		plumbaginifolia	Viviani	20
	Repandae	*repanda*	Willdenow ex Lehmann	48
		stocktonii	Brandegee	48
		nesophila	Johnston	48
	Noctiflorae	*noctiflora*	Hooker	24
		petunioides	(Griseback) Millan	24
		acaulis	Spegazzini	24
		ameghinoi	Spegazzini	?
	Acuminatae	*acuminata*	(Graham) Hooker	24
		pauciflora	Remy	24
		attenuata	Torrey ex Watson	24
		longibracteata	Philippi	?
		miersii	Remy	24
		corymbosa	Remy	24
		linearis	Philippi	24
		spegazzinii	Milan	24
Petunioides	Bigelovianae	*bigelovii*	(Torrey) Watson	48
		clevelandii	Gray	48
	Nudicaules	*nudicaulis*	Watson	48
	Suaveolentes	*benthamiana*	Domin	38
		umbratica	Burbidge	46
		cavicola	Burbidge	40
		debneyi	Domin	48
		gossei	Domin	36
		amplexicaulis	Burbidge	36
		maritima	Wheeler	32
		velutina	Wheeler	32
		hesperis	Burbidge	42
		occidentalis	Wheeler	42
		simulans	Burbidge	40
		megalosiphon	Heurck and Mueller	40
		rotundifolia	Lindley	44
		excelsior	Black	38
		suaveolens	Lehmann	32
		ingulba	Black	40
		exigua	Wheeler	32
		goodspeedii	Wheeler	40
		rosulata	(S. Moore) Domin	40
		fragans	Hooker	48
		africana	Merx and Butler	24

Figure 6.1. **World tobacco production**
(tonnes)



Source: TJI No. 3, 1991.

d) Genetic and cytogenetic characteristics

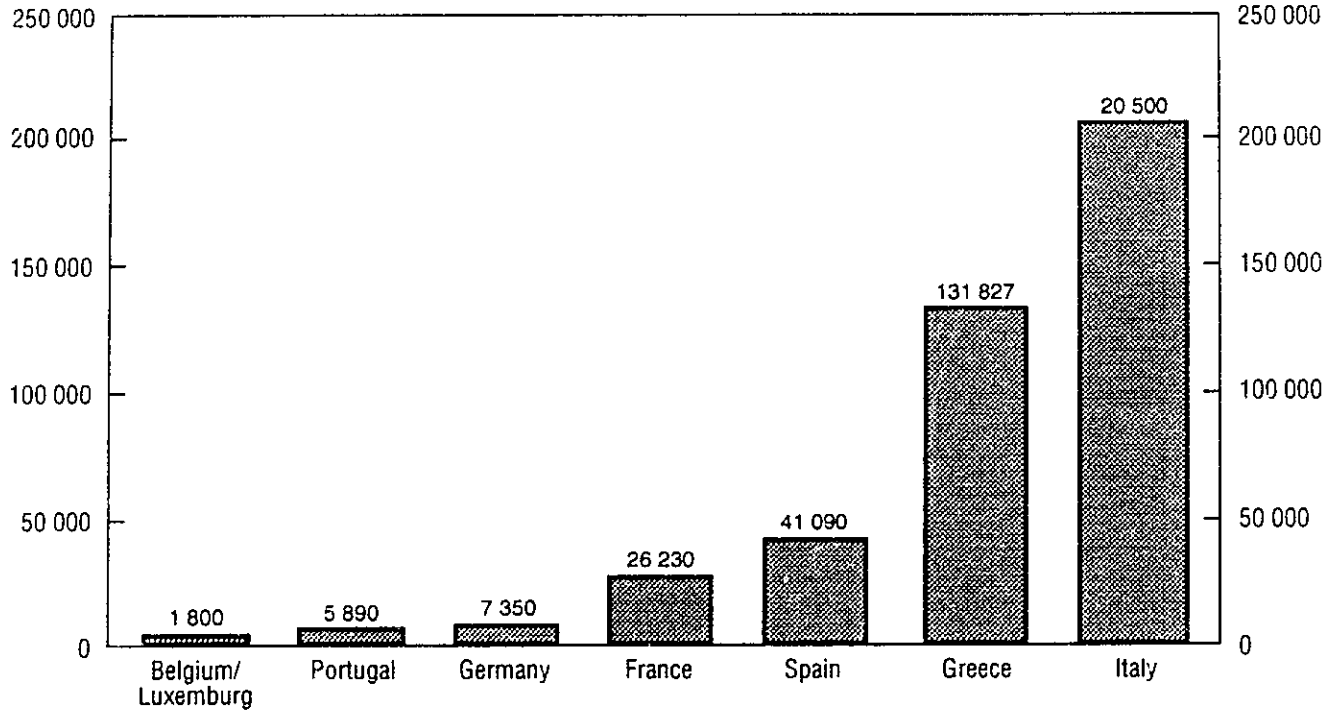
The commonest numbers of chromosomes in *Nicotianae* soma cells are 24 and 48, but 18, 20, 32, 36, 38, 40 and 46 are also encountered.

Interspecies affinity has been studied by many authors, including Goodspeed (1954) and Gisquet and Hitier (1961), who observed all the possible combinations, from total species incompatibility to hybrid plant creation, according to the chromosomes paired.

Two features of the *Nicotiana* genus have greatly contributed to extensive cytogenetic research. First, *N. tabacum* is a plant of allopolyploidal origin. Because of its economic importance, studies have been conducted to decipher its phylogenetic relationship with possible parent species. The phylogenetic relationships between other species of the genus have been deduced from karyological studies of the number of chromosomes, chromosome pairing and meiosis in hybrid combinations. Second, gene transfer may be performed in an effort to confer disease resistance, immunity to insect pests or new biochemical properties, and the chances of success of such transfers may be calculated.

Male sterility (CMS) is a germplasm character that can be transmitted to the commercial strains of *N. tabacum*; it can facilitate, for example, the production of F_1 hybrids without inducing castration (Delon, 1986). Different forms of male sterility may be distinguished, according to the species used as germplasm donor. In the case of *N. suave-*

Figure 6.2. **EEC tobacco production, 1990**
(tonnes)



Source: TJI No. 3, 1991.

olens or *N. megalosiphon,* CMS may be expressed as feminisation of the anthers (stigmatoids). On the other hand, *N. undulata* causes formation of petaloid anthers and a corolla from which the stigma protrudes. Gerstel made a comprehensive bibliographic survey of cytoplasmic male sterility in 1980.

e) Current phytosanitary considerations in movement of germplasm

Tobaccos may be moved as seed or as plants. Whereas in the case of seed there is virtually no risk of disease or parasite transmission – blue mould (*P. tabacina*), powdery mildew (*E. cichoracearum*), black root rot (*C. elegans*), black shank (*P. parasitica* var. *nicotiana*) are not seed-borne – the risk is high where plants are concerned.

There are no generally applied quarantine rules for importing tobacco seed into a country. Quarantine is advisable, however, to protect against the following:

- wildfire (*Pseudomonas tabaci*);
- anthracnose (*Colletotrichum tabacum*);
- blue mould (*Peronospora tabacina*).[1]

The usual form of disinfection involves soaking seed for 10 to 15 minutes (in a gauze bag) in a 0.1 per cent silver nitrate solution, then drying it in hot air (30-50°C) for 24 hours.

f) Current end use(s)

The best known and most commonly cultivated species of *Nicotianeae* throughout the world is *N. tabacum* and its multitudinous varieties. Tobacco is smoked in cigarettes or cigars, chewed, or taken as snuff. Cultivated tobaccos are classified into five main families according to method of growing, curing and processing:

- flue-cured: a hot air technique is used for curing bright Virginia-type tobaccos;
- light air-cured: bright Burley-type tobaccos are cured by natural ventilation;
- sun-cured: bright aromatic tobaccos are cured in the sun;
- dark air-cured: dark (French-style and cigar) tobaccos are cured by natural ventilation;
- fire-cured: dark (Kentucky-type) tobaccos are cured over fires.

Numerous strains of *N. rustica* are rich in alkaloids, but their cultivation – restricted to North Africa, eastern Europe, Pakistan, etc., – is declining.

Some other species, particularly those belonging to the sub-genus *Petunioides*, are cultivated as ornamental plants.

Tobacco may be used to produce nicotine and employed as an insecticide. The singular properties of tobacco proteins, in particular proteinic fraction I (Rubisco), could rekindle interest in leaf proteins for human and animal nutrition.

Tobacco could also be exploited, after extraction, as a natural source of various chemicals such as malic and citric acids, solanesol and essential oils (Tso, 1990).

Lastly, tobacco is a very valuable plant model in cell and molecular biology research.

Reproductive mechanisms

a) Mode of reproduction

The tobacco flower is hermaphroditic, usually complete and zygomorphous. The androecium is composed of five free stamens normally of unequal length. Anther dehiscence may occur before (*N. rustica*) or after the opening of the corolla. The pistil comprises two welded carpels generally forming two chambers. The style arises from the top of the ovary and terminates in a stigma that may take various forms.

b) Perennial vs annual?

Most *Nicotianeae* species are annual. Some, such as *N. glauca, N. tomentosa,* and certain strains of *N. tabacum,* flower only rarely the first year, or flower too late to produce seeds, except in places where the plants are not killed by frost. These *Nicotianeae* can survive and reproduce only in a greenhouse.

c) Mode of pollination

Tobacco is considered to be an autogamous plant. This is the case with *N. rustica,* in which flowers open only after pollination. If self-fertilisation is to be carried out on *N. tabacum,* it is advisable to cover inflorescences with a protective bag. Among the cross-pollinating agents, wind apparently carries pollen for no more than 20 m; a more

important role is played by bees, bumble-bees and certain butterflies, since the tobacco flower contains abundant nectar. In male-sterile flowers, hand-pollination is necessary.

d) Dispersal and survival mechanism of propagules

In areas of cultivation where sub-zero weather is common during the winter months, tobacco can survive only in seed form, the herbaceous parts being killed by frost.

e) Ability to cross with related species

Industrial tobacco (*N. tabacum*) will not cross spontaneously with species of a genus other than *Nicotiana*. Even within the genus, spontaneous interspecies crossing is extremely rare.

Toxicology

With the exception of nicotine, the principal alkaloid in tobacco, which has been widely studied, little research has been done on changing the amount of a chemical substance present in tobacco leaves, for the simple reason that it is difficult for a geneticist to decide which of the 3 500 constituents of tobacco to increase or reduce. As to nicotine, tobaccos yielding less than 0.1 per cent of nicotine in their dry matter – as against the 2-3 per cent in conventional varieties – have been obtained (Schiltz *et al.*, 1983). Efforts have also been made to reduce the tars produced by tobacco combustion, but not much variation can be achieved.

Environmental requirements for life cycles

a) Climatic restrictions to extension of the crop

The geographic limits on tobacco cultivation lie between 60°N and 40°S. Most producing countries are situated, however, between latitudes 45°N and 30°S. Tobacco is by nature a tropical plant that can be grown in more temperate climates. The vegetation period in these countries varies from 100 to 120 days. Given a near-optimum average temperature of 27°C, the growth period can be brought down to 80-90 days. Varietal improvement is aimed mostly at boosting tobacco's ability to resume growth after being transplanted in the cold, moist soils usually found in temperate regions in springtime.

B. Current breeding practices and varietal development research

a) Description of main breeding techniques

i) Germplasm maintenance

All varieties of *N. tabacum* and *N. rustica* may be maintained in reserve by using bags, thanks to natural self-fertilisation. Short-day types are reproduced in the greenhouse. Auto-fertilisation by hand may be necessary for some species.

ii) Basic breeding

Creative selection of *N. tabacum* relies on inter-varietal crossing and the isolation of a pure strain by self-fertilisation through genealogical selection (pedigree).

Backcrossing and recurrent selection are other techniques that may be applied to tobacco.

Both androgenesis and maternal haploids (obtained by crossing with *N. africana*) shorten the time needed for producing a stable homogeneous strain.

Somatic hybrids have been obtained from various species of the *Nicotiana* genus, and the first transfers of alien genes have been successfully achieved. These may make it possible to develop rapidly varieties of industrial tobacco resistant to specific herbicides (phosphinotricine, bromoxynil, etc.).

iii) Variety development

Step 1. The initial evaluation is carried out at the Tobacco Institute in Bergerac on F_4 to F_6 strains. Each variety is repeated four times in elementary plots of 250-300 plants according to a statistical arrangement in complete blocks. The weight yield is calculated, and a first assessment of the agronomic and physico-chemical properties of the raw material, including a taste test, is made.

Step 2. For later generations, similar trials are conducted on regional locations (in south-western, south-eastern, western and north-eastern France).

Step 3. Semi-industrial cultivation over an area of five to ten hectares provides a clear idea of the new varieties' viability and marketability.

b) Main breeding objectives

The main objectives of varietal improvement are determined by requirements emanating from producers who seek weight yield, disease resistance, harvesting facility, and curing suitability; and from manufacturers and consumers who seek alkaloid content (nicotine, nornicotine, etc.), tar yield, flavour and filling power.

As to disease resistance (fungus, bacterial, viral), the *Nicotiana* genus contains various anti-disease factors that have been successfully transferred (see Table 6.2).

In the case of such chemical properties as nicotine content, which involves two major genes, varieties have been obtained with a dry matter nicotine content of 0.1 to 5 per cent. Nornicotine, a nicotine derivative produced by demethylation, and also commanded by two major genes, has been entirely eliminated from some tobaccos by appropriate selection. Other chemical properties, such as tar yield, flavour precursors, polyphenol content and composition, can be modified through selection.

c) Testing for the most important breeding goals

Various methods for assessing strains during the selection process are available to the breeder:

1. The cotyledon test (Schiltz and Coussirat, 1969): under controlled conditions of light, temperature and nutrition, this method detects the properties of seedlings at the cotyledon stage (10-25 days).

Table 6.2. **Main species used for obtaining improved varietal resistance**

Disease and pathogenic agent	Source species									
	N. tabacum	*N. debneyi*	*N. excelsior*	*N. glutinosa*	*N. goodspeedii*	*N. megalosiphon*	*N. suaveolens*	*N. alata*	*N. longiflora*	*N. plumbaginifolia*
Wildfire *Pseudomonas tabaci*						×				
Blue mould *Peronospora tabacina*		×	×		×		×			
Powdery mildew *Erysiphe cichoracearum*	×	×		×						
Black root rot *Thielaviopsis basicola* = *Chalara elegans*		×								
Black shank *Phytophthora parasitica*	×								×	×
Virosis										
TMV				×						
PVY	×									
TSWV								×		

2. The leaf test (Schiltz and Genève, 1968): this consists of examining the newly formed root system on the stalks of single leaves detached from greenhouse plants. It is a way of assessing the development of the root system, checking whether it has been attacked by a parasite and, if so, measuring the extent of the infestation, thereby diagnosing the tobacco's reaction to the parasite.
3. Greenhouse or controlled atmosphere tests: in dealing with certain diseases – black root rot, black shank, viroses (PVY, VMT) – analyses can be performed by minibed cultivation in greenhouses or in controlled atmosphere rooms regulated for studying fungus or viral parasites.
4. Outdoor tests: the ultimate test is outdoor confirmation. In the case of diseases (blue mould, powdery mildew), it is especially important to select plots suited to natural inoculation. The degree of resistance is calculated by checking against a damage assessment chart, such as the ones devised by CORESTA for blue mould and powdery mildew.

d) Assessment of general performance

The final assessment of varieties is carried out in the open air by cultivation in plots of 250-300 plants, replicated four times according to a whole block system.

In the field, the plants' morphology (size and leaf implantation, shape and number; see Figure 6.3), budding performance, and flowering date, are analysed.

After harvesting and curing, the weight yield and quality index are determined, along with a number of characteristics. Some are physical, such as filling power, combustibility; others are chemical, such as total alkaloids (nicotine, nornicotine), total nitrogen, reducing sugars (Virginia), nitrates (Burley), and tar yield as calculated by a smoking machine.

Taste testing by a panel of experts is the final measure of whether a new variety has potential or not.

C. Seed multiplication for commercial use

a) Stages in seed production

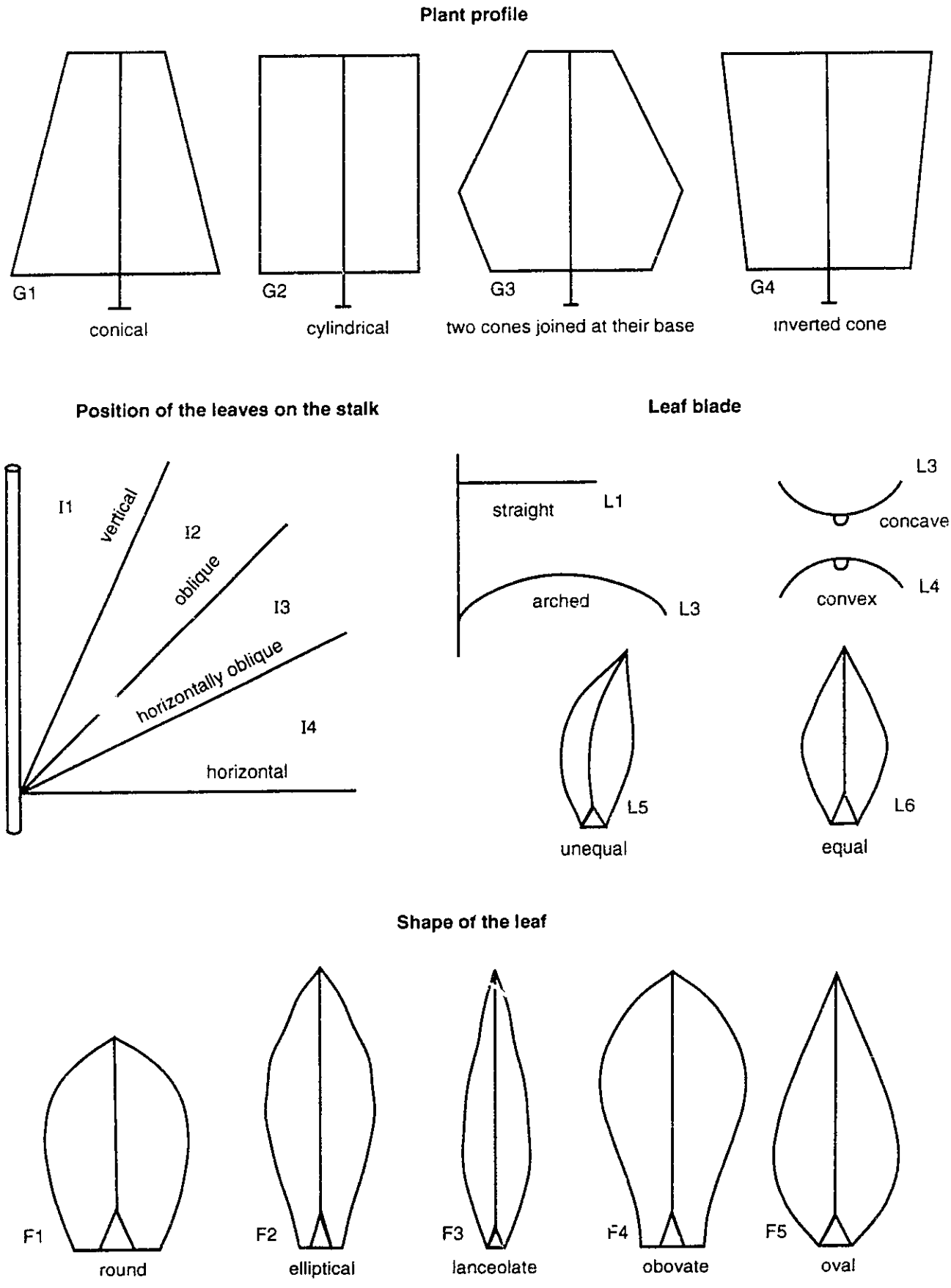
In view of their reproduction ratio of 150 000, tobaccos do not require large areas for seed production, and a single generation (or reproduction) is enough to go from seed stock to commercial quantities.

Reproduction in France is organised either by the Tobacco Institute in Bergerac (ITB) or by tobacco growers who have the requisite technique and land situated away from commercial tobacco cultures.

b) Isolation practices

Where the fertile varieties are concerned, the seed garden must lie at least 40 m away from commercial tobacco fields, except where seed heads are enclosed in bags and topping is done to prevent the neighbouring cultures from flowering.

Figure 6.3. **Plant morphology**



The tip of the leaf: very acute, acute, slightly acute, rounded.
The base of the limb: sessile, petiolate, narrow, winged, decurrent.

In the case of male-sterile F_1 hybrids, the male-sterile beds must lie no more than 50 m from fertile strains.

c) Role of seed certification and registration

While France still does not have an official catalogue of tobaccos, production is highly organised, and the Tobacco Institute in Bergerac carries out varietal tests (identification, purity, freedom from disease) and seed quality tests. Seed must meet the following minimum standards:

- purity, 99 per cent;
- germinating power, 80 per cent.

Germinating power is determined using 2 × 100 seeds exposed to light on wet blotting paper at 27°C in conditions of maximum humidity.

d) Lifespan and market spread

Varietal renewal is somewhat slow. Some varieties such as Dragon Vert, Paraguay and Nijkerk have been cultivated since 1870. The tobacco industry nevertheless takes a close interest in varietal development, particularly as it affects yield and quality. Fifteen or so different varieties are grown today in France (see Table 6.3), chief among them being the blue mould-resistant dark tobacco variety PB D6, first cultivated in 1968.

Table 6.3. **Tobacco culture in France by variety**

(estimated percentage)

Variety		1987	1988	1989
Dark tobaccos		9 612 ha	8 517 ha	7 573 ha
comprising:	• PB D6	86	84	92
	• Paraguay	6	5	3
	• ITB 19	2	2	1.6
	• Nijkerk	2	1.5	1.6
	• Dragon vert	1	0.7	1.0
	• GDH	3	6.8	0.4
	• Other	–	–	0.4
Burley		1 353 ha	1 139 ha	1 190 ha
comprising:	• BB 16	59	37.6	40
	• BB 16 A	23	31.7	36
	• B 217	15	30.7	24
Virginia		3 405 ha	3 013 ha	2 650 ha
comprising:	• Virgin D	97	89	85
	• MN 944	2	10	11
	• ITB 30	–	–	3
	• K 326	–	–	1
	• Other	1	1	–

The current trend away from dark tobacco towards Burley and Virginia provides the opportunity for developing new varieties, usually F_1 MS hybrids, some of which are covered by patents (ITB 1000, ITB 2001, ITB 2201).

Note

1. Seed-borne transmission of *Peronospora tabacina* is a highly controversial subject. The shipping of seed from countries where blue mould exists to others that are uninfested is regulated under the guidance of CORESTA (Centre de Coopération pour les Recherches Scientifiques Relatives au Tabac, 53, Quai d'Orsay, 75347 Paris Cedex 07, France). Treatment of seed is usually the minimum precaution taken.

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7. Tomato

by

Jacqueline Philouze and Louis Hedde

A. Characteristics of the crop

a) Geographic origins; centre of diversity

Wild species of tomato (genus *Lycopersicon,* Solanaceae family) originated in the western part of South America, in an area stretching from southern Colombia to northern Chile and from the Pacific coast (including the Galapagos) to the western foothills of the Andes, with some species growing at an altitude of up to 3 400 m. The cultivated tomato in its wild form is the *L. esculentum* var. *cerasiforme,* which originated in the same area. Unlike the eight other species of wild tomato which have remained within the confines of their native area, *L. esculentum* var. *cerasiforme* spread extensively into every tropical and subtropical part of America, even as far as Texas and Florida. One hypothesis, now widely accepted, is that the tomato became domesticated in Mexico, but it is not known when this took place. The tomato was introduced to Spain from Mexico in the first half of the 16th century, then moved from Spain into other European countries and on to other continents.

b) Geographic distribution of use: main production areas

For a long time, the tomato was viewed with suspicion. It was thought to be toxic like other species of Solanaceae (*e.g. Belladonna,* nightshade), and for a long time was cultivated for its ornamental or curiosity value. This deep-seated prejudice persisted until the 18th century, when the plant began to be grown as a crop. It took on more importance in the 19th and then the 20th century. The tomato is now cultivated in every country and at every latitude from the equator virtually to the polar circle. It is the most widely eaten vegetable in the world. Global output increases regularly from year to year, rising from 48 million tonnes in 1978 to 56 million tonnes in 1983 and 64 million tonnes in 1988 (FAO, 1989). Table 7.1 shows output in the top ten producer countries, and in the six EEC member states with the highest output.

Table 7.1. **World tomato output in 1988**
(the ten leading producer countries)

Origin	Surface area (× 1 000 ha)	Yield (t/ha)	Total output (× 1 000 t)
World	2 669	24.0	63 988
United States	166	50.0	8 301
USSR	400	18.0	7 200
China	341	16.1	5 474
Turkey	140	37.5	5 250
Egypt	172	29.1	5 000
Italy [1]	116	39.9	4 643
Spain [1]	61	42.6	2 596
Brazil	62	38.4	2 378
Romania	75	30.7	2 300
Greece [1]	35	54.6	1 929
Portugal [1]	16	53.1	865
France [1]	13	57.6	743
Netherlands [1]	2	239.1	550

1. One of the six main producer countries in the EEC.
Source: FAO (1989).

c) *Taxonomic status*

The genus *Lycopersicon* contains only nine species, namely the cultivated tomato *L. esculentum* (and its wild form *L. esculentum* var. *cerasiforme* with its small fruit, easily crossed with the tomato), together with eight wild species, all with small fruit:

- *L. pimpinellifolium,* with red fruit;
- *L. cheesmanii,* with orange fruit;
- *L. hirsutum*
- *L. parviflorum*
- *L. chmielewskii*
- *L. chilense*
- *L. peruvianum*
- *L. pennellii*

with green fruit

It is unknown whether there are any phylogenic links between these species. The hypothesis that *L. pimpinellifolium* might be an ancestor of *L. esculentum* is no longer accepted. The two species apparently derive from common ancestors situated further back. Molecular biology studies are expected to produce sounder hypotheses over the next few years.

d) *Genetic and cytogenetic characteristics*

The cultivated tomato, like other species of the genus *Lycopersicon,* is a diploid species with 2n = 24 chromosomes.

Silvering on the leaves of the tomato is due to cytoplasmic heredity. No other traits with similar heredity are known.

e) Current phytosanitary considerations in movement of germplasm

Pathogenic agents such as the tobacco mosaic virus, and the bacteria *Corynebacterium michiganense, Pseudomonas tomato* and *Xanthomonas vesicatoria,* which can cause extensive damage in tomato crops, are transmissible through seed. Treating seed in dry heat (24 hours at 80°C) or, better still, when dry or wet with sodium hypochloride (bleach), are effective cleansing methods.

In France, other diseases developed when, for instance, young tomatoes raised in contaminated compost were imported from nearby countries. This was the case with *Fusarium* infesting the roots (*Fusarium oxysporum radicis lycopersici*), which forced many glasshouse growers in the south of France to make a complete changeover to new varieties. Finally, there has been a rapid spread in several animal parasites (*e.g.* glasshouse whitefly *Trialeurodes vaporarorium,* the leaf miner *Liriomyza* ssp.) caused by the introduction of a range of different vegetable or floral species, but also by the development of glasshouses where these very harmful pests can survive the winter. In the Mediterranean basin, the worst of them can be vectors of viral diseases, and examples include aphids [vectors of potato virus Y (PVY), and cucumber mosaic virus (CMV)], the thrips *Frankliniella occidentalis* [vector of tomato spotted wilt virus (TSWV)] and the whitefly *Bemisia tabaci* [vector of tomato yellow leaf curl virus (TYLCV)].

f) Current end uses

Tomatoes are grown to be eaten fresh or processed. Industry offers a wide variety of preserved products including tomato paste, juice or ketchup, and peeled, crushed or powdered tomatoes.

Reproductive mechanisms

a) Modes of reproduction and pollination

Cultivated tomatoes, like the wild species of *Lycopersicon,* are multiplied using seed propagation. Although cuttings can be taken, in practice they are not, since the cost is higher than for seed propagation and the health of cuttings taken from adult plants often leaves much to be desired.

In the cultivated tomato *Lycopersicon esculentum,* the staminal cone completely encloses the pistil, and the stamens enclose the downward-facing stigma. Stamen dehiscence takes place through two lengthwise slits inside the staminal cone. Pollen is released precisely when the stigma is at its most receptive, and the flower's structure ensures strict self-fertilisation.

b) Perennial or annual

The cultivated tomato is considered an annual because it is sensitive to frost. Wild species are herbaceous annuals or short-lived perennials in their area of origin. They too are sensitive to frost.

c) Dispersal and survival mechanisms of propagules

In temperate countries, the cross-fertilisation rate is very low, at around a few per thousand, because of the flower's structure and the absence of nectar; few insects are likely to carry the pollen.

In tropical or subtropical countries, two factors combine to give a cross-pollination rate of a few per cent which may cause problems for breeders. First, the style's length depends on its environment and may be longer than the stamen cone; second, the presence of highly active insects ensures cross-pollination (*e.g. Exomalopsis* ssp. in the French Antilles). It is therefore advisable to put inflorescences into cellophane bags for controlled self-fertilisation or hybridisation.

Growers, in particular those using glasshouses, are seeking to improve fruit-setting in tomatoes. Consequently, inflorescences with flowers in full bloom are given a short (one-second) vibration to help them to release their pollen (when there is too little, or relative humidity is too high). This is done three times a week using an electric vibrator. For some years now, bumble-bees, *Bombus terrestris*, have been used for the same purpose, much to the satisfaction of glasshouse growers who obtain colonies of the bees from specialist suppliers. There is a very slight risk that some bees might escape from a glasshouse and visit nearby tomato plots, and this cannot be ruled out altogether.

If certain wild species of *Lycopersicon* are strongly autogamous, others are allogamous and, in some cases self-incompatible. Allogamy is ensured by the presence of pollinating insects in the area of origin and by the flower's structure, with a style much longer (up to 5 mm) than the stamen cone.

d) Ability to cross with related species

Interspecific cross-breeding only works if *L. esculentum* is used as the female. Some crosses are easy to obtain (with *L. pimpinellifolium, L. cheesmanii, L. chmielewskii, L. parviflorum, L. hirsutum, L. pennellii).* With *L. peruvianum* and *L. chilense* they are much harder; one way of overcoming this breeding obstacle is to grow immature F_1 embryos.

In nature, spontaneous crosses are only conceivable with *L. esculentum* var. *cerasiforme* in tropical and subtropical regions, and with some wild species in the area where the genus *Lycopersicon* originated.

Toxicology

Green tomatoes contain a low concentration of tomatin, an alkaloid that tastes bitter but disappears upon ripening. However, the fruit of certain tomatoes close to the wild form (*e.g.* "tomadoses" in the Antilles) still contain a little tomatin even when ripe. The alkaloid can easily be bred out.

a) Environmental requirements for life cycles

The best temperature for tomatoes ranges from 15° to 18°C at night and 20° to 25°C during the day. Pollen meiosis is completely disrupted at temperatures of over 33°C or under 7-10°C (depending on the variety). Plants stop growing when the temperature falls below +10°C.

Tomatoes are insensitive to the photoperiod. However, the species demands quite a lot of light, particularly when flowering begins. Breeders in northern Europe, especially in the Netherlands, have bred plant material adapted to short, dull days, and so can breed cultivars capable of bearing fruit under glass all year round, with very high yields.

B. Current breeding practices and variety development research

a) Main breeding techniques

i) Germplasm maintenance

The various genotypes of *L. esculentum* are maintained through self-fertilisation. For wild species, many of which are allogamous or even self-incompatible, it takes around ten plants to obtain one sample; each plant, taken individually as a female, is pollinated by hand with a mixture of pollen from the ten other plants. This is to make it as certain as possible that the variability and heterozygosity of the initial sample is maintained.

ii) Basic breeding

All tomato cultivars currently available were developed using controlled hybridisation and selection of the best plants in separate generations. These are the classic methods for improving an autogamous plant when good F_1 hybrids are increasingly being sought. The pedigree method is by far the most widespread: it is an efficient way to fix characters whose genetic determination is simple. These are numerous in the tomato and they help to eliminate genotypes with defects rapidly, especially those relating to fruit appearance, which would make it impossible to cultivate a particular variety. The backcross method has been very useful, since many vital characteristics in the tomato are monogenic. Single Seed Descent (SSD) is used to improve characteristics with low heritability (such as the fruit's soluble dry-matter content). Finally, recurrent selection, alternating between cross-bred and self-fertilised generations, is a way of combining genes that favour a given characteristic determined by a number of genes. The various methods supplement one another and may be alternated as part of a breeding programme.

iii) Variety development

The best lines can be propagated and distributed as true-breeding cultivars/varieties maintained by self-fertilisation.

Today, however, cultivars are increasingly F_1 hybrids. Many F_1 hybrids are tested and the general and specific aptitude of the lines to combine is evaluated. Seeds from F_1 hybrid cultivars are obtained manually, once flowers on the female parent have been castrated and pollinated with pollen from plants selected as males.

b) Main breeding objectives

The main objectives concern adaptation to different environments and growing methods, fruit quality, and resistance to parasites. Wild species of *Lycopersicon* have proved to be extremely valuable in all these respects. Rick (1986) sums this up very well.

Adaptation goals are met by breeding varieties that are well-adapted to various types of cultivation. Plants may be grown in the open field, whether staked or not (in which

case there will be a single harvest or several harvests), or grown under cover, *i.e.* under plastic or glass, with or without heating, and in the soil or in artificial media.

Breeding varieties adapted to a range of different growing conditions and to a range of sowing dates naturally requires the right breeding sites. Breeders therefore work in many stations across the world, wherever climatic conditions most resemble the targeted growing area. The genetic material used for breeding is therefore scattered world-wide in a wide range of areas. Knowledge of how this material will behave and react under widely differing environmental conditions is crucial if new varieties are to be a guaranteed success.

Breeders look for varieties adapted to extremely varied climate and soil conditions, particularly those which are constraints for today's growers. Work in the Netherlands has made it possible to grow tomatoes under glass all year round, thanks to new varieties bred to adapt to short, dull days. Work has been undertaken in several countries to increase adaptation to both high and low extremes of temperature. Higher-altitude ecotypes of the wild species *L. hirsutum* are particularly valuable parents where resistance to low temperatures is concerned (seed germination, growth, setting of fruit). The species *L. cheesmanii* is commonly used as a parent to breed plants that tolerate salinity.

For the fresh produce market, work has been undertaken to improve not only the appearance (colour, shape, size) but also the firmness and shelf life of tomatoes. Firmness and longer storage potential are increasingly required for varieties transported over long distances. Such varieties can now be found on the market. The genetic structure for long storage potential is complex and appears to be semi-dominant polygenic. Some hybrid varieties also possess single non-maturation genes (*rin* = ripening inhibitor, or *nor* = non-ripening) which enable heterozygous hybrids with these genes to mature more slowly. Unfortunately, such genes do not have a very favourable effect on taste.

It is possible to breed higher vitamin content (vitamin C, beta-carotene) into a variety, and taste can also be improved. But such work must be economically viable, and traders and consumers will have to pay prices that offset the probable loss of yield per hectare. The only examples currently on the market are cherry tomatoes, a very small market, and industrial tomatoes with a high dry-matter content. Here, the notion of yield in tonnes per hectare is gradually being replaced by that of dry-matter per hectare.

Since tomatoes are grown in a wide variety of environments, often under conditions that are far from ideal for their development, they can be affected by a very large number of pathogenic agents. Almost 200 diseases have been listed throughout the world. Wild species have proved to be an extremely valuable source of resistance to parasites; in fact, they have provided every form of resistance bred into cultivated tomatoes to date. Various forms of resistance found in wild species and currently used in breeding programmes are listed in Table 7.2.

c) Testing for important breeding goals

Tests on resistance to disease (artificial inoculation tests) and to various forms of stress (*e.g.* seed germination, growth, low temperature pollen-formation) are carried out in air-conditioned chambers. Breeding and agronomic tests are carried out in the glasshouse and in the open field.

Table 7.2. **Parasite resistance bred into tomato cultivars**

– L. pimpinellifolium		
O +++	I	*Fusarium oxysporum* f. sp. *lycopersici* pathotype 0 (ex race 1)
O ++	I-2	*Fusarium oxysporum* f. sp. *lycopersici* pathotype 1 (ex race 2)
O +++	Ve	*Verticillium* ssp. pathotype 0 (ex race 1)
	–	*Verticillium* ssp. pathotype 1 (ex race 2)
O ++	Cf-2	*Cladosporium fulvum* (= *Fulvia fulva*)
O +	Cf-5	*Cladosporium fulvum* (= *Fulvia fulva*)
O +	Cf-6	*Cladosporium fulvum* (= *Fulvia fulva*)
O +	Cf-9	*Cladosporium fulvum* (= *Fulvia fulva*)
O +++	Sm	*Stemphylium* ssp.
O +	Ph-2	*Phytophthora infestans*
O ++	Pto	*Pseudomonas tomato*
O ++	–	*Pseudomonas solanacearum* (tropical countries)
	–	*Corynebacterium michiganense*
O +	–	Tomato spotted wilt virus (TSWV)
– L. cheesmanii		
	–	*Liriomyza* ssp.
– L. hirsutum		
O +	Tm-1	Tobacco mosaic virus (TMV)
O +	Cf-4	*Cladosporium fulvum*
	–	*Oidium lycopersicum*
	–	*Corynebacterium michiganense*
– L. peruvianum		
O ++	Frl	*Fusarium oxysporum* f. sp. *radicis lycopersici*
O +	pyl	*Pyrenochaeta lycopersici*
O +	Cf-4	*Cladosporium fulvum*
	Lev	*Leveillula taurica*
O +	Tm-2	Tobacco mosaic virus (TMV)
O +++	Tm-2[2]	Tobacco mosaic virus (TMV)
O +	–	Tomato yellow leaf curl virus (TYLCV)
	–	Tomato spotted wilt virus (TSWV)
O +++	Mi	*Meloidogyne* ssp.
– L. pennellii		
	1-3	*Fusarium oxysporum* f. sp. *lycopersici*, pathotype 2 (ex race 3)

O Resistance bred into marketed cultivars, indicating current importance (+, ++ or +++) in crop-growing and gene symbol if identified.
– Resistance originally found in the wild species as named.

d) Assessment of general performance

Sets of marks have been developed for tests on resistance to disease. Glasshouse and field trials are monitored throughout the growing period, fruits are harvested, weighed and counted and their appearance or chemical composition is analysed (dry-matter content and acidity for industrial varieties).

C. Seed multiplication for commercial use

a) Stages in seed production

Seed propagation for true varieties and seed production for F_1 hybrids are carried out by a large number of private companies throughout the world. The total quantity of tomato seeds used world-wide every year ranges approximately from 300 to 500 tonnes, and 30 per cent of this is F_1 hybrid seed. It is difficult to state exactly how much land is covered by the crop, but a likely estimate is about 3 000 ha.

Seed production areas are found all over the world – in Europe, North and South America and Asia.

F_1 hybrid seed is produced by specialist firms, and for a long time certain countries like Bulgaria and Taiwan were pioneers in this field. Today, production areas for F_1 hybrid tomatoes are now much more widespread, in Asia, South America and under glass in northern Europe.

b) Isolation practices

Since the tomato acts like an autogamous species, production plots do not have to be isolated. Accidental cases of natural cross-fertilisation may occur, but the rate never exceeds 1 per cent. Seed breeders and producers must check the seed of true and F_1 hybrid varieties for compliance and good germination.

c) Role of seed certification and approval

In most developed countries, particularly in Europe and the United States, any authorisation to market seed is subject to the inscription of the new varieties on national or Community lists. The purpose of official inscription trials is to provide users with a guarantee that the variety is stable, homogenous and distinct from earlier varieties. However, inclusion of a varicty on a list is not an automatic acknowledgement of its agronomic value. For garden seed, there is no certification by bodies outside the production company. Each company assumes full responsibility towards its customers.

Tomato-seed breeders and growers are able to guarantee the quality of what they produce by complying with a whole series of controls. These apply to seed plants (basic seed) and commercial seed. For F_1 hybrids, the process is as follows:

For each parent line:

- homogeneity and resistance to disease is checked;
- breeders' seed is produced from a given number of lines (10 to 15) to prevent variance or genetic drift;
- foundation seed production is controlled using breeders' seed;
- plant checks, phytotests and RFLP on foundation seed.

Hybrid seed production:

- controls on production teams and fields;
- controls on harvested seed;
  - germination;
  - absence of seed-transmitted parasites;
  - occasional or systematic disinfection;

- purity checks: self-fertilisation, accidental cross-fertilisation, risk of mixing;

– identifying a variety:
  - checks on plants;
  - RFLP (restriction fragment length polymorphism).

Commercial tomato seed is not subject to the official certification regulations laid down by the authorities, but must comply with minimum quality standards set within the trade.

d) Surveillance of variety; lifespan after commercial release

Before they are launched onto the market, new varieties are given two years of outdoor trials by growers in order to check varietal characteristics in the field and determine the most appropriate growing methods. The information is then sent out to breeders who work with development specialists in seed companies and technicians in extension institutes and producer associations.

Once a variety is on the market, information exchange continues between the breeder, sales and marketing teams, crop technicians and producers. This collaboration is essential if new varieties are to be successful and breeders are to take on board the many new potential problems raised by crop adaptation (new growing techniques), quality (adapting to consumer markets) and plant health (new diseases).

Owing to extreme diversity in growing conditions and consumer markets, the tomato is certainly, among the market-garden species, the one with the greatest number of varietal types, be it for fresh produce or for the processing market. New varieties may appear very suddenly, and some varieties have a very short life span, particularly those grown in highly specialised production areas: these include Europe's hothouse varieties or export crops in Spain and Morocco, for instance.

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8. Sunflower

by

Felicity Vear and Jerry Miller

A. Characteristics of the crop

a) Geographic origins; centre of diversity

Helianthus annuus L., the cultivated sunflower, is one of the few crop species that originated in North America, in the southwestern states of the United States. It was first grown by the Indians, who used it as a source of edible oil. Sunflowers were introduced into Europe through Spain in the 16th century. It is known that they were used as food in Russia by 1779, and intentional breeding for oil content had started by 1860. Since then, sunflowers, used both as a source of oil and for confectionery purposes, have spread to almost all parts of the world, from the tropics to northern production areas in Canada and Russia.

Wild *Helianthus annuus* and its close relatives originated in North America. The principal centre of diversity of cultivated sunflower was Russia, but the type of old cultivated material, heterogeneous populations grown in widely different environments, led to considerable genetic drift, and secondary centres of variation have developed in Europe, Argentina, North America, North Africa, China and Australia.

b) Geographic distribution of use

Sunflower is the most important annual species grown specifically for its oil and is now the second leading hybrid crop grown in the world. In 1989, world production was about 21.6 million tonnes, on about 15.7 million ha. The main producers are Russia, Argentina, the United States, China and the European Community. In the EC, France and Spain each grow about 1 million ha. There are also significant areas in Australia, South Africa, Turkey, India, and many eastern European countries. The mean world yield is about 13 q/ha.

c) Taxonomic status

The genus *Helianthus* is composed of 67 species, mostly originating in North America, but a few originate in South America. The genus is divided into four groups (Heiser, 1978):

Annui: The cultivated sunflower, *H. annuus*, is found in this group. The 13 species are all diploid (2n = 2x = 34) annuals, originating in the southwest United States. Wild *H. annuus* is found, often as a weed, west of the Mississippi. It is 1 to 3 m high, branched and shows a great deal of variability. Sunflower can be hybridised with all the other species in the section. Those of most interest include: *H. petiolaris*, *H. bolanderi*, *H. praecox*, *H. neglectus* and *H. anomalus*, which are sources of cytoplasmic male sterility, and *H. argophyllus* and *H. anomalus*, which are resistant to drought.

Divaricati: The 30 perennial species, found in the eastern and central United States, spread and perpetuate by means of rhizomes or tubers. There are diploid (2x), tetraploid (4x) and hexaploid (6x) species. *Helianthus tuberosus*, the Jerusalem artichoke, belongs to this group. Some species, such as *H. occidentalis*, *H. rigidus* and *H. resinosus*, show disease resistance which it would be useful to introduce into cultivated sunflowers.

Ciliares: The six species are herbaceous perennial plants, found in Mexico and the western United States. They have deep or spreading roots. Chromosome number is 2n = 34, except for *H. ciliares*, for which tetraploid and hexaploid types are known.

Fruticosi: The 17 species found in South America are closely related to another genus, *Viguira*, and are quite different from the North American species. These species are classified in the genus *Helianthus* because of their involucre bracts in the form of scales. The plants are perennial and shrubby. Chromosome number is 2n = 34 (diploid) for the species studied.

d) Genetic and cytogenetic characteristics

Research on sunflower genetics is relatively recent (1960 onwards), and no classic chromosome map has been produced. Few linked genes are known, the main exception being a recessive gene giving male sterility, linked with only 1 per cent recombination to a gene controlling anthocyanin production (Leclercq, 1966). Chromosome mapping is now in progress, using both morphological and molecular markers.

The main cytoplasmic trait known and used is cytoplasmic male sterility (CMS). The first source, obtained from a cross between *H. petiolaris* and cultivated sunflower by Leclercq (1969), is used throughout the world for commercial hybrid production. From other interspecific or intraspecific crosses, about 20 other CMS have been obtained (Serieys and Vincourt, 1987). They constitute an insurance should any specific problem arise with the first CMS. Studies are in progress to determine their possible agronomic interest.

e) Current phytosanitary considerations

There has been considerable movement of germplasm in the last 20 years, including distribution of collections of genetic resources developed in all parts of the world and of modern genotypes from the main research centres in Europe, North and South America, South Africa and Australia.

Since sunflower is a crop of recent origin in many countries, phytosanitary measures to protect against introduction of disease are of great importance. The main disease concerned is downy mildew, *Plasmopara helianthi*, which probably moves between

countries on seed, although at a low frequency. This parasite originated in North America, and different races are known there and in Europe, South America, Africa and Asia. Efficient seed treatment is now available (metalaxyl) and it is important that it be used. Australia has a quarantine system.

Another pest which could be imported with seed is caused by the parasitic plant, *Orobanche koumana*, commonly known as broomrape. This is found mainly in eastern Europe and Spain. Many insect pests are found in the United States, and it is not known whether they could spread to other sunflower growing areas.

f) Current end uses

The most important use of sunflower is for its oil, which constitutes about 50 per cent of the seed. This oil is rich in polyunsaturated linoleic acid and is of high nutritional value, both as oil and margarine. At present, there is also great interest in a new oil type, which originated from a mutation in Russia and has up to 87 per cent oleic acid (mono-unsaturated). This oil could replace olive oil at a lower cost, but is of particular interest for industrial purposes, including lubricants, pharmaceuticals and cosmetics.

The seedcake or meal left after oil extraction can contain up to 40 per cent protein, depending on whether or not the seed is dehulled before oil extraction. Sunflower meal is lower in lysine, but higher in methionine than soybean meal. When not dehulled, it can be used as a ruminant supplement, but there is now increasing interest, especially in Europe, in dehulling, which provides a product that can be used in non-ruminant feeds.

Sunflower seed is commonly eaten as a snack, in particular in eastern Europe and North America. Special, large seeded, low oil, confection types have been developed in the United States, Spain and China. They are sold either whole or dehulled, for uses similar to those of peanuts.

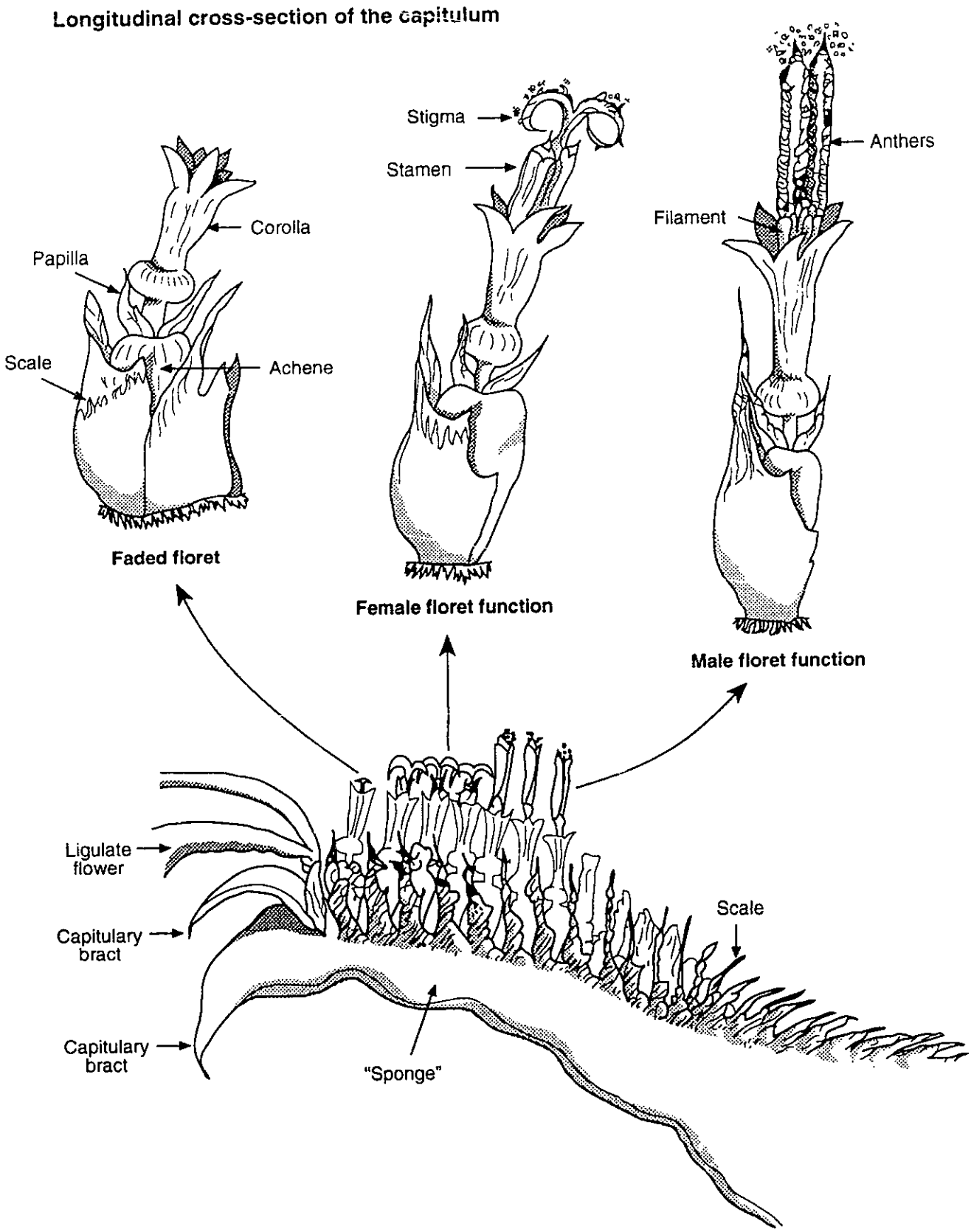
Reproductive mechanisms

a) Mode of reproduction

The sunflower flowers or florets are grouped on a head or capitulum. Until flowering, the terminal bud shows heliotropism (movement to face the sun throughout the day), but once flowering starts, the movement stops, with single headed types facing to the rising sun. The flowers are hermaphrodite, with both male organs (stamens) and female organs (pistils) (Figure 8.1). Sunflowers are cross-pollinated, with a complex system of sporophytic self-sterility. In wild forms of both *H. annuus* and other *Helianthus* species, cross-pollination is obligatory, whereas in cultivated types, self-pollination is common, if self-fertility mechanisms are incorporated into the genotype. The open-pollinated varieties were highly self-sterile since this was, in fact a favourable character: sunflowers show hybrid vigour and a good population was a mixture of vigorous hybrid plants. In order to maintain this vigour, there had to be a majority of cross-pollination, so that few plants showed inbreeding depression.

The effort to create hybrid varieties in modern breeding programmes required homozygous, inbred parental lines that could be maintained. This was a radical change, since it required selection of self-fertile genotypes. Nevertheless, hybrid vigour is still the objective, and cross-pollination occurs in the commercial field.

Figure 8.1. **Cross-section of a sunflower capitulum with details of the florets at different stages**



Source: Lamarque *et al.* (1984).

b) Perennial vs annual

No perennial sunflower has been developed. It should be noted that the Jerusalem artichoke, *Helianthus tuberosus,* is a perennial crop in the genus, and tubers are harvested for fodder and human consumption. Perennial *Helianthus* species can cause some problems in breeding fields. Once introduced, their rhizomes are difficult to eradicate (unless they are grown in some form of container).

c) Mode of pollination

Sunflowers are pollinated by insects, mainly by honey-bees and bumble-bees (*Apis mellifera* and *Bombix* ssp.) In areas where there are natural woodlands, for example, wild bees are sufficient. Elsewhere, hives of honey-bees may be introduced into sunflower fields, especially in the production of hybrid seed. Bees are attracted not only by the bright colour of the ligular florets (often known as ''petals''), but also by nectar and various aromatic compounds produced by the plant.

d) Dispersal and survival of propagules

The sunflower seed is an achene (dry indehiscent fruit). The cultivated type is often distinguished as the botanical variety ''macrocarpus''. The seed is very large, with a weight of 30 to 100 g per 1 000 seed. In contrast, the wild species have much smaller seeds, which very rarely reach 10 g per 1 000 seed.

Cultivated sunflowers have lost their dispersal mechanisms, and seed remain on the head at maturity. Incomplete feeding by birds may drop the seed on the soil. Most wild sunflowers shatter their seed at maturity, and seed may be spread over short distances by feeding animals to whose coats the rough achene surface attaches. Cultivated sunflower seed remains viable in the soil for five to ten years. With a normal dormancy period of three months, seed germinates when brought to the surface by ploughing. Wild sunflower seed probably lasts even longer in the soil and shows irregular dormancy, so that not all seeds germinate at one time.

e) Crosses with related species

As mentioned in the section on taxonomy, wild and cultivated *Helianthus annuus* can cross with all the other species of the *annui* section of the genus, but at a low rate.

The most common crosses would be between cultivated sunflower and the wild *H. annuus* and *H. petiolaris* species. Crosses with *H. argophyllus* are also possible; however, this species and cultivated sunflower rarely grow in proximity. Spontaneous crosses between wild species and cultivated sunflower are easy to eliminate, since they show the dominant branching of wild types.

Artificial crosses of cultivated sunflower with the perennial species are now becoming possible through the use of *in vitro* culture techniques, but such crosses do not occur in the wild. Artificial crosses with other species in this section generally produce a few seed.

Toxicology

No particular toxicants are known in sunflowers.

Environmental requirements for life cycles

a) Climatic restrictions to extension of the crop

Sunflowers require a minimum temperature of about 6°C for growth. The crop will support a slight frost when it is less than one month old. It is grown as a winter crop in mild areas and as a summer crop in temperate zones. If physiological maturity is reached, frosts before harvest can be an advantage, giving rapid death and drying and halting any pathogens. This often occurs in the northern United States, Canada and Russia.

To extend the crop in hot, dry areas, breeding programmes aim at improving drought resistance. This may either be direct, by selecting for tolerance to restricted water supply during growth, or indirect, by selection for growth at low temperatures, so that the crop can be sown earlier and escape summer droughts. The latter selection would also help to extend the crop to cool temperate areas.

b) Biological restrictions to extension of the crop

Sunflower shows little photoperiod dependence, and can be grown from the equator to at least 50°N or S.

B. Current breeding practices and variety development research

a) Main breeding techniques

i) Germplasm maintenance

The first sunflower varieties were heterogeneous, open-pollinated populations maintained by farmers. Then, from 1920 to 1970, more characterised populations were bred, especially in Russia, at the VNIIMK station at Krasnodar. The best known were VNIIMK 6540, VNIIMK 8931 and Peredovik. There were also Argentinian, French, Canadian and Romanian varieties. All these populations constituted the main genetic resources of cultivated sunflower until the mid-1970s. They are maintained by breeders either in isolated plots (where pollination is carried out by bees) or by a series of sib-crosses under paper tubes or cloth bags, with mixture of the seed of all plants harvested in order to maintain variability.

ii) Basic breeding methods

Mass or recurrent selection programmes permit improvement of basic breeding populations by increasing the frequencies of favorable genes. Then, to obtain inbred lines which are parents of hybrids, pedigree selection is used.

Mass selection: In mass selection, each selection cycle represents only one generation. Plants, grown in an isolated plot, are allowed to intercross, and those that should be retained are selected according to their phenotype, that is, their appearance. Such a method can be efficient for characters that can be observed before flowering, so that the uninteresting plants can be eliminated before they produce pollen.

Recurrent selection: One cycle of selection involves two or three generations, with two steps: intercrossing of material and testing of the progenies obtained. The first

programmes of recurrent selection on sunflowers were developed by Pustovoit, following what he called the "method of reserves". This method was based on the studies of offspring and the creation of new populations from the remaining seed of the best individuals. This system is efficient when there is good genetic variability and a high level of heritability (good prediction from one generation to the next). It is used for oil content and capitulum resistance to *Sclerotinia*, for example (Vear and Tourvieille, 1984).

With the development of hybrids, recurrent selection for yield is more complex, since it is necessary to make hybrids in order to determine the combining ability of inbred lines. The yield of a hybrid is not correlated with that of its parental lines. The system used at INRA in Clermont-Ferrand is given in Figure 8.2*a*.

Pedigree selection: This method is used to obtain fixed, homozygous lines from recurrent selection programmes and to combine interesting characteristics from complementary lines. In the latter case, crosses between two male-fertile lines are made, either using emasculation by gibberellic acid (possible on a small scale only) or by crossing two male-fertile plants and distinguishing the F_1 hybrids from the selfed inbreds by their vigour. Plants are self-pollinated at each generation by covering capitula with paper or cloth bags until they show complete fixation. Each progeny is followed separately, and selection can be practised at each generation. An example of this method is given in Figure 8.2*b*.

For the potential female lines, CMS must be introduced by backcrossing. This takes six to seven generations. Since it involves considerable work, it is usually started only after a test for combining ability has been completed. It is possible to accelerate this phase, using *in vitro* culture of immature embryos (Alissa *et al.*, 1986). In this way, it is possible to complete four to five generations per year, instead of the usual two or possibly three.

iii) Variety type: hybrids

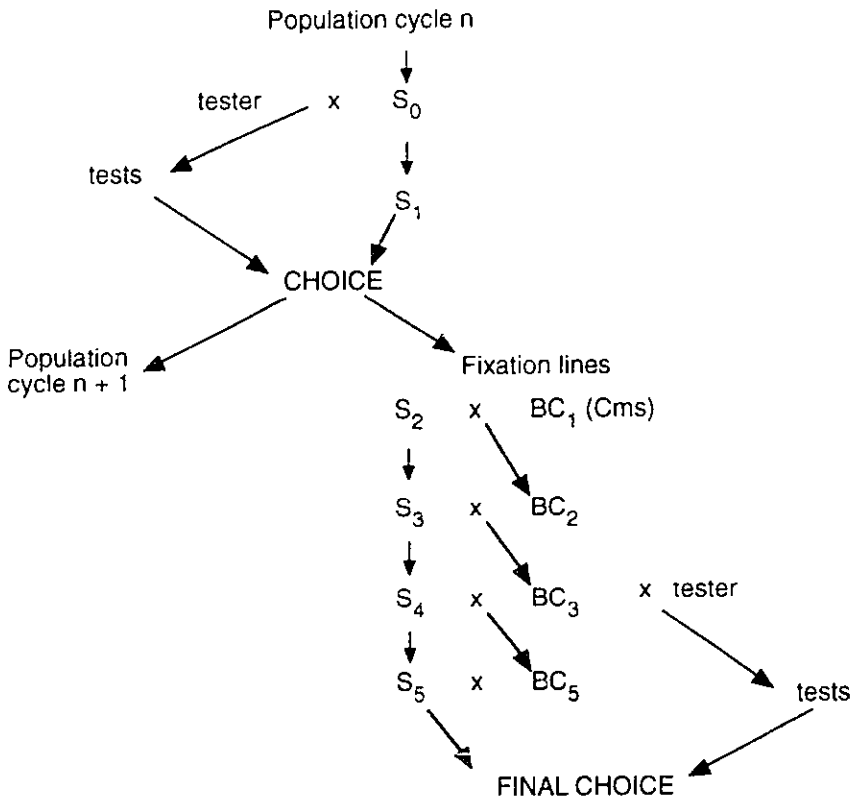
The first studies of hybrid vigour in sunflowers were made during the late 1940s by Canadians, who found an increase of 60 per cent in yield compared with populations. They produced some hybrid varieties using self-sterility to facilitate crosses between parental lines. However, the proportion of hybrid plants was often only 50 per cent, for it was impossible to use highly self-sterile parents since they could not be multiplied.

To be useable in hybrid production, a recessive gene giving male sterility must be distinguishable before flowering. The gene linked with anthocyanin production, reported by Leclercq in 1966, fulfilled these conditions and made possible the first production of true hybrid sunflower varieties (see Figure 8.3). The male parents were normal sunflower lines without the male-sterile gene. These hybrid varieties were developed by INRA from 1969 (INRA 6501) to 1975 (Airelle). However, their production gave rise to two problems:

1. The 1 per cent recombination gave rise to male-fertile plants not showing anthocyanin, which produced pollen within the rows of females. The pollen from these male-fertile female plants was transferred by bees and pollinated up to 30 per cent of the male-sterile plants.
2. The need to eliminate half the plants of the female parent made necessary a very dense sowing, followed by thinning, which was costly and irregular.

Figure 8.2. **Diagram of sunflower breeding**

a. Diagram of recurrent breeding (female population)



b. Diagram of genealogical breeding (female lines)

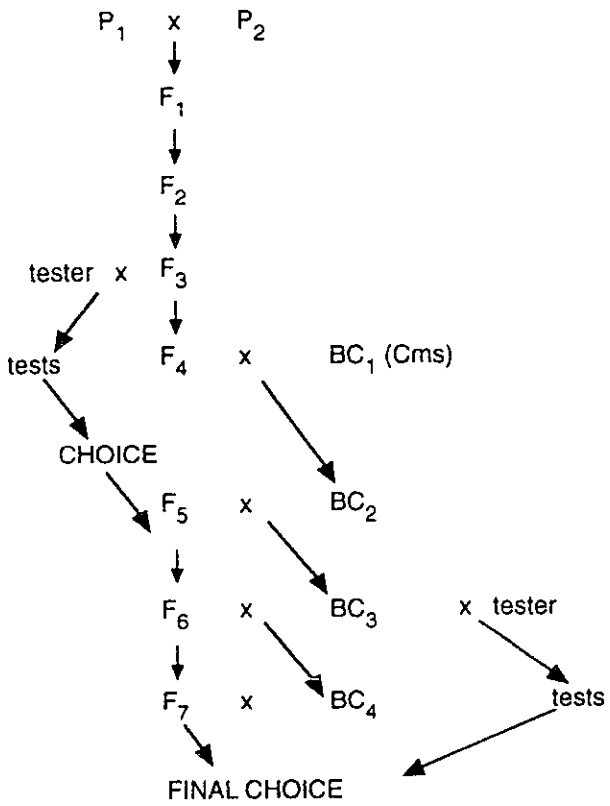
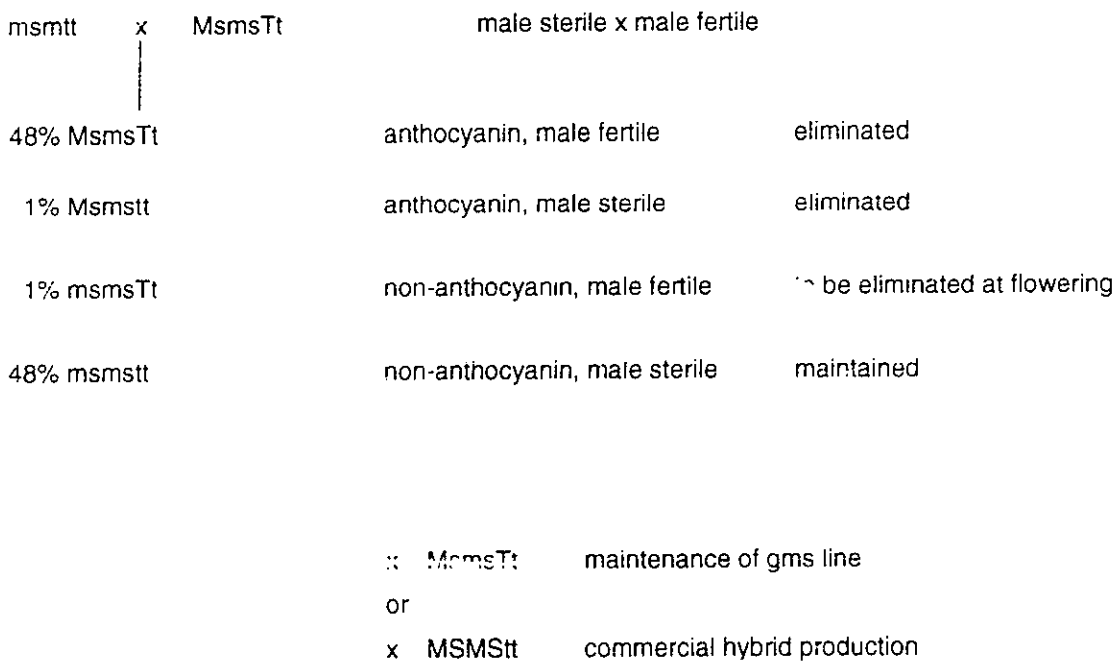


Figure 8.3. **Diagram of the use of marked genetic male sterility in sunflower (genes: Ms/ms = male fertile/male sterile: T/t = anthocyanin/non-anthocyanin)**



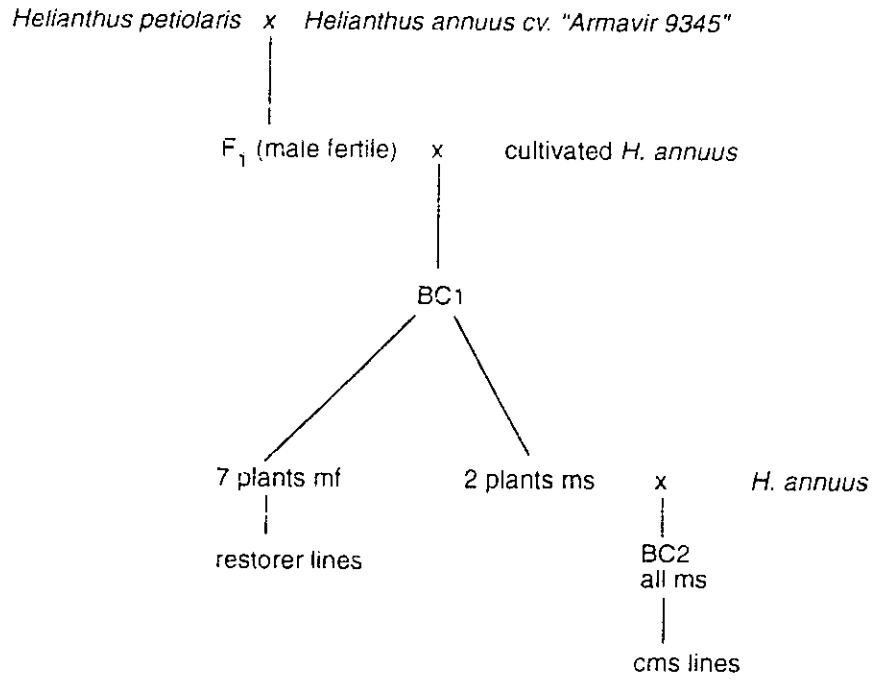
The discovery of a cytoplasmic male sterility by Leclercq (1969) (in a cross between *H. petiolaris* and *H. annuus*) simplified hybrid production, since all female plants are male-sterile (Figure 8.4). Restorer genes were found in progenies from the same cross and also in wild *H. annuus*. The first hybrids (Fransol and Relax) were registered in 1974, and cytoplasmic hybrids have been widely grown since 1978. Several studies have shown increases from 105 to 140 per cent in yield of hybrids over those of open-pollinated varieties.

b) Breeding objectives

Sunflower seeds provide not only oil but also a high-protein seed cake. In some countries, farmers are paid according to their oil yield per hectare, but in France and the United States they are paid according to their seed yield, with a variable premium according to oil content (the base being 44 per cent). Present breeding criteria are thus the following:

Seed yield has a low level of heritability (Fick, 1978), that is, it is highly influenced by the environment, and specific reactions of different hybrid combinations are apparent. It is therefore the character, for any genotype, that is the most difficult to describe accurately. The yield of sunflowers is determined by the number of seeds per head and the weight of 1 000 seed. These characters generally have a higher level of heritability than yield itself, but they may be negatively correlated, and the better predictor of yield varies according to trials. Thus, in most breeding programmes, grain yield of hybrids is measured directly.

Figure 8.4. **Origin of the "traditional" cytoplasmic male sterility in the Sunflower**



Source: Leclercq (1969).

The highest *oil contents* known (60 to 65 per cent) are probably close to biological limits. Oil content is determined by the plant on which the seeds are borne; the pollen has little effect. Oil content is a highly heritable character (Fick, 1975), which can be selected in early generations of a breeding programme. Generally, hybrids have higher oil content than their parents (heterosis), but this is not the case when inbred lines have 50 per cent or more oil.

Oil content is now measured by nuclear magnetic resonance (NMR), a rapid non-destructive method which only requires 2-3 g of seed. It is therefore one of the easiest characters to select.

i) Factors determining yield stability

The *earliness at harvest* of a genotype can be defined in several ways. The date of flowering is important for determining the period when the plant is most susceptible to drought and, under humid conditions, to *Sclerotinia* capitulum attack. However, for the farmer, the most important character is the date at which sunflowers can be harvested. Physiological maturity (maximum yield and oil content) is reached when the seed has about 35 per cent water content. There is a certain independence between drying of the seed and drying of the capitulum. The most usual measure of earliness is therefore the humidity of seed at, or slightly before, harvest, when the range between early and late varieties is at least ten points.

Humidity of seed at harvest depends on the whole growth period, but more especially on the length of time between flowering and maturity (Chervet and Vear, 1990). Heritability is moderate, and general effects of inbreds are found in their hybrids (general combining ability).

Resistance to disease is one of the main factors determining the success of a sunflower crop in different parts of the world. The problems differ in different countries and environments. In addition, the situation is not the same in North America, where sunflower and its main pests are endemic, and in countries where sunflower is a recent introduction. The relative newness of the crop also means that new diseases can appear, as parasites from other plant species become adapted.

Some diseases are of worldwide importance; others are more localised. Those most commonly found are discussed below.

Downy mildew (Plasmopara helianthi). This disease, of world-wide importance, is maintained in the soil up to ten years and transported occasionally on seed. The hypocotyls and roots of young plants are infected in humid conditions. The plants become dwarfed and produce no seed.

Resistance to downy mildew has been obtained from both cultivated sunflowers and other *Helianthus* species. It is determined by one or a few major dominant genes and is functionally complete. However, it is race-specific, and different *Plasmopara* races are known in North America (up to six races) and Europe (three races), for example (Gulya *et al.*, 1991). Resistance can be tested on seedlings in a growth chamber, using a test that lasts two weeks. Breeding progammes to introduce resistance into modern genotypes are rapid and, at present, keep pace with the appearance of new races.

White mould (Sclerotinia sclerotiorum). Of worldwide importance, except in very dry zones, *Sclerotinia* causes a soft humid rot on different parts of the plant: roots, stem base, terminal bud, leaves, capitulum. Although these different attacks are by exactly the same fungus, they may almost be considered different diseases, of different importance in different parts of the world: root and stem base in North America and Europe; terminal bud in Europe and North Africa; capitulum in Argentina, China and Europe.

With the possible exception of resistance to terminal bud attack, resistance is horizontal (no parasitic races), partial (all levels of attack found), and under polygenic control. It is generally additive, with a moderate level of heritability (Castano *et al.*, 1992). It is therefore necessary to carry out long-term breeding programmes to assemble in one genotype many additive factors, the sum of which provides appreciable resistance to one or several forms of *Sclerotinia* attack. Breeding tests are available, but they generally have to be carried out in the field on adult plants.

Progress is thus much slower than against downy mildew, but it can be considered that gains in resistance are permanent. Resistance factors in cultivated sunflower are used at present, but in the future, it is hoped to introduce additional factors from perennial *Helianthus* species.

Phomopsis (Diaporthe helianthi). This is a new fungal species, discovered in Yugoslavia in the early 1980s. It has since been found in neighbouring countries and in France. The parasite attacks through leaves, spreads to the stem and causes wilting, premature drying and stem breakage. It overwinters in the remains of sunflower stems, and is thus particularly important in areas where sunflower crop residues are not ground and ploughed in in the autumn.

Resistance is horizontal, polygenic and partial, although with greater distinction between genotypes than for *Sclerotinia* resistance. Breeding tests on adult plants and selection in naturally infected fields are possible. Resistance is found in cultivated sunflower and also in the *annui* section of the *Helianthus* genus.

Grey rot (Botrytis cinerea). This disease is important in temperate zones with humid autumns. *Botrytis* causes a soft rot of the capitulum at maturity. When it is very severe, the seeds are also rotten. Most frequently, *Botrytis* causes problems at harvest and reduces oil quality (excessive acidity). Resistance is of the same type as for capitulum attacks of *Sclerotinia*, but less work has been carried out on this disease. Observations of natural attack are used more frequently than breeding tests.

Verticillium dahliae wilt. This disease is important principally in North America and Argentina. *Verticillium* infects through leaves, and on reaching the stem, produces a toxin which kills the upper part of the plant. Resistance is mainly oligogenic, but at present no races of the pathogen are known.

Alternaria ssp. This disease is found mainly in hot countries with high humidity during the later stages of growth (Australia, Africa, India, northern Argentina). It causes large brown necrotic spots, which can limit photosynthesis and translocation, on all aerial organs. Resistance is polygenic.

Rust (Puccinia helianthi). Important rust attacks are observed in North America, Australia, and South America, and sometimes in relatively mild climates, such as that of Spain. Black pustules appear on the leaves of sunflower plants, limiting photosynthesis. Resistance is "vertical" (gene for gene), oligogenic and complete. A large number of races are known, but breeding is still for race-specific resistance.

Broomrape (Orobanche koumana). Broomrape is a plant parasite found mainly in eastern Europe, Russia, Turkey and Spain. It grows on sunflower roots, absorbing nutrients and so weakening the plants. Its seed is a very fine powder which can easily be windborne. Resistance is vertical and oligogenic. Breeding programmes require the introduction of resistance genes. Some control is obtained using herbicides.

Other diseases. Other diseases which may require control in certain areas, on certain genotypes or in certain years include: *Septoria helianthi, Rhizopus* ssp., *Macrophomina phasioli, Albugo trageopogonis, Phoma* ssp. and certain bacterial infections. Few viruses or nematodes are known on sunflowers.

Sunflower is a host to a number of *insect pests.* In North America, approximately 15 species of sunflower insects cause plant injury and economic loss, depending upon the severity of infestation (Schultz, 1978). The existence of this number of insect pests coincides with the evolution of wild sunflowers in North America. To date, these insect species have not been transferred to other production areas of the world. However, two species of insect attack sunflower outside North America. These are the European sunflower moth in Europe and Russia, and the Rutherglen bug in Australia.

Head-infesting species of insects that produce economic damage include: sunflower moth, *Homoesoma electellum* (Lepidoptera: Pyralidae); European sunflower moth, *Homeosoma nebullella* (Lepidoptera: Pyralidae); banded sunflower moth, *Cochylis hospes* (Lepidoptera: Cochylidae); sunflower budworm, *Suleima helianthana* (Lepidoptera: Tortrichidae); seed weevil, *Smicronyx fulvus* and *S.sordidus* (Coleoptera: Curculionidae); and sunflower midge, *Contarinia schulzi* (Diptera: Cecidomyiidae). Foliage and stem feeding species include: sunflower beetle, *Zygogramma exclamationis* (Coleoptera:

Chrysomelidae); painted lady, *Cynthia cardui* (Lepidoptera: Nymphalidae), and stem weevil, *Cylindropterus adspersus* (Coleoptera: Curculionidae).

The most important insects in North America are the sunflower moth, banded sunflower moth, seed weevil and sunflower midge. Resistance to the sunflower moth and European sunflower moth has been associated with the armoured layer, a pigmented substance between the outer layer and the adjoining sclerenchyma tissue in the sunflower hull. Few resistance mechanisms have been determined for the other species. Other mechanisms for decreasing damage have been investigated and include: biological control, chemical deterrents derived from wild *Helianthus* species, pheromones to trap insects, chemical feeding deterrents and cultural controls.

When sunflowers suffer from *drought*, photosynthesis and grain-filling are reduced, so breeding programmes seek to find genotypes which make the best use of available water. Factors such as root penetration, stomatal regulation, and epidermal permeability are studied. It is not well known what genetic variability exists in cultivated sunflower. Species such as *H. argophyllus* may be important as sources of resistance.

Lodging may be at stem base or mid-stem, but it is always catastrophic. As a resistance factor, reduced height may be selected, but often the two characters are independent. Breeding consists in eliminating the genotypes which show lodging. The level of heritability is quite high.

ii) Factors determining quality

Oil quality: The "normal" composition of sunflower oil varies according to climate: in temperate conditions, it contains up to 75 per cent linoleic acid and 20 per cent oleic acid, whereas in hotter climates, up to 60 per cent oleic acid and 30 per cent linoleic acid are common. With no modification, this oil is valuable for direct use and in margarine production.

Breeding work is being carried out to obtain genotypes with up to 85 per cent oleic acid. This character is probably determined by a small number of genes (Miller *et al.*, 1987), but their effects may be temperature dependent. High oleic oil is used for food and for industrial purposes.

Dehulling aptitude: Cultivated sunflowers vary for this character. It appears to depend on the structure and thickness of the hull. Generally, large seeds with thick hulls are most easily dehulled, but some small seeds with high oil content are also satisfactory.

Protein content: At present, sunflowers are not bred for their protein content, which varies between 11 and 30 per cent. In recent years there has been a drop in protein content, due to selection for high oil content, as these two characters show a close negative correlation. If high protein is required, it will be necessary to develop specialised varieties with lower oil content.

c) Testing methods

The study of most breeding criteria involves field trials. Downy mildew resistance tests are carried out in growth chambers, but so far, no characters can be measured *in vitro*.

d) General performance monitoring

Certain morphological characters are used throughout the world to describe sunflower genotypes. On a more local scale, multi-site trials are used to determine the agronomic value of hybrids. Other than this, each breeding centre uses its own data system. It may be noted, nevertheless, that for the cross-pollinated sunflower crop, the description of a fixed, homozygous inbred line must include not only data on the inbred line itself but also data concerning its combining ability (value of its hybrids) for the main agronomic characters.

C. Seed multiplication for commercial use

a) Stages in seed production; isolation distances

The rate of multiplication of sunflower is about 1 to 400 for inbred lines (10 q/ha). Hybrid variety seed is produced by farmers under contract to breeders in isolated fields (these are often grouped to produce one variety around a village or group of farms). For Europe, minimum isolation from another sunflower genotype is 500 m. The basic parental seed is also multiplied in the field with an isolation of 3 km. The prebasic seed is produced either in the field with an isolation of 5 km or under insect-proof cages. All earlier generations are produced under paper or cloth bags.

b) Variety registration (Europe)

In Europe, to be offered for sale, a sunflower variety must be registered in a national or European catalogue. For this, it must undergo two years of trials in about 15 locations and be found to have no prohibitive fault, to be uniform and reproducible, to be distinct from any known variety and to show some agronomic improvement compared with control hybrids, which are the most widely cultivated varieties at that time.

c) Surveillance of seed behaviour

This varies according to country. In some places, it is the breeders' sole responsibility. In France, a base collection of all parents of hybrids is maintained and used for control of all hybrid productions (by the SOC = Société officielle de contrôle). When this organisation is satisfied that the variety produced is accurate, certificates verifying authenticity are attached to the bags of seed sold to farmers.

After release, the breeder generally monitors the behaviour of a variety. For example, in France, one of the new downy mildew races was first reported by a private breeder. Where they exist, technical and extension organisations are active, probably because sunflowers are a new crop and the inter-profession is well structured.

In North and South America and Europe, there is no doubt that any new problem will be rapidly identified, with feedback to authorities and breeders. The delay will be longer in developing countries, where farmers still grow open-pollinated varieties and keep their own seed or buy it in the open market. In this case, it is necessary to carry out specific enquiries in the field to determine possible problems and their importance.

d) Lifespan and market spread

In Europe, during the first year after registration, a new variety is generally tested only in large-scale trials carried out by the breeder and by extension services. Large-scale commercialisation will start the following year. (A breeder will produce large quantities of hybrid seed only when the variety is sure to be registered.) At present, about half the varieties registered in the French catalogue have a commercial career. They generally last about four or five years, and an exceptional variety will last eight to ten years at the most

In recent years, the sunflower market in each country has been dominated by four or five varieties, with another 20 having a small level of sales. This may be due to the fact that breeding programmes are recent, so that large specific advances are found in a few varieties. It is possible that, in the future, advances will be smaller and more frequent, so that there will be more varieties of equal value and a more fragmented market.

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9. Maize

by

William S. Niebur

A. Characteristics of the crop

a) Geographic origins; centre of diversity

At present, maize is thought to have a multicentred origin. Randolph (1959) and McClintock (1959) have used morphological and physiological characters as well as chromosome knob distribution to support this theory. The data suggest that independent domestications occurred in teosinte throughout Central America. Galinat (1983) has suggested a two-stage development of maize as we know it today. The first stage involved the selection of the races within teosinte (Inadvertent Stage), while the second was aimed at creating the maize we know today (Deliberate Stage). Thus, experts propose the general area of Mexico and Central America as the centre of origin of maize.

b) Geographic distribution of use; main production areas

Corn, or maize *(Zea mays* L.), is the world's third leading cereal crop, following wheat and rice. About 17 million ha of maize were grown in Europe in 1992, including, very roughly, about 100 000 ha of maize seed. In Latin America approximately 25 million ha of maize are planted, Brazil, Mexico, and Argentina being the most important countries. In 1992, 32 million ha of maize were planted in the United States. Maize is cultivated over a broad range of climatic conditions. Very early varieties that require 60 days to reach maturity and those varieties that require an entire year to produce grain are sometimes grown in the same geographical regions.

c) Taxonomic status: relationship to neighbouring species

Maize is an example of a crop so domesticated it cannot survive without human help. This domestication was accomplished by the indigenous peoples at the centre of origin of maize, the Americas, prior to the arrival of Europeans.

The potential for transfer to wild or weedy relatives is non-existent in Europe since no wild relatives exist there. Teosinte is the only wild relative of maize with which it can easily cross-pollinate. Teosinte grows in Mexico and Guatemala, where it is frequently found growing in maize fields. Since it crosses readily with maize and forms fertile hybrids, the potential for transfer of traits from maize to teosinte is real.

d) Genetic and cytogenetic characteristics

Improvements based on single gene changes will be especially amenable to the newer techniques. At this point, engineering maize plants with the newer techniques is in the very early stages of technical development. It is likely that in the future, the so-called recombinant techniques will greatly enhance the ability to generate new cultivars with specific traits. However, the newer techniques are already facilitating analysis of traditional lines. Using a process known as restriction fragment length polymorphism (RFLPs), breeders can make a genetic fingerprint of a plant. Once genes are mapped and identified, scientists can carefully read the genetic structure of the plant. The use of RFLPs is enabling faster analysis of desirable progeny. RFLP analysis has also enabled companies to analyse competitors' hybrids and to learn which are genetically similar, thereby reducing the number of hybrids that have to be planted for comparison performance trials.

McClintock (1929) first characterised the ten chromosomes of maize using mitotic studies. Classification was based on chromosome length and centromere position. Presently cytologic research is being conducted on chromosome staining techniques, meiotic mutants, examination of the B chromosomes and the better understanding of the events involved during synapsis. The class of cytological mutations that appear to be most interesting today are those that occur after meiosis. Many post-meiotic male sterility mutants have been identified, and these are generally female-fertile. Most of these mutants behave as a recessive in the heterozygous condition. These mutants have been extremely important in commercial seed production. During the period 1950-70, Texas cytoplasmic male sterility (CMS-T) was used in place of mechanical castration. The epidemic of Southern corn leaf blight in 1970 occurred primarily because of the susceptibility created by the use of this character. A quick conversion to normal cytoplasm was made and the problem was corrected. Today, other forms of cytoplasmic sterility are being used in seed production (CMS-C and CMS-S). In North America in 1987, 66 per cent of seed production used 100 per cent normal cytoplasm, 22 per cent used CMS-C cytoplasm and 12 per cent used CMS-S. Thus, a mixture of techniques is being used in order to avoid the kind of situation that occurred in 1970.

e) Phytosanitary considerations in movement of germplasm

Maize germplasm moves freely around the Western hemisphere (North, Central, South America and Europe) with appropriate phytosanitary documents. There exist certain countries, such as Italy, Spain, and Portugal, that require documentation to ensure that bacterial diseases such as *Erwinia stewartii* (E.F. Smith) are not present in the seed being transported. Seed exchange between Africa, Asia, Australia, New Zealand and the countries mentioned above is strictly controlled and often requires quarantine for one generation to guarantee freedom from disease.

f) Current end uses

Most of the maize currently produced in Europe is sold for animal feed. Maize for feed is generally bulked for sale, rather than sold on the basis of cultivar quality characteristics. Therefore, breeders of maize for animal feed focus on improving agronomic traits such as yield and resistance to disease, insects and various environmental stresses. Another significant portion of the maize produced in Europe is used to produce

high fructose sweeteners, starch and cooking oil. Certain breeding programmes focus on improving or developing specialty maize cultivars for characteristics such as increased polyunsaturated fats for food oil; increased oil production for poultry and swine or industrial uses; higher protein content or quality (such as increased lysine content); improved wet milling extractable starch; and food-grade dry milling qualities. Improved protein quality, if it does not require greater management practices, such as increased fertiliser use, may be of special value in those parts of the world where maize is the primary food grain, *e.g.* Mexico, Central America and parts of South America, China and Africa.

Reproductive mechanisms

a) Mode of reproduction and pollination

During development in nurseries, all pollinations are made by hand. Genetic isolation is maintained by putting paper bags over the reproductive structures of the plants or by detasselling rows serving as female (seed) parents where a common male is present. For self-pollination, the bags are put over both the male tassel and the female ear silks. At the time of pollination, ear silks may be cut back to allow exposure of fresh silks. Pollen is taken from the tassels and put on the silks. For cross-pollination, only the silks of the females and the tassels of the males are bagged, or the crosses are made in isolated blocks (200-400 m from other maize).

Maize is a monoecious species which has pistillate flowers on the ear shoots and staminate flowers in the tassel (Kiesselbach, 1949). The main stem or stalk of the plant terminates in a staminate inflorescence or tassel that provides the source of male gametes. Each node above the soil can produce a branch that will potentially terminate in a pistillate flower or ear. Usually all but one degenerate and the upper branch dominates the lower ones. The staminate inflorescence is comprised of many spikelets which hold the stamens. The stamens (anthers) are pushed out of the protective plant parts when pollen maturation is complete. The pollen matures in the anthers, forming microspores capable of pollination. The extruded anthers break open at the tip, and this allows the pollen to escape. Very little pollen will be lost until the tassel is moved or shaken by the wind. The pollen is easily carried by the wind to adjacent plants. Each plant is capable of producing more than 15 million pollen microspores. The development of the pistillate inflorescence occurs at an auxiliary branch bud. As stated before one to two buds will dominate while all others deteriorate. Each spikelet contains two flowers of which one will generate a kernel. For this reason the kernels occur in double rows, and there is always an even number of kernel rows on the ear. The style or silk of the flower grows from the florets and will emerge from the flower to be pollinated. The pollen is caught by the silk and is given moisture, causing the pollen to germinate and creating the pollen tube. The pollen tube penetrates to the micropyle and then continues to the embryo sac where fertilisation occurs.

b) Dispersal and survival mechanisms of propagules

The likelihood of maize being manipulated so that it could establish itself in the wild is essentially nil. It is possible for maize to be manipulated so that volunteer plants are more likely to occur, but this would simply be a function of the management practices

used to avoid or control volunteers. If, for example, volunteer plants are controlled by use of a herbicide, and the maize has been made resistant to that herbicide, then a different herbicide or practice would have to be used. The common practice of crop rotation, alternating, for example, soybean and maize plantings in the same field, avoids problems with volunteer maize plants and, in addition, gives higher maize yields.

c) Ability to cross with related species

Because the risk of transfer is real, it becomes important to analyse whether a particular trait would cause a significant problem if transferred to teosinte. The likely rate of transfer to teosinte and the likely rate of spread throughout the teosinte population once transferred are important parameters when calculating the potential significance of such transfer.

If, for example, herbicide resistance were transferred, one would want to know whether the resistance would be selected for in the general teosinte population. If the herbicide was not used within the agricultural environment, then the trait might become significant or widespread only within the agricultural environment. If so, one should then examine whether such resistance would be a problem on farms. For example, if a specific herbicide resistance were introduced to maize in order to selectively kill teosinte in maize fields, then transfer of that resistance to teosinte would be undesirable, although it might simply leave the situation unchanged. One might next ask whether the cost of introducing the resistance would be justified given estimates of the likelihood, frequency, and time-scale for transfer of the resistance to teosinte, as well as the costs of other means of removing teosinte, such as mechanical tillage.

If the new trait is pest resistance, one might perform a similar analysis, focusing on the likely significance of the transfer of that trait to teosinte. If the pest is a major controlling factor in the survival or spread of teosinte, then resistance to that pest could significantly affect the environmental competitiveness of the plant. If, on the other hand, the pest does not play a major role in the control of teosinte, then transfer of that trait would likely have little environmental impact.

Environmental considerations

Traits for disease and pest resistance, including traits introduced by molecular methods from diverse sources, raise issues such as the potential for selection and the consequent development of pests resistant to pesticide. Such issues have been repeatedly dealt with in the past for many conventionally introduced traits. It is important to keep in mind the risk/benefit issues regarding the use of such traits in plants to replace other pest management practices, such as spraying of chemical pesticides and use of non-chemical controlling practices.

Environmental requirements for life cycle

Maize is primarily limited by an inability to grow under very cold conditions. World distribution of maize is essentially from the equator to 45° latitude. New areas in the Russian republic (56°N) are being developed for maize hybrids that will be used for animal consumption (silage). Maize requires an adequate amount of precipitation or irrigation in order to survive. The lower limit during the growing season is at or near

40 cm. Under more water-limited climates maize is often replaced by sorghum (*Sorghum bicolor* L.)

B. Current breeding practices and variety development

There are about 100 active maize breeding programmes in Europe. Maize seed is produced almost exclusively by commercial breeders. Development of new inbred lines of hybrid maize is dominated by commercial breeders, but foundation seed companies also develop inbred lines which they lease to commercial companies for use in hybrid production or breeding. Public institutions develop inbred lines, which are then used by commercial breeders as part of their breeding programmes, but this source is of less importance than it was 20 years ago. A large commercial company may sell 20 to 50 different hybrid cultivars, each having its own advantages and disadvantages for different climates, soil types, insect and disease incidence. However, except for specialty and food-grade cultivars, the seed harvested from the different cultivars are all bulked together and sold as yellow dent number 2 for feed, industrial and export uses.

a) Main breeding schemes/techniques

i) Parent line breeding

The first five or six generations (F_5 or F_6) of breeding and purification occur primarily in the breeders' nurseries. Yield testing of experimental hybrids is carried out in two to five locations in the area that the breeder serves. Thousands of genetically distinct lines are grown near each other; genetic isolation is maintained and found to be sufficient for breeding purposes by putting paper bags over the reproductive structures of the plants. In any one season, a nursery may have some 20 000 rows, about ten hectares, devoted to all stages of inbred development and hybrid production. Lines will be evaluated *per se* for parental traits, disease resistance, and insect resistance; in hybrid combination they will be evaluated to determine which have the greatest genetic potential. At stages F_3 through F_8, the lines will be tested more extensively for properties as a parent of a hybrid. They are crossed with known elite lines in the nurseries. The resulting seeds are planted in small two-row plots at several test locations on breeding stations and on farms near breeding stations. Because the test plots are for performance evaluation and not for generating pure seed, the plants do not require isolation. Isolation is designed to prevent contamination of test seed needed for further breeding. Test plots for evaluation of performance may be freely pollinated, because the seed will ultimately be destroyed.

To speed development, one to two selfing generations are frequently planted in winter nurseries in a tropical or subtropical area: Hawaii, Chile, southern Florida, Puerto Rico, Mexico or New Zealand. These nurseries can also be used to generate seed for yield testing and further breeding work.

ii) Hybrid breeding

Lines found to be promising are then crossed with various other lines in an attempt to discover good hybrid combinations. The seed is subjected to performance trials in different parts of the country over two to four years, allowing exposure to various environmental conditions. A large breeding programme may have some 35 000 plots,

totalling 30 ha, of advanced hybrid yield trials, three-fourths of which are planted in commercial fields located 50-100 kilometres from the commercial maize breeding station. Product harvested from these trials is commonly left with the farmer for commercial use for animal feed on the farm. Successful new hybrids based on F_9 inbreds will be tested in eight-row strips on up to several hundred farms. Two to three hybrids may be planted on commercial farms throughout the geographic area targeted by the breeding programme. While these hybrids are being evaluated in strip trials and on the farm the first year, the hybrid seeds of the same combinations are produced for sale and for further strip testing the following year. The volume of production is controlled. The seed harvest from the maize test plot is blended with other maize for commercial sale.

b) Main breeding objectives

The development of new commercial hybrids can be divided into two parts. First, breeders must establish phenotypically homogeneous lines with desired growth characteristics: resistance to different stress factors, growth-to-maturity time, yield, acceptability as parents, etc. At the same time, the lines must be tested in various combinations to find suitable hybrids for commercialisation. A breeder may evaluate some 50 000 genetically distinct lines each year. By the end of the evaluations, some six to ten years later, perhaps one or two new inbreds will be available for use as a parent in a commercial hybrid.

During selfing generations (purification process), plants are subjected to stress tests. Parent lines of new hybrids must exhibit good seed yield under a variety of growing conditions. Plants may be inoculated with corn borer eggs, with leaf blight spores, or with common maize viruses, depending on the disease and insect pest pressure in the area being served. Tests are also conducted to evaluate tolerance to environmental stress such as drought and cold.

With traditional breeding techniques, lines have been developed with resistance to such pests as first and second brood corn borer and northern and southern corn leaf blight, with tolerance to herbicides, and with improved seed production characteristics.

If a hybrid has a yield significantly higher under optimal conditions than other commercially sold varieties, breeders may tolerate somewhat greater loss under some stress conditions, but stability of performance across environments is very important. However, factors that farmers (and therefore breeders) tend to notice and dislike are loss under predictable stress conditions, failure to germinate properly, and premature dropping of the ears. Given the highly competitive nature of the maize seed industry, a breeder cannot afford more than slight differences in overall yield. Therefore, yield under stress is very important. Some 3 000 rows (about 15 ha) may be used for stress screening. Hybrids are extensively tested across a wide array of yield levels to determine those that give consistently higher yields across the range of environments the farmer can expect.

c) Testing for the most important breeding goals

Drought and cold tests are performed using randomised block designs, or nested randomised block designs. In the latter, regions within a block are planted with plants of similar height to avoid shading problems. Within a nest site, the plants are randomised.

C. Seed multiplication for commercial use

a) Stages in seed production

Once a parent line is established, bulk of pure uniform parent seed will have been turned over to foundation increase. A bulk of the genetically pure line will then always be the fixed parent source for the inbred. Foundation increase will be selected for about three generations to prevent genetic drift.

Once enough seed of a hybrid has been generated for performance trials, it is no longer produced by hand-pollination. To generate seed for early strip tests, small isolated fields of one to five hectares are planted with adjacent rows of the inbred parent (*i.e.* four rows female to one row male). The female rows will be detasselled and the block will be isolated from other corn, using a minimum distance of 200-400 m.

For extensive strip tests and commercial introduction, one to several hundred acres will be planted by commercial contract farmers to generate the F_1 seed. One common practice is to alternate four rows of a female line with one row of a male line. The female plants are detasselled to avoid self-pollination.

b) Isolation practices in variety production

Accepted practice is for foundation seed production fields to be isolated by 200 m. This distance may be lessened if there are adequate rows of male plants surrounding the field.

The potential for transfer of traits to other maize plants is of minimal significance in Europe because the vast majority of commercially grown maize is hybrid. Therefore, the farmer does not save seed for the following season and pollen transfers are not passed on to future generations. The commercial breeder follows isolation procedures to maintain the genetic purity of his foundation lines. New lines are unlikely to require new isolation procedures. In the unlikely event that they did, breeders could adapt as necessary.

c) Testing and evaluation of experimental hybrid varieties

In early strip tests, seed will be distributed to 50 to 600 or more farmers, who plant it alongside favourite hybrids in a strip four or 12 rows wide. Farmers may devote one to two hectares to planting experimental hybrids. In later strip tests, the hybrid will be planted in thousands of such strips, at the same time as it is beginning to be marketed as hybrid seed. Because commercial seed is sold as a specific F_1 hybrid, great care must be taken not only to avoid contaminating seed from foreign parent lines, but from its own female parent. Therefore, lines designated as females must be detasselled or made male-sterile. Detasselling is the principal method, used on 60-80 per cent of all European maize hybrids. Detasselling may be done manually and/or mechanically.

d) Seed certification

In Europe, most new cultivars are developed by commercial breeders. They must undergo government certification; this normally requires that hybrids be evaluated over two to four years of testing for hybrid performance and parental purity and stability. Thus, before submission to officials, companies conduct extensive performance trials of

new hybrids, comparing them over two to three years with their own and their competitors' hybrids in many environments. It is common practice for official agencies to conduct trials of commercial hybrids sold in their country and for farmers to test samples of seed before planting extensive acreage. This surveillance continues after registration by both officials and breeders to ensure that performance is as expected.

e) Surveillance of seed production

For sale of seed there are established purity standards for each country. Most companies set standards preventing the sale of seed that contains 5-6 per cent or more of self-pollinated plants or 6-8 per cent or more of foreign-pollinated plants. Thus, the standard is for genetic purity rather than percentage of pure seed (as in the case of small grains, soybeans and forage grasses).

f) Lifespan of varieties and adoption by the farmer

The average commercial life of a maize cultivar is five to ten years, primarily because replacement cultivars are continually introduced with progressively better yield. The annual average yield has increased by about one per cent since the 1930s, and continues to do so at 1.5 to 2 per cent per year. Almost all maize seed sold in Europe is hybrid and 70 per cent of that is single-cross hybrid. The F_1 seed gives much higher yield than do the subsequent generations, which is why the farmer goes back to F_1 seed each year rather than growing his or her own F_2 seed.

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10. Sugar Beet

by

Nils Olof Bosemark

A. Characteristics of the crop

a) Geographic origins; centre of diversity

The wild relatives of sugar beet originated in Asia Minor, but some forms are widely distributed throughout the Mediterranean area and Europe. All cultivated beets, both leaf-beets and those with swollen roots, are likely to have originated from wild maritime beets through simple selection by man.

The history of sugar beet began in 1747 when the German chemist Marggraf found that the sweet substance in beets was sucrose, until then thought to exist only in sugar cane. At the end of the 18th century, his student, Achard, began to study sugar beet cultivation and beet sugar manufacture. In 1801 he built a factory in Silesia and started to select beet types suitable for sugar production. His work produced the White Silesian beet. Later, several other researchers attempted to improve the beet material. Louis de Vilmorin began selection work based on the Silesian beet, and in 1837 he introduced progeny testing as a selection method. As a result, and following the introduction of the measurement of specific gravity in 1850 and the polarimeter in 1862, sugar content increased. It rose from 7 to 9 per cent in 1830 to 11 to 13 per cent in the so-called "*Beta imperialis*", considered to be the first true sugar beet, developed by the German breeder Knaur in 1854. While some sugar beet researchers believe that "*Beta imperialis*" originated from spontaneous crosses between Achard's "Silesian beet" and forms of the wild beet *Beta maritima,* others believe that sugar beet is the result of repeated selection in populations of the "Silesian beet", itself considered to have originated from natural crossings between various types of cultivated root and leaf beets.

b) Main production areas

Sugar beet is the sole or main crop for sugar production in the temperate zones of the northern hemisphere. Since the Second World War sugar beet has also been grown as a winter crop in countries with hotter climates such as Morocco, Algeria, Tunisia, Egypt, Syria, Iraq and Iran (see Table 10.1).

Table 10.1. **World sugar beet acreage 1991**

Area	Acreage (1 000 ha)
Countries in the EEC	2 043
Remaining Western Europe	713
Former USSR	3 190
Remaining Eastern Europe	910
United States and Canada	570
South America	55
China	440
Middle/East Africa	261
Japan	72
Total	8 254

c) Taxonomic status

Sugar beet belongs to the genus *Beta*, family Chenopodiaceae, which, besides beets and spinach (*Spinacea oleracea*), contains only a few cultivated plants with limited distribution. The genus *Beta* consists of four sections: *Beta, Corollinae, Nanae,* and *Procumbentes* (Table 10.2). To the section *Beta* belong all cultivated beets and a range of wild forms, all of which are sexually compatible and give fertile offspring with each other. They may thus all be considered to belong to the same collective species, *B. vulgaris*, L.

Table 10.2. **Taxonomic division of the genus *Beta***

	Species		2n
Section 1:	*Beta*		
	B. vulgaris L.		18, 36
Section 2:	*Corollinae*		
	B. macrorhiza	Stev.	18
	B. lomatogona	Fish et Mey.	18, 36
	B. corolliflora	Zos.	36
	B. trigyna	Wald. et Kit	45, 54
Section 3:	*Nanae*		
	B. nana	Bois et Held.	18
Section 4:	*Procumbentes*		
	B. procumbens	Chr. Sm.	18
	B. webbiana	Moq.	18
	B. patellaris	Moq.	36

d) Genetic and cytogenetic characteristics

All members of the section *Beta* are diploid with 2n = 2x = 18 chromosomes, with the exception of the wild form *B. macrocarpa,* a variety of the subspecies *B. maritima* which has both diploid and tetraploid populations (2n = 4x = 36), the latter confined to the Canary Islands.

Artificially induced autotetraploid sugar beet were introduced in sugar beet breeding in Europe in the early 1940s and gave rise to so-called polyploid or anisoploid sugar beet varieties, consisting of a mixture of tetraploid, triploid (2n = 3x = 27) and diploid plants. Beginning in the mid-1960s these varieties were very largely replaced by triploid hybrid varieties.

The development of hybrid varieties in sugar beet was made possible by the discovery of cytoplasmic male sterility (CMS) in sugar beet (Owen, 1945) and the subsequent development of hybrid breeding techniques (Owen, 1948). As in other plants, CMS in sugar beet is the result of interaction between nuclear genes and changes in the mitochondrial genome (Powling, 1982; Halldén *et al.,* 1990). To obtain entirely male-sterile offspring, CMS plants must be pollinated with so-called maintainer plants, which carry the appropriate nuclear genes for male sterility but a normal, unchanged mitochondrial genome. Plants having the appropriate maintainer genotype may be more or less scarce in different populations and can be identified only through test-crossing to a CMS plant.

In nuclear male sterility (NMS), first described in sugar beet by Owen (1952), the sterility depends on a single recessive nuclear gene. Since such a system does not permit production of a population that is 100 per cent male-sterile – a requisite for large-scale hybrid seed production – its use is restricted to facilitating crossings in self-fertile materials.

Sugar beet seed normally consists of a seed-ball formed by two to four true seeds. In 1948 the Russian sugar beet geneticist V. F. Savitsky found a few monogerm plants in a seed field of the Michigan Hybrid-18 variety in the USA. Seed increases of one of them, designated SLC 101, have been used to introduce the monogerm seed character almost everywhere. The monogermity in SLC 101 is conditioned by a single recessive gene *m* (Savitsky, 1952). Due to segregation for genes modifying the expression of the monogerm gene, the frequency of good monogerm plants in the F_2-generation is usually considerably lower than the expected 25 per cent.

Together, monogerm seed, permitting drilling to a stand, and effective sugar beet herbicides have made possible complete mechanisation of the spring work in sugar beet growing.

e) Current and possible new end uses

By far the largest part of the sugar produced from beets is used for human consumption and only a smaller part as feed stock in fermentation processes. Although it is an excellent raw material for the chemical industry, the high price of sugar, compared with oil or other fossil fuels, severely limits its use in such contexts. The same applies to the use of sugar beet as a feedstock for production of bio-ethanol. Developments in sugar beet breeding and increased costs of fossil fuels may change the situation in coming years.

Reproductive mechanisms

a) Mode of reproduction and pollination

Sugar beet, like the wild members of the section *Beta,* is sexually propagated by seeds. Apomixis occurs in the genus *Beta* but only in the polyploid species of the section *Corollinae.* Sugar beet is normally strongly self-incompatible and sets few or no seeds at all under strict isolation. Studies by Larsen (1977, 1978) have shown that self-incompatibility is caused by four interacting S-loci, each carrying two S-alleles. Incompatibility occurs when each S-allele carried by the pollen is matched by an identical allele in the pistil. However, self-incompatible plants may set some seed after selfing. This "pseudo-compatibility" or "pseudo-self-fertility" due to a breakdown of the incompatibility mechanism, is more or less pronounced in different genotypes and is highly influenced by environmental conditions, especially temperature. Almost obligatory self-fertility is caused by the presence of a special dominant self-fertility gene (SF). Plants having the SF gene in single or double dose usually set 90 to 95 per cent selfed seed even if flowering openly and surrounded by unrelated, self-sterile or self-fertile plants.

Sugar beet is largely wind-pollinated; insects play a minor role. Since the pollen can be carried over long distances, breeding stock and commercial seed production fields must be isolated by distance (see below).

b) Growth habit

Sugar beet is normally biennial and develops a large succulent root the first year and a seed stalk the second year. It requires a period of low temperature (thermal induction) to change over from the vegetative to the reproductive stage. The length of thermal induction required is genetically determined, and if short enough, seed stalk development may be induced by low spring temperatures in the first year, a phenomenon referred to as bolting. As day length is also important for flower induction, the term "photo-thermal flower induction" is used, especially when biennial genotypes are induced to flower and set seed in the first year through manipulation of temperature and day length. The genetics of bolting resistance in biennial beets is still unclear. Some studies suggest that it is governed by several genes with different degrees of dominance (Le Cochec and Soreau, 1989), others that it is largely recessive (Mc Farlane *et al.*, 1948).

The majority of wild Mediterranean *Beta* beets (*B. maritima, B. macrocarpa, B. atriplicifolia*) are annuals, but biennial types also occur. North Atlantic *B. maritima* types, on the other hand, are frequently perennial. The annual growth habit is governed by a dominant gene *B* (Owen, 1952), which causes plants that carry it to run to seed very quickly under conditions of long days and reasonably high temperature. The gene may be used in breeding to obtain a quick succession of seed generations, but it can cause considerable problems if allowed to contaminate breeding stocks or commercial seed fields (see below).

c) Dispersal and survival mechanisms

If seed plants are cut too late, some loss of seed will occur through shattering. However, sugar beet seed plants do not shatter as easily as some wild *Beta* species, which may drop their seeds as they ripen.

Seed falling on the ground usually does not germinate the same season, partly because of the presence of germination inhibitors, partly because of poor soil contact. This seed is thus generally plowed down to below germination depth in the autumn, but may be brought up to the surface at a subsequent plowing and then germinate. Sugar beet seed may be stored in the soil for ten years or more and still retain its germination capacity.

d) Ability to cross with related species

As mentioned above, sugar beet hybridises freely with all wild members of the section *Beta*, and hybrids are usually fully fertile. Artificial hybrids can be produced with the species in the *Corollinae* section, but such hybrids are mostly sterile and only set a few seeds when backcrossed to sugar beet. Hybrids with the apomictic species are an exception and often set an abundance of apomictic seed. Artificial hybrids between sugar beet and members of the section *Procumbentes* usually die at the seedling stage. They can be saved by grafting onto sugar beet, and they then develop into vigorous plants. These hybrids are also almost completely sterile and set few seeds upon backcrossing. No hybrids between sugar beet and *B. nana* of the section *Nanae* are known to the author.

Toxicology

The sugar beet root does not contain any toxic or harmful substances. However, beet leaves contain oxalic acid, which can cause problems if fresh, unwilted sugar beet tops are used as cattle feed.

Environmental requirements for life cycle

Sugar beet is a crop best suited to temperate regions, but it is grown as a winter crop in some hotter areas. In colder areas, where spring comes late and winter sets in early, the growing season is too short to result in adequate yield. It pays the farmer to irrigate sugar beet, even in fairly humid areas.

Although the growth of sugar beet benefits from long days, the vegetative stage must still be considered day-length neutral. This contrasts to the reproductive stage where long days play a role both before and during seed stalk development.

B. Current breeding practices and variety development

a) Main breeding techniques

i) Germplasm maintenance

Although relatively few cycles of recurrent mass and half-sib selection in sugar beet × *B. maritima* crosses can result in populations sufficiently adapted and domesticated to be useful as germplasm resources (Bosemark, 1989), few sugar beet breeders engage in this kind of work, or they do so in a very limited way. However, most sugar beet breeding organisations participate in the Beta Genetic Resources Network, organised with the assistance of the International Board for Plant Genetic Resources (IBPGR), which aims at organising co-operative, pre-competitive research and pre-breeding using primitive or wild germplasm resources.

The gene pool normally maintained by sugar beet breeders consists of old varieties and a wide range of broad-based populations. Populations in current use, such as diploid or tetraploid pollinator populations, and populations used as sources of inbred lines are repeatedly reselected using mass selection, a combination of individual selection and family selection, or some other method of recurrent selection.

ii) Basic breeding

The simplest method of selection is mass selection: desirable individual roots are selected on the basis of appearance, size, sugar content and technical quality. The selected plants are allowed to flower and set seed together, and the seed is bulked without a prior progeny testing. In half-sib family selection, the progenies are tested in replicated yield trials; only the best families are intermated to form the new improved population (Figure 10.1). Progenies may also be developed through pair crosses between selected roots, so-called full-sib selection. In S_1-progeny selection, selected roots (or a random selection of plants) are selfed and the selfed progenies selected on the basis of their performance in yield trials. If repeated, these methods are referred to as recurrent mass selection, recurrent half-sib or full-sib selection, and recurrent S_1 selection.

Populations improved with one or the other of these methods may be used as such as variety components, they may serve as sources of superior families, or they may be used in inbred line development. With the type of self-fertility used (see section on reproductive mechanisms) it is not necessary to bag plants or to use isolators since virtually 100 per cent of the offspring will be selfed on unprotected plants. To facilitate the development of inbred lines that are maintainers for CMS, self-fertile populations segregating for nuclear male sterility and homozygous for the maintainer genotype may be developed (Figure 10.2). Such a system permits efficient population improvement via S_1 selection and extraction of outstanding S_1 families for the development of inbred lines (Bosemark, 1971). This is done much as for maize, through repeated selfing combined with selection between and within lines.

iii) Variety development

Traditional sugar beet varieties were multigerm, diploid synthetic populations based on a number of populations improved via repeated mass and family selection. Components were usually selected on the basis of a test of general combining ability (GCA). In Europe, beginning in the early 1950s, the diploid synthetics were gradually replaced by polyploid or anisoploid varieties. These varieties, which aimed to exploit the superiority of triploids over diploids and tetraploids, were produced by intermating diploid and tetraploid component populations grown in mixture. The resulting seed was a mixture of diploids, triploids and tetraploids, roughly in the proportions 25 : 50 : 25 (Figure 10.3). The change from diploid to anisoploid sugar beet varieties meant not only that a large number of tetraploid populations had to be created through chromosome doubling, but that these had to be selected and handled separately from the existing diploid populations.

With the almost simultaneous discovery of cytoplasmic male sterility and the monogerm seed character, it became possible to produce different kinds of monogerm hybrid varieties (Figure 10.4). European breeders considered that triploid monogerm hybrids, based on an F_1 hybrid between an inbred male-sterile line and an unrelated maintainer line as the female parent, with a tetraploid population as the pollinator parent [(A × B) × 4 × pop.], offered the quickest way to monogerm varieties with acceptable

Figure 10.1. **Half-sib selection with sugar beet**

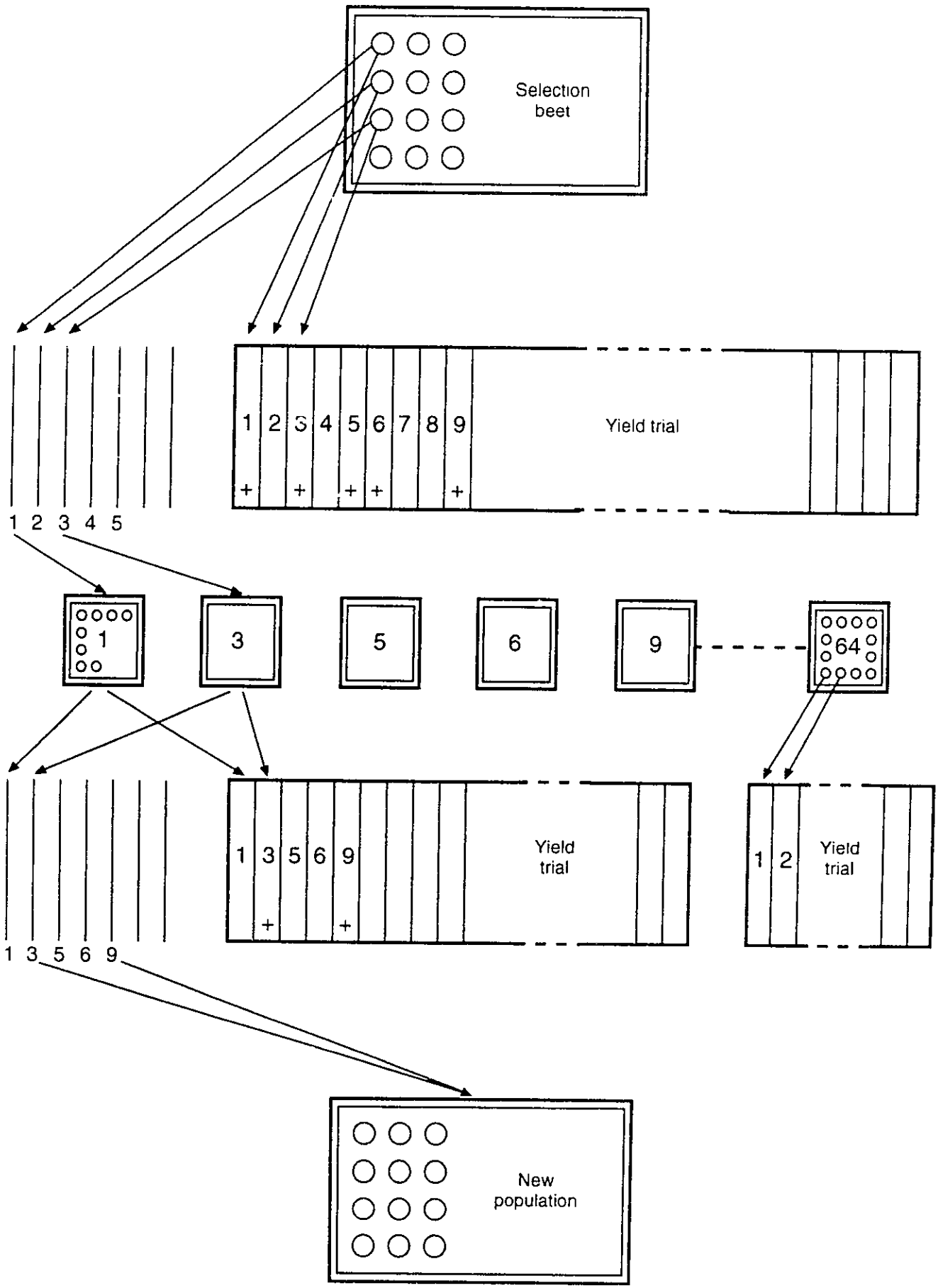
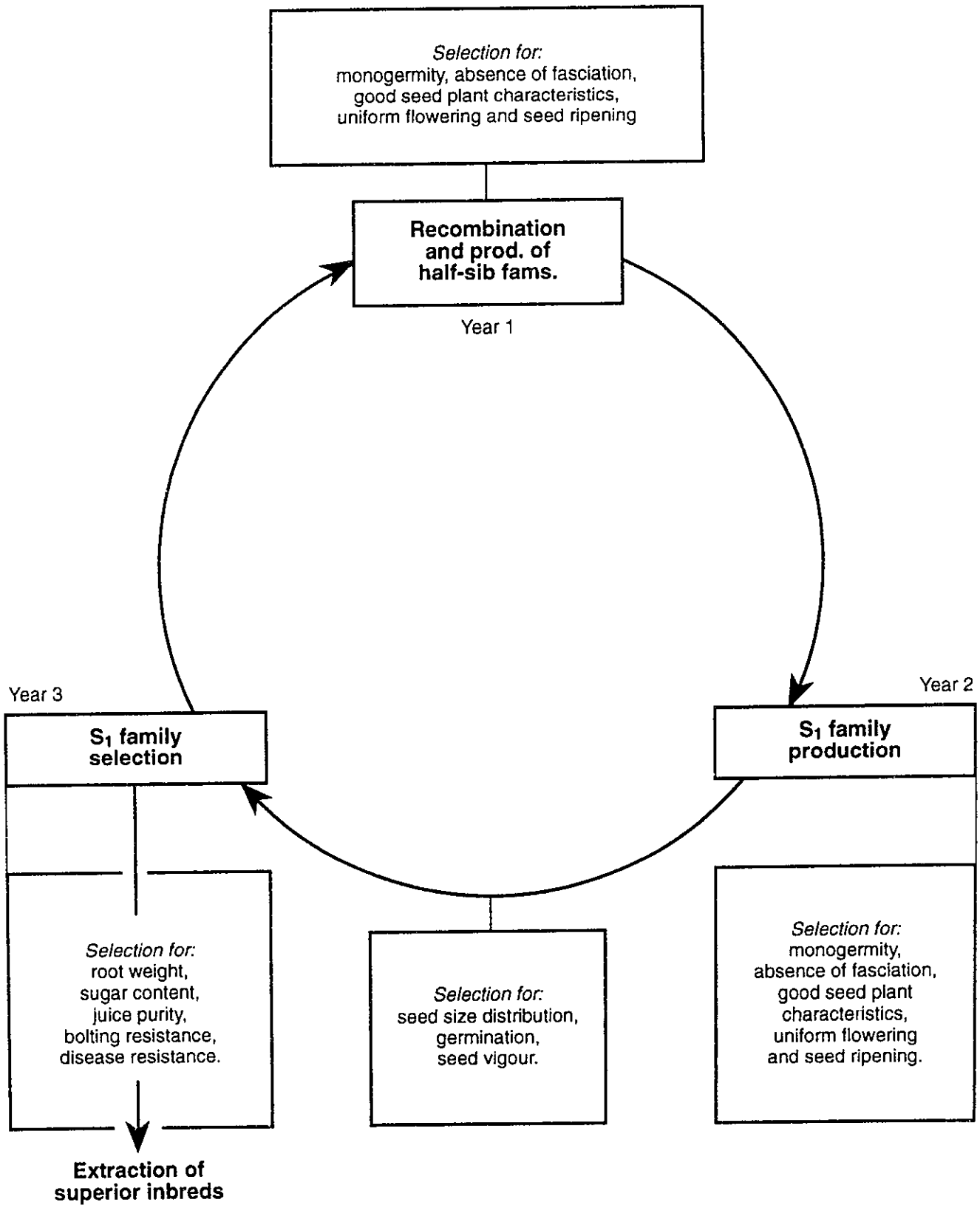
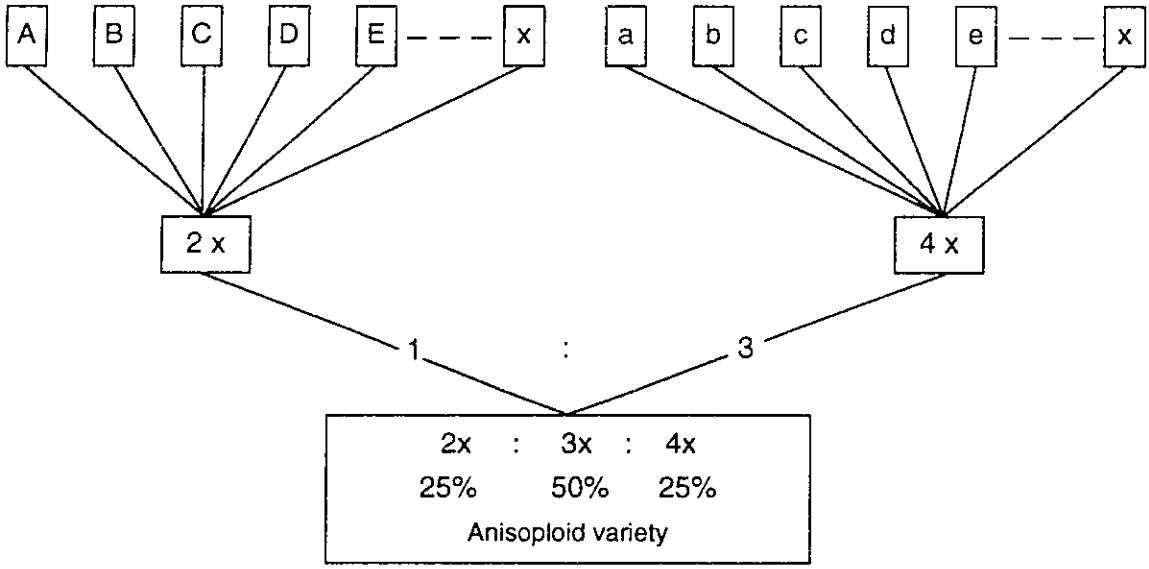


Figure 10.2. **Simple reccurent selection (SRS) in a self-fertile, monogerm, maintainer genotype sugar beet population segregating for nuclear male sterility**



Note: The figure illustrates the characters that may be selected for in each of the three years of a selection cycle.

Figure 10.3. **Mode of producing an anisoploid synthetic sugar beet variety**



Note: Since the tetraploids produce both a smaller amount of pollen and pollen that is less effective than that of the diploids, the mother seed lots of the diploid and tetraploid components have to be mixed in the proportion 1:3 to arrive at a commercial seed containing roughly the proportions of diploids, triploids and tetraploids indicated.

Figure 10.4. **Kinds of hybrids and their corresponding pedigrees**

Kind of hybrid	Pedigree
1. Single cross	A x B
2. Three-way cross	(A x B) x C
3. Double cross	(A x B) x (C x D)
4. Top-cross	A x open pollinated population
5. Top-cross	(A x B) x open pollinated population
(A, B, C, and D stand for inbred lines)	

yield and quality characteristics. In the USA, where breeders had not engaged in developing polyploid sugar beet, they instead produced diploid monogerm top-cross hybrids [(A × B) × 2 × pop.]. Today, there are both diploid and triploid monogerm hybrids in

Europe as well as in the USA, although triploids still dominate in Europe and diploids in the USA. However, there is mounting evidence that, in the long run, specific diploid single-cross hybrids (A × B) may replace existing complex diploid as well as triploid hybrid varieties.

b) Main breeding objectives

The objectives of sugar beet breeding are to create stable, dependable varieties that give the highest possible yield of white sugar per unit area and in relation to production costs, and that also meet the demands of growers and the sugar industry in other respects. These breeding objectives can only be reached through selection for a variety of agronomic and technological characters, some complex, others relatively simple in nature.

The characters subject to selection may be divided into:

- morphological and anatomical characters that affect harvest or factory operations, including root size, shape, fanginess and fibrosity;
- physiological characters, such as bolting resistance, seed and field emergence capacity, disease and pest resistance as well as resistance or tolerance to various abiotic stress conditions;
- chemical characters that affect white sugar recovery in the factory, including sugar content and content of impurities (*i.e.* sodium, potassium and α-amino nitrogen).

c) Testing

All selection work involves visual assessment and assessment by weighing or measuring. While selection of individual roots is based on visual selection in the field followed by weighing and chemical analysis in the laboratory, selection of progenies, from single roots or from different family structures, is based largely on the performance of the progenies in replicated field experiments. Such field experiments are planned and carried out so that they follow good farming practices as much as possible. The trial sites should also be representative of the potential growing area. During the growing season, observations are made on field emergence, general vigour, and diseases and pests. Observations are entered into a field book or a portable computer and later fed into the main data base. At harvest all the beet in the plot, except for guard rows, are lifted and transported to the beet laboratory for washing, weighing and chemical analysis. In recent years some breeding organisations have introduced more or less sophisticated mobile beet laboratories, which are taken into the trial fields. All trial data are processed and compiled by computers which also carry out the necessary statistical calculations.

C. Seed multiplication for commercial use

a) Seed production

As already mentioned, current hybrid sugar beet varieties are almost always diploid or triploid top-crosses in which the female is a single hybrid between a CMS inbred and an unrelated maintainer inbred (CMS-A × maintainer B). Production of basic seed of the

female hybrid parent [CMS(A × B)] thus requires seed of three elite lines: the CMS-line A, its equivalent maintainer line A, and the unrelated maintainer line B.

Where winters are severe, stecklings (small beets for seed production) sown in the early summer are harvested in the autumn, stored over winter and transplanted the following spring when they will develop a seed stalk, flower and set seed. With the overwintering method of seed production, used in the USA as well as in southern France and Italy, stecklings remain in the field all winter. With this method seed production fields may be directly sown (*in situ* seed growing) or the stecklings may be transplanted from over-wintered beds to the seed production fields in the spring.

The commercial seed production fields usually consist of strips of six rows of the CMS parent alternating with two rows of the pollinator. The entire field is surrounded by a few rows of the pollinator. After flowering, but well before seed ripening, the pollinator rows are cut and destroyed, leaving only the CMS rows to be harvested. With about 20 000 CMS plants/ha, the yield of clean but unprocessed seed is 2.5-3.0 tonnes. This results in 500-600 units of processed seed (1 unit = 100 000 seeds), equivalent to 600-720 kg.

b) Isolation practices

According to the OECD beet seed scheme of 10 October 1988, basic seed production must be at least 1 000 m away from any pollen source of the genus *Beta*. For production of certified seed, the minimum isolation distance varies from 1 000 m to 300 m, depending on the chromosome number of the intended pollinator and the chromosome number of a neighbouring pollen source (see Table 10.3). A seed production field is accepted only if there is assurance that there are no volunteer plants of the genus *Beta*.

c) Seed certification

Certified seed must be the first multiplication of basic seed of the cultivar. Satisfactory conditions for the production and processing of certified seed must be ensured by

Table 10.3. **Minimum isolation distances for the production of basic and certified seed of sugar beet under the OECD seed scheme**

Basic seed:		
Distance to any pollen source of the genus *Beta*		1 000 m
Certified seed:		
Ploidy of intended pollinator	Ploidy of neighbouring pollen source	Distance (m)
2x	4x	600
4x	2x	600
2x or 4x	Unknown	600
2x	2x	300
4x	4x	300
2x or 4x	Any other *Beta* species	1 000

field inspection and appropriate tests by the designated authorities. According to the scheme, only seed of cultivars that have shown satisfactory results in official tests in at least one country and been placed on the national list of cultivars of that country are eligible for certification.

Certified monogerm sugar beet seed must have a minimum analytical purity of 97 per cent (by weight), a minimum of 80 per cent germination and a maximum moisture content of 15 per cent. A weighted average sample of the total seed production of a cultivar is made up and used for the annual agronomic trials, in order to determine if any modifications have occurred as a result of maintenance breeding. Cultivars are no longer maintained on the list if the conditions of acceptance are no longer fulfilled.

Both basic seed and certified seed are produced under the responsibility of the breeder/seed producer who continuously surveys his seed crops and tests the purity and quality of the harvested seed during seed processing. As mentioned above, derivatives of wild annual *Beta* beets grow as weeds in fields or on wasteland in many parts of the Mediterranean area. Stray pollen from such weed beets had very limited possibilities for contaminating seed crops of the old diploid synthetic varieties, since these were well protected by an abundance of their own pollen. However, with the introduction of hybrid varieties, where 75 per cent of the plants in the seed production fields are male-sterile, contamination sometimes became a problem, especially since the tetraploid male parent plants usually open their flowers and release pollen later in the morning than do diploids. Thus, diploid male-sterile flowers were open to pollination by stray pollen for a period of time almost every morning during flowering. As soon as this was realised, breeders moved the seed production out of areas with known weed beet occurrence. Although this has virtually eliminated the problem, many sugar beet seed companies test the seed from every grower for the presence of annual weed beet hybrids, and they discard all seed lots that contain such hybrids above a certain very low level.

d) Surveillance of variety behaviour after release; lifespan of varieties

A variety's performance after commercial release can be followed by studying the results of the official trials conducted in the countries in which the variety has been accepted. The results of these trials as well as the advice of the sugar factory field hands and extension service staff guide farmers in their choice of variety and alert them to outbreaks of pests and diseases.

The commercial life of a sugar beet variety may vary considerably but is rarely longer than ten years. With the steadily increasing number of new varieties being introduced each year, the life cycle of sugar beet varieties tends to get shorter and shorter.

D. Role of emerging technologies

In recent years the advantages offered by cell and tissue culture have been widely recognised by sugar beet breeders. Thus, *in vitro* vegetative propagation is now used both as a means of preserving genotypes and in the development of improved tetraploid populations.

Although sugar beet is a recalcitrant plant, several organisations have developed transformation-regeneration systems for sugar beet and introduced genes for resistance to

various herbicides as well as to virus diseases. Resistance in sugar beet to the new low-dose, broad-spectrum herbicides is of considerable interest, both because satisfactory weed control with current sugar beet herbicides often requires three to four applications, and because under certain conditions the growth of the beet may be retarded. Resistance to a low-dose, broad-spectrum herbicide with short residual time may thus result in fewer applications, better weed control and fewer negative environmental effects. Most of the work on virus resistance concerns beet necrotic yellow vein virus (BNYVV), which is potentially the most damaging sugar beet disease in southern and central Europe. Most attempts to engineer resistance to the BNYV-virus have been through introduction of the virus coat protein gene. So far relatively little information has been released about this work.

As a hybrid crop of considerable economic value, sugar beet is one of the prime candidates for plant genetic engineering. However, due to the complexity of current sugar beet varieties, the introduction of a gene through genetic engineering techniques and the subsequent development of a variety may take nearly as long as sexual introduction from an unadapted gene source.

However, other benefits from advances in molecular biology will, in the long run, be as important to plant breeding as the transfer of genes across sexual barriers. Of particular interest is the access to DNA markers, such as restriction fragment length polymorphisms (RFLPs). When these are found to be genetically correlated with a qualitative or quantitative trait, they can be used as indirect selection criteria for that trait. As understanding of the physiology, biochemistry and genetic control of important plant processes increases, conventional and new plant breeding techniques will progress more and more in concert. This will create the potential for developing sugar beet varieties that not only give higher yields of white sugar and other useful compounds, but do so with less input of agrichemicals and are thus better adapted for sustainable and economical growing systems.

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11. Alfalfa

by

D.K. Barnes

A. Characteristics of the crop

a) Geographic origins; centre of diversity

Common alfalfa is believed to have originated in the area that includes Asia Minor, Transcaucasia, northwestern Iran, and northeastern Turkey. The climate in these areas alternates cold winters with hot and dry summers. The soils are typically well drained, near neutral in pH, with high lime subsoils (Michaud *et al.*, 1988).

b) Geographic distribution

Alfalfa or lucerne (*Medicago sativa* L.) is the world's most important forage crop. It was recognised and domesticated by early man and is now found growing wild from China to Spain and from Sweden to North Africa to Yemen. It has become acclimatised in South Africa, Australia, New Zealand, and North and South America. More than 32 million ha are grown throughout the world (Michaud *et al.*, 1988).

c) Taxonomic status

The genus *Medicago* contains more than 60 species, two-thirds of which are annuals and one-third perennials. The basic genomic number of Medicago is x = 8, except for a few annuals that have x = 7. Three ploidy levels (diploid, tetraploid and hexaploid) are found among the *Medicago* ssp. *Medicago sativa,* which is the primary species of commerce, is a 32 chromosome autotetraploid species. The origin and evolution of *M. sativa,* which often is referred to as the *M. sativa* complex, has been described by Quiros and Bauchan (1988).

d) Genetic and cytogenetic characteristics

McCoy and Bingham (1988) summarised the cytology and cytogenetic descriptions of alfalfa. More recently, McCoy and Echt (1992) discussed the current status of chromosome manipulations and genetic analyses in alfalfa. They reported that alfalfa tissue is easily cultured and efficiently transformed. Once isolated, genes from virtually any organism can be transferred into alfalfa. Molecular techniques are being used to develop

gene maps of alfalfa. The future for improving traditional and non-traditional traits in alfalfa appears to have few limitations.

e) Current end uses

Alfalfa has the highest feed value for farm animals of all commonly grown hay and pasture crops. It produces more protein per ha than grain or oil seed crops. It has a high mineral content and contains at least ten vitamins, particularly vitamin A. These characteristics make its use as hay, meal, and silage a desirable ration component for most farm animals, especially for dairy cattle (Barnes and Sheaffer, 1985). Alfalfa is also used as high quality pasture for all classes of livestock. Greater rates of gain have been reported for sheep and cattle on alfalfa and on alfalfa-grass pastures than on grasses alone.

Alfalfa is also a primary honey crop in the United States where it accounts for about one-third of the annual production by honey-bees. Honey is frequently a by-product of seed production. A significant quantity of alfalfa seed is used for growing sprouts, a specialty food for human consumption. To date no varieties have been developed specifically for use in either honey or sprout production.

Alfalfa has also gained recognition because of its high rates of biological nitrogen (N_2) fixation. In association with the bacteria *Rhizobium meliloti,* alfalfa can remove more than 200 kg N_2 per ha per year from the atmosphere (Vance *et al.*, 1988). The fixed N_2 is one reason why alfalfa can increase subsequent crop productivity when it is used in rotations. In addition, crops grown after alfalfa benefit from improved water-holding capacity, increased soil organic matter, and reduction of some pathogens. Alfalfa also minimises pollution by reducing water run-off and soil erosion and by removing soil nitrogen from greater depths than can be reached by annual crops (Barnes and Sheaffer, 1985).

Reproductive mechanisms

a) Modes of reproduction and pollination

Development and pollination of the alfalfa flower have been described by Viands *et al.* (1988). Their findings are summarised here.

Alfalfa has a highly specialised papilionaceous flower with a unique tripping mechanism that limits the types of insects that can effect pollination. Perennial alfalfas are primarily cross-pollinated, because the tripping mechanism is combined with a stigmatic cuticle that usually prevents contact of self pollen with stigmatic secretion prior to tripping of the flower. Partial self-incompatibility and various sterility mechanisms also reduce self-pollination in some alfalfa genotypes (plants), but not in all.

Most alfalfa genotypes can be self- and cross-pollinated by hand. Cross-pollination can be done either with or without prior emasculation (removal of pollen from the flower without fertilisation). Cross-pollination with bees may produce both self and hybrid seed in the absence of sterility mechanisms. Only a few genetic markers are available to distinguish hybrid from self seed.

Cross-pollination usually results in higher seed set (seeds per flower and pods per flower tripped) than does self-pollination. Modest evidence suggests that a partial self-

incompatibility system results from interactions between the pollen tube and various parts of the pistil, especially the ovary and ovules.

Two male-sterility systems, genetic and cytoplasmic, have been identified. Two types of genetic male-sterility, each controlled by different recessive genes, have been reported. Genetic male-sterility will not be very useful in field production of hybrids in the absence of a system controlled by a dominant gene. Cytoplasmic male-sterility offers the best mechanism for the production of hybrid seed. Two systems have been identified, both conditioned by single recessive nuclear genes interacting with cytoplasmic factors. Hybrid production is not at present economical, because of generally low seed yields on male-sterile plants. The random distribution of male-sterile plants and pollinator (pollen-producing) plants in the field (rather than in rows), the increased ratio of pollinator plants to male-sterile plants, and the use of high seed setting or high pollen producing pollinators are important for increasing seed yield of male-sterile plants.

An alternative method of producing hybrid seed would be to use female-sterile plants for pollinating male-sterile plants. Large-scale application of this system is presently limited because female-sterility is controlled by a single recessive gene. Female-steriles would have to be maintained either by outcrossing to female-fertile plants or by vegetative propagules.

b) Ability to cross with related species

There is no crop plant that alfalfa can cross-pollinate with. Alfalfa is unlikely to pollinate with related species, because these only occur in a few parts of the world, and even in those areas naturally occurring hybrids are extremely rare. Progeny from natural crosses between *M. sativa* and related species should be similar or better than the related species, in forage quality, adaptation and usefulness to the environment.

Toxicology

For the ruminant animals that consume most alfalfa forage, lignin and bloat are the principal anti-quality factors (Howarth, 1988). The lignin content of alfalfa increases with the maturity of the forage. Increased lignin content decreases digestibility of cell-wall fibre, causing reduced animal performance and very great economic losses. Harvesting forage at the early to late bud stage prevents the detrimental increase of lignin in alfalfa.

The risk of ruminant bloat prevents greater use of alfalfa in pastures, especially for beef cattle production. Research has been conducted on breeding a bloat-safe alfalfa, but no varieties with this trait are available. Attempts are being made to incorporate forage tannin production from birdsfoot trefoil into alfalfa via molecular genetics techniques.

Environmental requirements for life cycles

Alfalfa grows under very diverse environmental conditions. However, high temperatures can inhibit growth, reduce yield, and shorten stand longevity. Cold temperatures can limit adaptation through sub-lethal or lethal winter injury (McKenzie *et al.*, 1988). Alfalfa has the ability to go into dormancy when exposed to unfavorable periods of cold, heat, or drought, and unlike many tree species, it can be forced into or out of dormancy at any time by favorable or unfavorable environmental conditions.

Photoperiod can affect dormancy responses and the development of resistance to cold. A short photoperiod (long night period) is necessary to initiate the development of cold tolerance in cold-tolerant varieties (McKenzie *et al.*, 1988). Light quality may also influence cold tolerance. Alfalfas adapted to southern latitudes are cold-sensitive and lack photoperiodic sensing mechanisms.

Germplasm is available to develop alfalfas adapted to most environmental conditions found in the world's agricultural production areas. However, difficulties can arise when breeding and evaluating alfalfa cultivars for a combination of environmental adaptation factors and resistance to multiple biotic pest problems. There often appears to be a close association between high stress tolerance and low crop yield. The lack of dependable laboratory test procedures to evaluate many types of stress tolerance often requires plant breeders to depend on the unpredictable occurrence of test environments in the field.

B. Current breeding practices and variety development research

a) Main breeding techniques

i) Germplasm maintenance

For alfalfa, germplasm maintenance has been ensured by several international groups which have collected *Medicago* plant introductions (PIs) from most countries where the crop has been grown for a long time. These collections include local ecotypes and randomly collected genotypes. Most basic germplasms are maintained by individual countries as PIs. The two largest collections are held by the VIR Institute in St. Petersburg, Russia (3 700 entries) and the PI collection in Pullman, Washington, USA (2 340 entries). Canada, Australia, France, and the International Centre for Agricultural Research in the Dry Areas (ICARDA) in Syria also have sizeable collections. All of the alfalfa basic germplasms are accessible to scientific exchange.

Efforts are underway to evaluate the basic germplasms in the United States collection for a series of pest resistances, physiological stress tolerances, and agronomic traits. This information is being computerised so that it can be accessed via telephone. The PIs are also being increased and stored under favorable conditions.

ii) Basic breeding

Rumbaugh *et al.* (1988) have described basic breeding population development. Most alfalfa breeding programmes are based on the development of elite populations and/or strains. A population – a group of plants having a similar origin, adaptation, or trait – can be a variety strain, ecotype or any germplasm source. Populations can be maintained as separate entities, or they can be combined. Intra-population improvement usually consists of growing large numbers of plants from a population, selecting 200-300 plants with the target traits, and then recombining (intercrossing) them. The procedure can be repeated for improving a series of traits. Inter-population breeding and/or strain building can be used to increase the genetic diversity of alfalfa populations; open breeding allows genes to flow from one population to another and reach an equilibrium.

iii) Variety development

Variety development procedures vary according to plant breeder. Some procedures are: population improvement, strain crosses, synthetic varieties, and hybrid varieties. Population improvement is often used in cultivar development when breeding for an increased level of pest resistance. A large number of plants can be screened and the most resistant plants saved and intercrossed. The same procedure may need to be repeated two or three times to raise the mean pest resistance of the population to the desired level. Progeny testing is frequently used when selecting for increased yield.

iv) Techniques used

Strain crosses are used for quickly developing cultivars with multiple traits. One population (variety or germplasm source) with a given set of desirable traits can be crossed with a population with a different set of traits. The resulting population will have the combined traits of both populations. The procedure is fast, and it can provide some increased yield from heterosis (hybrid vigour). The primary shortcoming is that the level of expression for most traits will be about the average of the traits in the two parental populations. For some traits, this may be less than the desired level.

The most frequently used method for developing varieties has been the use of synthetics. Tysdal and Crandall (1948) defined an alfalfa synthetic "variety" as a variety developed by crossing, compositing, or planting together two or more strains or clones, with bulk seed being harvested and replanted for further seed increase. It is assumed that a synthetic variety will be advanced one to three generations through open pollination. Prior to 1965, most synthetic varieties had fewer than 40 parent plants. More recently developed synthetic varieties usually have had larger numbers of parent plants (40-200) in order to minimise changes in gene frequencies and to reduce inbreeding during the generations of seed increase (Hill, *et al.*, 1988).

Hybrid alfalfa varieties are a possibility because a cytoplasmic male-sterility system has been reported and alfalfa is an insect-pollinated crop. At present, hybrid alfalfa is not practical, because pollinators do not produce large amounts of seed on male-sterile plants and the increases in forage yield from hybrids do not justify the increased seed costs.

b) Main breeding objectives

The main breeding objective for alfalfa in the last several decades has been the development of resistance to pests. Future varieties will likely combine multiple pest resistances with tolerance to abiotic stress factors, increased forage quality, and new traits that respond to environmental, agricultural, and industrial concerns. The increased number of desired traits in new varieties has required alfalfa breeders to develop extremely broad-based basic breeding populations that "pyramid" the necessary pest resistances and adaptation factors. It is rarely possible to use plant introductions directly in variety development. Some form of pre-breeding or molecular gene transfer procedures will be needed to ensure use of genes from PIs in new varieties.

c) Testing for the most important breeding goals

Testing alfalfa for important traits requires comparing experimental alfalfas to recognised (resistant and susceptible) check varieties in standards tests. Standard test

procedures for the most important pest resistances and physiological traits have been organised and described by the NAAIC, the North American Alfalfa Improvement Conference (Fox *et al.*, 1991). No specific government tests are conducted in the United States for any trait. However, locations are usually available where breeders can have their materials evaluated if they cannot conduct the test themselves. All test data are adjusted with respect to the resistant check cultivar. This allows for easy comparison of data from several tests. A summary of pest resistance data in the United States is presented each year by the Certified Alfalfa Seed Council. The susceptible varieties are those with 0 to 5 per cent resistant plants; those with 6 to 14 per cent resistant plants have low resistance (LR); those with 15 to 30 per cent have moderate resistance (MR); those with 31 to 50 per cent have resistance (R); and those with more than 50 per cent have high resistance (HR). MR and R levels of resistance are sufficient to provide maximum yields during exposures to most pests.

d) Assessment of performance/behaviour

Procedures and check varieties for testing forage yields vary according to the location. Generally, the plot size is about 1 m × 6 m, with four replications. Tests are conducted from two to five years after the year of seeding. Harvests are made at late bud, and the yields are expressed according to weight (tonnes per acre or MT/ha) of dry matter or 12 per cent moisture hay. Persistence is usually measured as a percentage of ground cover by alfalfa in the yield tests. All programmes include check cultivars, but the check cultivars vary among locations. Countries have different requirements for comparing the performance of new varieties in official tests. Nevertheless, the same format is used in essentially all forage yield testing programmes.

C. Seed multiplication for commercial use

a) Stages in seed production

Alfalfa seed production is very complex and requires a high level of grower management and the proper weather conditions. Rincker *et al.* (1988) reported that successful alfalfa seed production is favoured by clear, sunny, warm summer days in combination with little or no rainfall. Those climatic conditions favour both flowering and the pollinating activity of bees. Other critical production concerns include: control of detrimental insects, the supply of effective pollinators, and skillful application of irrigation water during flowering. Dry weather during seed harvest is also critical.

b) Isolation practices

Fields selected for the production of certified alfalfa seed in North America must not have been planted for a period of at least one year to other cultivars or "common" alfalfa (Rincker *et al.*, 1988). Field isolation requirements necessary to prevent genetic contamination are published by the Association of Official Seed Certifying Agencies (AOSCA). The minimum isolation distances from other blooming alfalfa for foundation, registered and certified seed production is 183 m (600 feet), 91 m (300 feet), and 15 m (50 feet) respectively. The minimum isolation distances were based on research initiated by the NAAIC to measure the percentage of contamination (outcrossing). It was learned that the

shape and size of the seed field were important. If 10 per cent or less of a field fell within the 50 m isolation distance, then no isolation was required for certified seed except for a 3 m border between fields. If more than 10 per cent of the field fell within the isolation zone, then that part of the field could not be harvested for certified seed.

c) Seed certification and registration

The release of new alfalfa varieties by both public and industry breeders in the United States is based on the review of standard test evaluation data. The review is conducted by the National Alfalfa Variety Review Board (NAVRB), which was organised in 1962 as the result of meetings by the NAAIC, the American Seed Trade Association (ASTA) and AOSCA. The board consists of four voting members and their alternates, a non-voting chairperson and a secretary. The voting members are a plant breeder from ASTA, a representative at large from ASTA, a public plant breeder from the NAAIC, and a plant breeder from ARS-USDA. The secretary and chairperson represent AOSCA. The board reviews and evaluates information on breeding history, seed multiplication, yield, persistence, pest resistance, and any unique claims. The board reports varieties that are distinctive and merit certification to AOSCA. The characteristics of all varieties receiving favourable action by the NAVRB are described in publications from the Certified Alfalfa Seed Council and from private companies and many state agricultural experiment stations.

Alfalfa variety recognition in the United States can also be obtained by receiving plant variety protection (PVP) from the PVP office in Beltsville, Maryland. The information required is similar to that of the standard test data requested by the NAVRB (Fox *et al.*, 1991). However, the PVP office compares each new variety with its alfalfa data base to ensure that the variety is distinctive in performance, whereas the NAVRB assumes uniqueness on the basis of the breeding background. Most varieties that receive a PVP certificate are also submitted to the NAVRB so as to ensure maximum recognition of the variety and its characteristics. The PVP certificate is most useful for international marketing and for limiting the sale of seed of a non-recognised generation in the United States.

d) Surveillance of released varieties

At present, there is no routine surveillance of released alfalfa varieties in the United States. More than 95 per cent of the currently used varieties are released by industry or developed by a public agency and contracted to a company for seed increase and sales. The owner of a variety has control over both the basic seed and the marketing of certified seed. The certification system ensures varietal purity. The characteristics of each variety approved for certification are well publicised; therefore, there is little reason for a variety to be intentionally misrepresented.

The public and private North American alfalfa research communities work together to monitor and research current problems. Information interchange is fostered by the NAAIC and its three regional conferences. The North American conference meetings occur in even-numbered years and regional meetings in odd-numbered years. The emergence of new pests or new pest races or biotypes is discussed at those meetings. NAAIC committees of public and industry scientists are formed to develop methodologies for breeding or for evaluating new types of pest resistances. As soon as the methods are

proved valid, they are considered standard tests and are published by the NAAIC (Fox *et al.*, 1991).

Another source of feedback on varietal performance in the United States is the interaction in each state between forage extension specialists and growers and industry representatives. Few problems of variety performance go undetected or unheeded. The rapid turnover of new varieties is added insurance that performance problems will be addressed. Generally a variety is submitted to the National Alfalfa Variety Review Board either in the year in which certified seed production is established or in the subsequent year. This ensures a rapid release to growers. The North American alfalfa market is highly fragmented, with many independent seed dealers who market their own varieties exclusively. From 30 to 40 new varieties have been approved by the NAVRB in each of the last five years. The large numbers of new varieties is due to the development of new traits *i.e.,* multifoliolate leaves, to new pest resistances and to the many companies marketing alfalfa seed. The trend is expected to continue for the next few years.

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12. Oilseed Rape

by

M. Renard, J.H. Louter and L.H. Duke*

A. Characteristics of the crop

a) Geographic origins and taxonomy

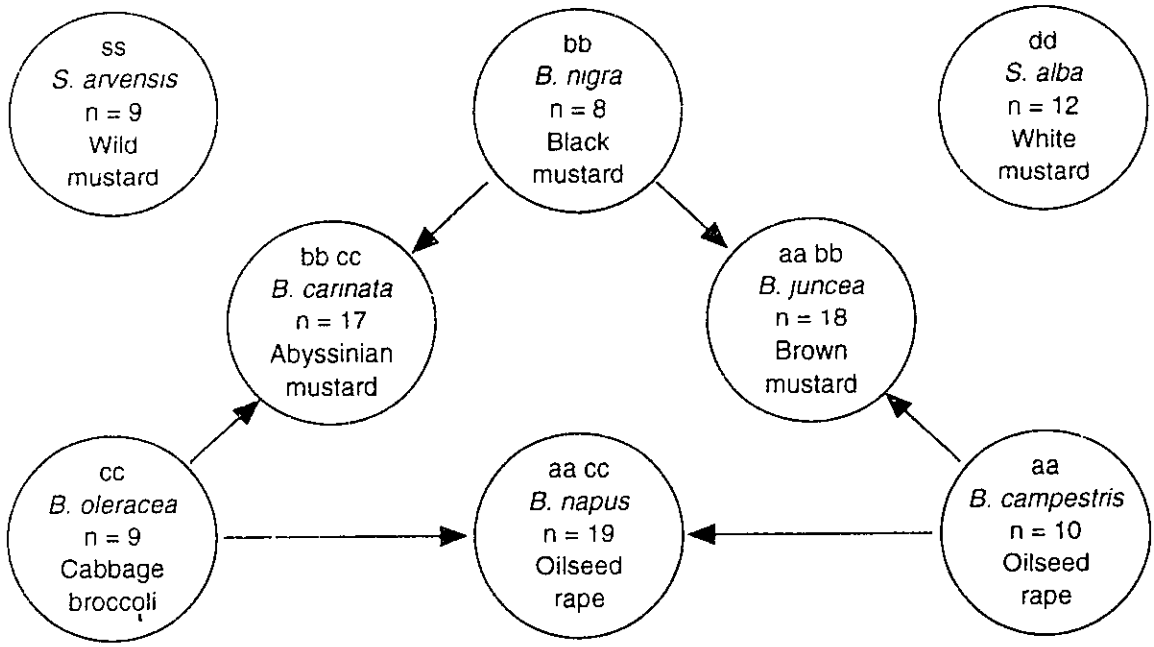
Oilseed rape has been cultivated for thousands of years in Asia and the Indian subcontinent and then later in Europe. Species of oilseed rape include *Brassica napus* L., *Brassica rapa* L. ssp. *oleifera* Metz. [= *B. campestris* L. ssp. *oleifera* (Metz.) Sink.], *Brassica juncea* L. and *Brassica carinata* Braun. All the evidence suggests the Mediterranean area as the centre of origin for these species. *B. napus* is the amphidiploid derived from hybridisation between *B. rapa* and *B. oleracea*; similarly, *B. juncea* was derived from hybridisation between *B. rapa* and *B. nigra*, while *B. carinata* was derived from *B. nigra* and *B. oleracea* (Figure 12.1). Some have postulated that *B. napus* has been formed at various times and places from cultivated forms of *B. oleracea* and *B. rapa* (Rudorf, 1950; Olsson, 1954; both cited in Tsunoda, 1980). On the basis of chloroplast and mitochondrial DNA, Song and Osborn (1991) determined that *B. napus* formed from at least four independent hybridisation events; generally different forms resulted from hybridisations with different maternal parents.

b) Geographic distribution of use; main production areas

Oilseed rape species are well adapted to cool, moist growing conditions and are grown extensively in China, the Indian subcontinent, Canada and northern Europe (Downey and Rakow, 1987). In Canada, northern Europe and China, *B. napus* and *B. rapa* are the predominant species grown. In India and in some parts of China, *B. juncea* is the dominant oilseed crop; *B. carinata* is widely used in Ethiopia. Both the latter two species have potential as major oilseed crops in areas outside their current zones of cultivation.

* The authors wish to thank R.K. Downey and W. Beversdorf for many helpful comments.

Figure 12.1. **Genome relationships of some economically important *Brassica* and *Sinapis* species**



Source: After U (1935, cited in Downey and Rakow, 1987).

c) *Taxonomic status*

There has been some controversy regarding the use of the name *B. campestris* for *B. rapa*; the latter is recommended by Toxopeus *et al.* (1984) on the basis that it was used first to describe the species by Metzger in 1833. *B. rapa* can be subdivided into three subspecies based on morphology and end use, *i.e.* oleiferous, leafy and rapiferous; the oleiferous types can be further subdivided into spring and winter forms. *B. napus* can also be subdivided into oleiferous and rapiferous forms; the oleiferous types can also be subdivided into spring and winter forms. *B. juncea* can be divided into oleiferous and leafy (rugosa) types (Prakash and Hinata, 1980; cited in Bing, 1991).

d) *Genetic and cytogenetic characteristics*

Research on *Brassica* genetics is relatively recent and no classic chromosome map has been produced. Chromosome mapping is now in progress, using molecular markers (Landry *et al.*, 1991). The main cytoplasmic traits known and used are triazine resistance and cytoplasmic male-sterility. From inter- and intra-specific crosses, various cytoplasmic male-sterility (CMS) systems have been obtained (Renard *et al.*, 1992). The "polima" system is the most widely used, but the "ogura" system improved by protoplast fusion seems to be more promising and is expected to be used for hybrid seed production in the short term in Europe and North America.

The genetic relationships among the various *Brassica* species are best illustrated using the "triangle of U" (Figure 12.1). In Figure 12.1, the small letters at the top of the circles symbolise the genome, and "n" indicates the haploid number of chromosomes.

According to Downey and Rakow, (1987) interspecific crosses are more successful if an allopolyploid species such as *B. napus* or *B. juncea* is used as the female parent, especially if the allopolyploid has one genome in common with the pollen parent Hybrids between monogenomic species are more difficult, with success rates of 0.002 to 0.03 hybrids per pollinated flower (Downey *et al.*, 1980; cited in Downey and Rakow, 1987).

e) Current phytosanitary considerations in movement of germplasm

In the past 20 years, there has been considerable movement of germplasm around the world. Generally, on a global basis, phytosanitary or import permits are not required but may be helpful if submitted (Ronikier, personal communication). In Canada, import permits are not required except for seed of transgenic oilseed rape (Prange, personal communication). Seed exchanges between China, India, Australia and New Zealand with Europe and North America are strictly controlled and often require quarantine to guarantee freedom from disease. In countries where phytosanitary certificates are required, seed may be inspected for the presence of *Leptosphaeria maculans* (rarely more than 2 per cent of seed carries this fungus, according to Martens *et al.* 1984), *Albugo candida, Alternaria brassicae* and sclerotia of *Sclerotinia sclerotiorum* or other obvious seed defects and pests. In Canada, breeder (pre-basic) seed of oilseed rape must be tested for *Leptosphaeria maculans* and must meet certain germination and quality standards.

f) Current end uses

The predominant use of oilseed rape is for the oil pressed from the seed and used for human consumption either as a cooking oil or for further processing or as a fuel for lamps. The oil content generally varies from 41 to 44 per cent depending on the variety (Thomas, 1984). The fatty-acid composition of the oil is presented in Table 12.1.

The oil-free meal can be used as a high protein feed supplement for livestock and poultry; the protein content of the meal may vary from 36 to 44 per cent (Anjou *et al.*, 1977 cited in Salunkhe *et al.*, 1992). The amino-acid profile of the meal is given in Table 12.2.

In Canada, oilseed rape varieties that meet the requirements of less than two per cent erucic acid in the oil and less than 30 µmoles glucosinolates per gram of oil-free meal (sometimes referred to as "double low" or "double zero") can be called "canola" varieties to distinguish them from varieties that do not meet these standards (Thomas, 1984).

In 1997, the definition of canola will be further refined: *canola seed* shall be of the genus *Brassica* which contain less than 18 µmoles of total glucosinolates per gram of whole seed at a moisture content of 8.5 per cent; the oil content of the seed shall contain less than 1 per cent of all fatty acids as erucic acid; *canola meal* will be used to describe a protein meal derived from seeds of the genus *Brassica* containing less than 30 µmoles of total glucosinolates per gram of meal at a moisture content of 8.5 per cent; and *canola oil* will be used to describe an oil derived from the seed of the genus *Brassica* with less than

Table 12.1. **Fatty acid composition of oils in rapeseed cultivars**

Species	Cultivar	Country of origin	Percentage of fatty acids						
			C16:0	C18:0	C10:1	C18:2	C18:3	C20:1	C22:1
B. napus	Major	France	3.5	1.2	14.2	13.8	9.1	10.9	46.9
	Primor	France	4.5	1.5	60.5	20.5	10.3	0.9	0.2
	Altex	Canada	3.5	1.3	60.7	20.5	10.4	2.5	0.5
	Tower	Canada	3.5	1.4	60.5	20.7	10.4	2.5	0.6
	Regent	Canada	3.4	1.4	61.7	20.0	10.2	2.5	0.7
B. rapa	Torch	Canada	2.8	1.3	57.4	20.1	10.8	3.1	3.3
	Candle	Canada	3.0	1.3	57.1	22.1	12.6	2.5	1.0
	R-500	Canada	1.5	0.7	12.1	11.4	8.3	5.6	58.6

Source: Daun and Bushum (1983); cited in Salunkhe *et al.* (1992).

1 per cent of all fatty acids as erucic acid (as determined by the latest officially accepted International Standards Organisation method).

High erucic acid oil is used mainly for specific industrial purposes, *e.g.* in the production of synthetic rubber and plastic and as a lubricant in marine engines and in the cold-rolling of steel.

Table 12.2. **Amino acid composition of rapeseed and soybean meals**

Amino acid	Rapeseed meal (%)	Soybean meal (%)
Alanine	1.6-1.7	1.9
Arginine	2.1-2.2	2.9
Aspartic acid	2.5-3.1	5.0
Cystine	0.2-0.5	0.3
Glutamic acid	6.4	8.1
Glycine	1.8-1.9	2.1
Histidine	1.0	1.1
Isoleucine	1.3-1.5	2.1
Leucine	2.5-2.7	3.4
Lysine	2.1	2.8
Methionine	0.7	0.6
Phenylalanine	1.4-1.5	2.2
Proline	2.3-2.7	2.3
Serine	1.6-1.7	2.3
Threonine	1.6-1.7	1.7
Tryptophane	0.4	0.5
Tyrosine	0.8-0.9	1.3
Valine	1.8-1.9	2.3

Source: Clandinin *et al.* (1978); cited in Salunkhe *et al.* (1992)

Reproductive mechanisms

a) Mode of reproduction and pollination

B. rapa is cross-pollinated because of a sporophytic incompatibility mechanism in the stigma that prevents pollen germination or pollen tube growth if the pollen is from a plant of the same genotype as the stigma (Downey and Rakow, 1987). *B. napus* is mainly self-pollinated but has been reported to outcross at an average rate of 30 per cent (Rakow and Woods, 1987; cited in Downey and Rakow, 1987).

The stigma is generally receptive for a period of three days before and after flowering. Once fertilisation occurs, the ovary elongates and forms pods (siliques) containing 25 or more seeds. Many pods are produced per plant resulting in a high multiplication factor.

B. rapa and *B. napus* have relatively large, bright-coloured flowers with four nectaries. The flower is well adapted for insect pollination and is very attractive to bees. Tasei (1978) found that bees visited the crop when 20 flowers per sq. m. were in bloom and reached a density of two foraging bees per sq. m. All worker bees take nectar; up to 20 per cent take nectar and pollen but none takes pollen alone. Neither wind nor insects are required for species that can self-pollinate (*B. napus*), although wind is probably important; for species that are self-incompatible (*B. rapa*) both wind and insect pollination are important.

b) Dispersal and survival mechanisms of propagules

Seed is the sole means of propagation in nature. Both species disperse mature seed through pod shattering; while seeds remain viable in the soil for more than ten years, seed dormancy is not considered a problem with the spring form of *B. napus*. The characteristics of pod shattering and seed dormancy contribute to the weediness aspect of these species. Efficient management techniques can reduce seed dispersal to a minimum. Shattering of pods tends to occur if the mature pods are harvested late. Therefore, if harvested at the right time, seed will only be dispersed by farm machinery or local fauna. Farm machinery must be kept clean and the cleaning must be conducted in a controlled area. Volunteer plants will need to be controlled using common weed control practices.

c) Ability to cross with wild species

Crosses between oilseed rape species and some weedy relatives occur with varying levels of difficulty depending on the cross (Figure 12.1). As was mentioned previously, interspecific crosses are more successful if an allopolyploid species is used as the female parent and there is one genome in common with the male parent. Bing (1991) investigated the crossing potential among several oilseed *Brassicae* by attempting hand pollinations and by planting crossing blocks in the field (Table 12.3).

B. napus and *B. rapa* can produce hybrids but of much reduced fertility. *B. napus* can also cross with *B. juncea,* and these hybrids can produce a small amount of seed and fertile progeny. Crosses between *Brassica* species and species in the genera *Raphanus* and *Diplotaxis* have also been observed.

Table 12.3. **Crossing potential between oilseed *Brassicae* and some weedy relatives, including *Sinapis arvensis***

Species used as female	Viable seed per 100 hand-pollinated buds Species used as male				
	B. napus	*B. rapa*	*B. juncea*	*B. nigra*	*S. arvensis*
B. napus		1 490.5	NT	0.9[1]	0[2,3]
B. rapa	933.8		NT	0.5	0
B. juncea	401.9	NT[3]		3.1[1]	2.5[1]
B. nigra	0.1[3]	0[3]	0.5[3]		77.4[4]
S. arvensis	0[3]	0	0[3]	7.0	

1 Dependent on parental genotype.
2 One plant from ovule culture.
3 No naturally occurring hybrid plant found when planted in the field.
4 Highly sterile
NT Not tested by Bing (1991).
Source: Bing (1991).

Toxicology

In the 1950s, it was recognised that the erucic acid content of rapeseed oil was nutritionally undesirable, because animal feeding studies had shown an abnormal fat build-up. At high feeding rates, growth retardation and heart lesions could result. This effect has not been recorded in humans (Ward *et al.*, 1985, p. 44). Plant breeders were successful in using traditional plant breeding methods to reduce the erucic acid content of the oil to less than 2 per cent. There are some niche markets for high erucic acid oil for use in lubricants and in plastics manufacture.

The oil-free meal could have been used as a high-protein feed supplement for livestock, were it not for the presence of compounds known as glucosinolates which can cause thyroid problems in non-ruminant animals and palatability problems in ruminant animals. Again, plant breeders were successful in reducing the amount of glucosinolates while maintaining a low erucic acid content in the oil. Varieties that meet the "canola" standard (double low) do not have these compounds in significant amounts and are commonly used for livestock consumption.

Sinapine and phytic acid are present at 1 and 1.5 per cent, respectively, and are undesirable. Sinapine is undesirable because it gives a "fishy" odour in brown-shelled eggs from hens fed the oil-free meal and phytic acid due to its chelating activity (especially zinc). The effect of phytic acid on zinc availability is easily mitigated by adding zinc to foods or by vitamin supplements (Shah *et al.*, 1979; cited in Downey and Rakow, 1987).

Environmental requirements for life cycles

a) Climatic restrictions to extension of the crop

Oilseed rape is an annual or winter biennial and is adapted to agricultural regions of cool, moist temperate zones or higher altitude areas of subtropical zones; where condi-

tions permit, the winter form of *B. napus* is the most productive and is widely grown in Europe and China. Winter forms of *B. napus* survive best in zones with winter survival requirements between those of winter oats and winter barley (Downey and Rakow, 1987). As latitude or altitude increase, the winter form of *B. napus* is supplanted by the summer form of *B. napus* or the winter or summer form of *B. rapa*. *B. juncea*, the dominant species grown on the Indian sub-continent, is well-adapted to drier conditions and is relatively fast maturing. *B. carinata* may perform well under long season growing conditions in many parts of the world but is currently grown principally in northeast Africa.

b) Biological restrictions to extension of the crop

B. napus and *B. rapa* require large amounts of water; water requirements peak during the period from flowering to ripening of seed when up to 8 mm of water per day is transpired (Thomas, 1984). Depending on the moisture stored in the soil, the soil type and other environmental conditions, *B. napus* and *B. rapa* may require 250 to 350 mm of rain during the growing season. The optimum temperature for growth of these species is about 20°C (Thomas, 1984).

B. Current breeding practices and variety development research

a) Main breeding techniques

i) Germplasm maintenance

The centres of origin of oilseed *Brassicae* have been mentioned previously. Germplasm accessions of oilseed *Brassicae* number about 500 in the gene bank maintained by Agriculture Canada in Ottawa; however, a more extensive collection with about 7 000 accessions is in the planning/implementation stage at the Agriculture Canada Research Station in Saskatoon. There are also extensive collections maintained in the United States, Germany, Spain and Japan.

ii) Basic breeding

Breeding activities in oilseed rape are generally directed at the development of improved populations or lines, although in recent years there has been some effort directed at the development of inbred lines for the production of hybrids. In the largely self-pollinated *B. napus*, backcrossing, pedigree and haploid breeding systems have been used successfully (Downey and Rakow, 1987; Beversdorf, personal communication). Breeding material is evaluated as the progeny of F_2 and F_3 plants in single rows, followed by replicated yield trials. For *B. rapa*, which is self-incompatible, backcrossing and recurrent selection have been employed most frequently (Downey and Rakow, 1987).

iii) Hybrid varieties

Besides self-incompatibility (*B. rapa*), various systems have been proposed for the production of hybrid *Brassica* cultivars: treatment with male gametocides, nuclear male-sterility (NMS) and cytoplasmic male sterility (CMS) systems. Since the sterility induced by gametocides is generally low and variable, and phytotoxic effects are observed,

gametocides do not have immediate practical utility. Recently, a NMS was obtained using *Agrobacterium tumefaciens* transferring a male-sterile gene coding for a ribonuclease and a restorer gene inhibiting the RNase gene. Male-sterile (MS) plants with the MS gene linked to a herbicide resistance gene can be selected by treating with that herbicide. Several male-sterility inducing cytoplasms, which could be developed into CMS systems for use in *Brassica* hybrid production, have been discovered. The "ogura" CMS system improved by protoplast fusion appears to be the most promising CMS system for *B. napus* in the short term.

iv) Variety development

In Canada and Europe, all pertinent agronomic and quality traits are evaluated and the best lines are tested in co-operative trials over a broad range of locations in the intended area of adaptation over a number of years. To receive approval for registration and sale, the line must equal or exceed the performance of the best current varieties. It may take eight to ten years from the time the cross is made to the time the variety is released for sale (Downey and Rakow, 1987).

b) Main breeding objectives

Seed yield is the major objective of most breeding programmes; in the 1960s and 1970s however, this objective was secondary in importance to the development of low erucic acid and glucosinolate varieties. Once double low varieties were a reality, seed yield again became prominent as a breeding objective. Current breeding objectives include: disease resistance (*Leptosphaeria maculans, Albugo candida, Sclerotinia sclerotiorum, Alternaria brassicae, Verticillium dahliae, Peronospora parasitica*); maturity (to fit the area of intended cultivation); oil and protein content; fatty acid composition of the oil; reduced fibre content (by crossing with Indian yellow sarson); reduced anti-metabolites; and reduced free fatty acid content.

Triazine tolerance was first observed in a weedy form of *B. rapa,* known as bird rape, by Souza-Machado *et al.* (1978) and was successfully transferred to *B. napus* by backcrossing (Beversdorf *et al.*, 1980). Thus triazine tolerant oilseed *Brassicae* could be grown on land previously treated with triazine herbicides or on land infested with triazine sensitive cruciferous weeds.

The fusion of biotechnology and agriculture has created new possibilities for plant breeders by introducing new and sometimes exotic traits into oilseed *Brassicae.* The possibilities are many but the earliest products of plant biotechnology will probably include tolerance to herbicides, insect resistance and changes in oil quality.

C. Seed multiplication for commercial use

a) Stages in seed production

In Canada, breeder (pre-basic) seed plots are sown in wide rows to allow roguing of off-types and weeds (Downey and Rakow, 1987). Breeder seed is then distributed to members of the Canadian Seed Growers' Association (CSGA) who produce foundation seed for their own use or for sale to other seed growers. Foundation seed is sold to other members of CSGA who produce certified seed for sale to farmers (CSGA, 1988). All

seed in the Canadian pedigreed seed system is inspected by government inspectors for off-types, weeds, and disease and must meet germination and "canola" quality standards.

In Europe, varieties of *B. napus* are maintained by selfing single plants, growing progeny rows and eliminating off-types (Downey and Rakow, 1987). The plant uniformity requirements for *B. rapa* varieties are less stringent than those for *B. napus* because it is an obligate outcrossing species. The trend towards plant breeder rights in recent years has caused breeders to be more concerned with the genetic purity of their breeder seed so that claims of distinctness, uniformity and stability can be verified.

b) Isolation practices

Pedigreed seed of oilseed *Brassicae* is produced in Canada on land without a history of oilseed *Brassica* or mustard production during the preceding five years for foundation seed or three years for certified seed (CSGA, 1988). *B. napus* plots for foundation seed production must be 200 m from another variety of oilseed *Brassica*; *B. rapa* must be 400 m from another variety of *B. rapa*. Isolation distances for these species for certified seed production is 100 m (CSGA, 1988). In Europe, breeder seed must be isolated from other oilseed *Brassicae* by 1 000 m, basic seed by 400 m and variety seed by 200 m.

c) Surveillance of variety behaviour; lifespan

During multiplication, varieties continue to undergo evaluation for performance and quality (erucic acid and glucosinolates) attributes. Following commercial release, evaluations continue through co-operative public and/or private variety evaluation trials. In Ontario, Canada, oilseed varieties remain on the recommended list as long as they yield 97 per cent of the yield of standard check varieties; varieties that do not meet this standard are deleted from the recommended list. The recommended list is a list prepared for farmers suggesting which varieties will perform best in their area.

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13. Cole Crops

by

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A. Characteristics of the crop

a) Geographic origin

Cole crops belong to the species *Brassica oleracea* L., of which cabbages are the most important. *B. oleracea* originated in southern Europe, where it grows along the coasts of the Mediterranean Sea.

b) Main production areas

White cabbage is grown on a large scale world-wide over a total estimated area of more than 1 million ha. In western Europe the second most common cole crop is cauliflower with an area of about 100 000 ha, but in most other regions it is grown on a small scale. Brussels sprouts are only important in northwestern Europe; an area of about 40 000 ha is used for this crop. In the Netherlands and the United Kingdom, the production value of Brussels sprouts is higher than that of cabbages. Kohlrabi, sprouting broccoli, curly kale and red and savoy cabbage are mostly grown on a limited scale.

c) Taxonomic status; genetic and cytogenetic characteristics

There are many different types of *Brassica* species. However, the great diversity of forms can be reduced, on the basis of their chromosome number, to three elementary monogenomic species, with single sets of chromosomes n = 8, 9 or 10, and three digenomic species, with single sets of chromosomes n = 17, 18 or 19, which originated in nature from crosses between the monogenomic species (Table 13.1). The cole crops belong to *Brassica oleracea,* a monogenomic species with 18 chromosomes (2n = 18) in the diploid stage.

The different botanical varieties of *B. oleracea* are given in Table 13.2. *B. alboglabra,* a type with white flowers and with 2n = 18 chromosomes, also belongs to *B. oleracea.*

Table 13.1. **Monogenomic and digenomic *Brassica* species**

	Cultivated types	Number of chromosomes (n)	Constitution of genome
Monogenomic species			
B. nigra	Brown mustard	8	b
B. oleracea	Cole group	9	c
B. campestris	Turnip group Chinese cabbage	10	a
Digenomic species			
B. carinata	Ethiopian mustard	17	bc
B. juncea	Brown mustard Leaf mustard	18	ab
B. napus	Swede group Rape	19	ac

d) Current end uses

Cole crops are mainly used for fresh consumption. In some countries part of the autumn-harvested cabbage is stored for several months at low temperatures. White cabbage may be fermented to sauerkraut. Sprouting broccoli, Brussels sprouts and, to a lesser extent, other cole crops are also quick-frozen. They are also used for canning, drying and pickling, but only on a small scale.

In many countries, 20 to 30 per cent of the area cultivated with vegetables is used for this crop. Cabbage yields can be high, sometimes over 50 tons per ha, and consumer prices are mostly low. Cole crops contain much more vitamin C than many other vegetables and have intermediate to high protein content. Leafy types, such as kale, are also rich in carotene and calcium. Consequently, cole crops play an important role in human nutrition, and it is important that plant breeders improve this cross-pollinated crop. Since the 1960s hybrid varieties have become far more prevalent and in some countries replace almost entirely the old open-pollinated varieties.

Table 13.2. **Cultivated vegetable types of *Brassica oleracea***

Latin name	Common name
B. o. var. *capitata* f. *alba*	White cabbage
B. o. var. *capitata* f. *rubra*	Red cabbage
B. o. var. *sabauda*	Savoy cabbage
B. o. var. *botrytis* subvar. *cauliflora*	Cauliflower
B. o. var. *botrytis* subvar. *cymosa*	Sprouting broccoli
B. o. var. *gemmifera*	Brussels sprouts
B. o. var. *gongylodes*	Kohlrabi
B. o. var. *acephala*	Curly kale

Reproductive mechanisms

a) Mode of reproduction and pollination

During flower differentiation the flower develops four sepals, six stamens, two carpels and four usually yellow petals. Flowers are pollinated by insects, particularly bees, which collect pollen and nectar. Seeds are globular, 2-3 cm in diameter, and greyish black to reddish brown. The fruits, which contain 10 to 30 seeds, are siliques (pod-shaped, breaking open at maturity both at dorsal and at ventral side, shedding the seed freely) and sometimes over 10 cm long.

Brassica crops are cross-pollinators, and fertilisation by self-pollination is prevented by an incompatibility system. Pollen on stigmas of flowers of the same plant either fails to germinate or only produces short pollen tubes that do not invade the stigmas, due to deposits of callose produced by the stigmatic papillar cells in direct contact with a pollen grain a few hours after pollination. The incompatibility reaction is maximal in flowers that have just opened. Seed setting is much higher when selfing is carried out two to four days before flowers open, a method used to overcome the incompatibility mechanism in order to self plants for breeding purposes (bud pollination). Self-incompatibility may decrease again some days after the flowers open, thereby making mass propagation of parent lines of hybrids possible by insect pollination.

All cole crops possess an active self-incompatibility mechanism, except the early and summer cauliflower varieties grown in temperate climates.

The self-incompatibility system of cole crops is controlled by a series of alleles (S-alleles) located at one locus. Each S-allele produces its own specific protein, which accumulates in pollen walls and pistils. When identical S-proteins are present in pollen and pistil, a self-incompatibility reaction is initiated. Over 50 S-alleles have been identified in cole crops, and plants are normally heterozygous for them.

The reaction between S-alleles is determined by a sporophytically determined incompatibility system. This means that pollen behaviour is determined by both S-alleles of the pollen-producing plant. S-alleles may act independently of each other, or one S-allele may be dominant, in pollen or style or both. Recessive S-alleles, which do not induce a high degree of self-incompatibility, are very common in many cultivars, while active dominant S-alleles are rare. Table 13.3 offers a simplified picture of the effect of the relationship between the S-factors on the incompatibility reactions. The effects of S-alleles may be influenced by environmental factors and genetic background, as dominance in many cases may be partial and mutual weakening of S-factors may also occur.

b) Growth and development

In temperate zones cole crops for seed production are sown in the summer, flower bud initials are produced in the autumn and winter, and plants start bolting and flowering in the spring. In temperate zones cole crops are biennial, with the exception of the annual types of cauliflower and broccoli. In the first year after sowing, plants develop vegetatively, first producing a number of petiolate leaves (juvenile stage) and subsequently sessile leaves (adult stage). Plants must have reached the adult stage before they can

Table 13.3. **The effect of the relationship between S-factors in style and pollen on the incompatibility reactions in the F_1 of a selfed SxSy plant**

Relation between S-factors[1]		Incompatibility reaction[2]
Style	Pollen	
Sx = Sy	Sx = Sy	SxSy SxSx ⟷ SySy
Sx = Sy	Sx > Sy	SxSy SxSx ⟷ SySy
Sx > Sy	Sx = Sy	SxSy SxSx ⟷ SySy
Sx > Sy	Sx > Sy	SxSy SxSx ⟷ SySy

1. Sx = Sy: independent relation; Sx > Sy: Sx dominant to Sy.
2. —— : incompatible reaction; ⟶ : compatible reaction in the direction indicated.

move into the generative stage. This normally takes about three months. Flower bud initials are laid down after exposure to temperatures below 10°C for at least two months. After this period, plants start bolting and flowering at higher temperatures. Day length does not influence flower formation and bolting.

Toxicology

In the subspecies of *B. oleracea*, glucosinolates and the amino acid S-methyl cysteine sulphoxide are found. When large amounts of cole crops are consumed, breakdown products of these compounds may be harmful to livestock. With the restricted daily intake of the human diet, no toxicity problems occur. A considerable heritable variation has been found for the concentrations of these compounds.

Environmental requirements

Optimal temperatures for flowering are 15 to 20°C; at higher temperatures flower drop may occur. In the tropics, temperature requirements for flowering are not usually met, although seed production may be possible at high altitudes. Varieties with higher maximum temperatures for flower initiation might prove useful in these areas. The annual types of cauliflower and broccoli produce flowers in the first year of growing, but temperatures must remain below 25°C.

B. Current breeding practices and variety development research

a) Main breeding techniques

i) Germplasm maintenance

Large collections of old open-pollinated varieties and wild relatives of *B. oleracea* are maintained in different gene banks, so the risk of loss of valuable gene material seems limited.

ii) Hybrid breeding by means of self-incompatible parent lines

Since the beginning of the 1960s, hybrid breeding of cole crops has been accomplished by means of self-incompatible lines on a large scale. The hybrids are produced by cross-pollination of two mutually compatible, but self-incompatible parent lines.

iii) Production of parent lines

Selected parent plants for hybrid breeding programmes are first checked for self-incompatibility by self-pollinating flowers that have just opened. When more than two seeds per pollinated flower are produced, plants are discarded. Incompatibility can also be assessed easily and rapidly by preparing detached styles one or two days after self-pollination with aniline-blue. Fluorescing pollen tubes can be observed under the microscope, using ultraviolet light. If less than three pollen tubes per style are present, the degree of self-incompatibility is considered sufficiently high.

Parent plants and plants of successive generations are selfed by bud pollination. After five to seven generations almost completely homozygous, very uniform inbred lines are obtained, although they have a much lower growth vigour (slower growth, smaller plants) than the original parent plants (inbreeding depression).

In cole crops a rapid large-scale production of homozygous lines is possible by *in vitro* anther culture. Embryo yields obtained in this way show a large variation, from zero to more than 200 embryos per 100 anthers in exceptional cases. Some genotypes are very responsive to this technique, and others are completely unresponsive. Cultures of isolated microspores also offer possibilities. Spontaneous chromosome doubling during anther culture often results in a high percentage of diploid plantlets.

The production of inbred lines by means of anther culture results in a large number of lines. To reduce this number, selected parent plants should first be propagated for one or two generations by bud pollination and plants selected from the most promising self-incompatible lines for anther culture.

Lines with an extremely low growth vigour and deviating characters (loose heads, weak stalks, deviating flower colour, extremely early or late flowering) are rejected. The remaining lines are selected on the basis of results of test crosses. To determine general combining ability, the lines are crossed with one or two tester lines. When crossing inbred lines, growth vigour is virtually restored. Sometimes hybrids are found with better performance than the parent populations (heterosis). Among a restricted number of the most promising lines, all possible crosses are made (diallel cross) to determine the specific combining ability of these lines and to trace those that give the best hybrids.

iv) Hybrid breeding by means of male-sterile parent lines

Male-sterile plants have been found in several cole crops. Male-sterile flowers produce short filaments and shrivelled anthers that do not produce pollen. Male sterility is usually determined by a single recessive gene: msms plants are male-sterile, and MsMs and Msms plants are male-fertile. Male sterility may also be controlled by a series of recessive genes. A monogenic dominant male-sterile mutant has been obtained by mutagenesis. Sometimes male sterility is clearly influenced by temperature. At low temperatures (10°C) plants may be male-fertile and at higher temperatures partially or completely male-sterile.

To date, no cytoplasmic-genetic male-sterility (CMS) has been found in cole crops, so it is not feasible to produce completely male-sterile parent lines. Only lines with 50 per cent male-sterile plants can be produced (crossing msms with Msms produces 50 per cent msms plants and 50 per cent Msms plants). CMS can be induced by transferring the genome of *B. oleracea* into the cytoplasm of *B. napus* or *Raphanus sativus* (radish). Species crosses have been made, and the F_1 hybrids backcrossed for a number of generations with *B. oleracea.* Male-sterile lines and maintainers can be obtained in this way. However, results so far have been disappointing, due to chlorosis of seedlings and young leaves (in crosses with radish), partial and unstable male sterility, floral abnormalities, poor seed set and lack of nectaries. Protoplast fusion of CMS *B. napus* and *B. oleracea* has led to the production of male-sterile cybrids with the plasm of *B. napus* and the genome of *B. oleracea.* Male-sterile cybrids of CMS *Raphanus sativus* and *B. oleracea* have been developed in the same way. Such cybrids seem to be male-sterile independent of their pollinators and offer good prospects for producing hybrid varieties of cole crops by means of male-sterile lines.

b) Main breeding objectives

In the last 20 years almost all the efforts of breeders of cole crops have been concentrated on the production of hybrid varieties. For a number of reasons, these varieties are replacing the old open-pollinated ones. Open-pollinated varieties of cross-pollinated crops, such as cole crops, consist of a mixture of genotypes, which are more or less heterozygous for a large number of genes. Consequently, plants of an open-pollinated variety show a large variation for many characters. By hybrid breeding, varieties are produced of which all plants possess the same favourable genotype for yield and other characters. For this reason, most hybrid varieties give distinctly higher yields than open-pollinated varieties; they are also very uniform, in particular when single-cross hybrids are introduced. This is important for mechanical harvesting. For breeders as well as for growers, it is important that hybrid varieties be genetically fixed, whereas open-pollinated varieties may vary by genetic drift, caused by changes in frequency and loss of alleles. Through hybrid breeding, physiological disorders such as internal tipburn of cabbage can also be controlled more easily than by selection in open-pollinated varieties which may show a large genetic variation for such deviations.

Breeding of varieties with resistance to disease is a main breeding objective in many crops. In cole crops, decisive results have only been obtained for resistance to yellows, caused by *Fusarium oxysporum f. conglutinans.* Research on resistance to this disease began at the beginning of the century in the United States, and many resistant open-pollinated varieties have been introduced.

Much research has also been done in the last 40 years on resistance to clubroot, caused by *Plasmodiophora brassicae*. Although in *B. oleracea* several sources of resistance to this disease are available, not much progress has been made, partly due to the mutability of the organism and the complex inheritance of the resistance.

In various accessions of *B. oleracea* differences in resistance to other diseases have been assessed. Diseases include black spot (caused by *Alternaria* ssp.), downy mildew (*Peronospora parasitica*), stalk rot (*Sclerotinia sclerotiorum*), white rust (*Albugo candida*), black leg (*Leptosphaeria maculans*), and those caused by turnip mosaic virus and cauliflower mosaic virus. Practical breeders have so far paid little attention to breeding resistance to these diseases, mainly because most resistances are incomplete and polygenetically inherited. Some seed firms are trying to produce varieties with resistance to black rot caused by the bacterium *Xanthomonas campestris*.

Hybrids continue to be produced for special purposes, such as processing, and for special conditions, such as high temperatures and humid or dry conditions. However, breeding of disease-resistant and pest-resistant hybrid varieties should be the main objective of hybrid breeders of cole crops. The prospects for developing such varieties, with the exception of those for yellows resistance, are not favourable. More research is needed before resistant hybrids will replace non-resistant varieties, but this is unlikely to occur before the 21st century.

C. Seed multiplications for commercial use

a) Production of hybrid seed

The best performing hybrid is the single-cross hybrid (F_1 hybrid), which is produced when two mutually compatible inbred lines with an outstanding combining ability flower together. Seed of the self-incompatible parent lines must first be produced on a large scale (for production of 1 ha of hybrid seed, about 250 g seed of each parent line is needed). This can be accomplished by bud pollination, although the procedure is very laborious. Lines can also be mass propagated in the field or in isolated rooms, by making use of the decrease in self-incompatibility of ageing flowers. Pollination is then carried out by insects, but when the lines are highly self-incompatible seed yields are low. Physical and chemical treatments to prevent the incompatibility reaction of inbred lines have also been proposed. The most effective procedure involves supplying isolated rooms containing flowering plants of an inbred line with about 5 per cent CO_2 gas. This method is now applied on a large scale in practical breeding and generally gives good results. To overcome problems in the production of hybrid seeds, research has also been carried out on the introduction of other types of hybrids, such as F_2 hybrids (mass-propagated F_1 hybrids), three-way cross hybrids [hybrids obtained by crossing an F_1 hybrid and an inbred line: $(A \times B) \times C$] and double-cross hybrids [crosses between F_1 hybrids: $(A \times B) \times (C \times D)$]. Such hybrids are always less uniform than F_1 hybrids and usually slower growing with lower yields.

Problems of producing seed of parent lines can also be solved by selection of parent lines consisting of two isogenic sublines, which only differ for their S-allele. When lines are produced by selfing for a number of generations, only plants that are heterozygous for the S-alleles in each generation are propagated. However, in the last generation of selfing a subline homozygous for one S-allele and a subline homozygous for the other are

selected. The sublines are maintained by bud pollination. For large-scale production of seed of the parent line, both sublines flower together and pollination is carried out by insects. The identification of the plants heterozygous for the S-alleles in every generation is very laborious, even when the ultraviolet method is used.

When producing hybrid seed, pollen is transported by insects, mostly bees. Good cross-pollination demands that the parent lines flower simultaneously and that plants of both lines are similar in size and produce the same number of flowers, with exactly the same colour, as bees do not visit flowers of different colours.

Seed yields from cole crops vary roughly from 100 to more than 1 000 kg/ha. Seed yields of single-cross hybrids are usually considerably lower than those of open-pollinated varieties.

b) Surveillance of variety behaviour

When producing hybrid seed, a proportion of the seed is always obtained by self or sister-brother fertilisation (sib seeds), as the parent lines are not completely self-incompatible and the effectiveness of many S-alleles is highly variable. Sib seeds produce off-types, reducing yield and uniformity, and therefore their proportion in hybrid seed lots must be determined. This can be done by raising young plants from a hybrid seed sample and comparing them with plants of the parent lines sown at the same time. Sometimes differences can only be seen in the adult plant stage, after 10 to 12 weeks.

The proportion of non-hybrid seed in commercial hybrid seed lots can be determined rapidly by electrophoretic separation of isoenzymes of acid phosphatase in extracts of single seeds. This enzyme is genetically controlled by one locus and five independently acting alleles, which correspond to five different bands on the electrophoregram. Plants of homozygous inbred lines are characterised by a single band and hybrid plants from crosses between parent lines with different single bands by a double-band pattern. Thus, it can easily and rapidly be known if seeds are produced by crossing. When the parents carry the same allele pair for the production of that particular enzyme, sib and hybrid seed cannot be separated, but use can be made of isoenzyme patterns of other enzymes.

Producing hybrid seed with self-incompatible parent lines is a risky affair, as genetic and environmental factors can lead to large annual fluctuations in frequency of non-hybrid plants. In many cases hybrid seed lots are unsuitable for sale. These complications can be completely avoided when hybrids of which one parent line is male-sterile are available. Prospects for developing entirely male-sterile lines by means of cybridisation are promising. It can be expected that hybrids produced with male-sterile parent lines will begin to replace present-day hybrids by the end of this century. Problems such as insufficient seed set due to insufficient pollination of male-sterile flowers by insects and instability of male sterility may retard this development.

c) Timeline on adoption of new varieties

In the near future, hybrid varieties of cole crops will completely replace open-pollinated varieties, as the evolution in several Western countries shows. This rapid change is due to the fact that the hybrids used are single-cross hybrids, with a high yield capacity and high uniformity. Seed costs of hybrid varieties are only a small percentage of the total production costs, as less than 0.5 kg seed per ha is usually needed when seeds are first sown on seed-beds. Replacement of open-pollinated varieties by hybrids is

slowest with cauliflower, as many cauliflower types are highly self-compatible and active S-alleles must be introduced by backcrossing. In many self-incompatible cole types the frequency of active S-alleles is low and that of weak S-alleles high, so that selection of sufficiently self-incompatible parent lines may be difficult.

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14. Onion

by

Q.P. van der Meer

A. Characteristics of the crop

a) Geographic origins

The geographic area including Turkey, Iran, northern Iraq, Afghanistan, mid-west Asia (including Kazakhstan) and western Pakistan is considered to be the main centre of *Allium* species. The ancestral group from which *A. cepa* probably originated includes wild taxa of the *oschanini* alliance of section *cepa, i.e. A oschanini* (including *A. praemixtum*) and *A. vavilovi.*

The domestication of *A. cepa* probably started within contemporary Tajikistan, Afghanistan and Iran, and this south-western Asian area is acknowledged as the primary centre of variability. Other regions where onions exhibit great variability, such as the Mediterranean basin, are secondary centres (Hanelt, 1990).

b) Geographic distribution of use; main production areas

Very probably some of the oldest onion types are grown in Egypt. Onions were depicted on ancient offering tables as early as 2700 BC (Tackholm, 1954; van der Meer, 1986). The present varieties Giza 6 Mohassan, grown south of Cairo, and Behairy, grown in the Delta, are undoubtedly straightforward generatively propagated descendants of these ancient onions. In India as well, onions are an old crop (6th century BC) They were introduced to central and northern Europe by the Romans but only became widespread during the Middle Ages. Introduction in Russia took place in the 12th-13th centuries (Hanelt, 1990), in the Americas only after 1492, in Indonesia before 1700 and in Japan probably only in the 19th century, via the United States.

Presently, large acreages of *A. cepa,* either onions or shallots, are found almost all over the world, from near the polar circle in Finland to the equator in Indonesia. Leading producers are the United States (2 087 000 tonnes), India (2 450 000 tonnes), Japan (1 160 000 tonnes), Turkey (1 950 000 tonnes) and Spain (1 146 000 tonnes) (FAO, 1988).

c) *Taxonomic status; genetic and cytogenetic characteristics*

The taxonomic status is still somewhat unclear, especially the degree of relationship to species like *A. galanthum, A. oschanini, A. fistulosum, A. farctum, A. pskemense* and *A. roylei.* Crosses have been reported with *A. fistulosum, A. vavilovi, A. pskemense, A. galanthum, A. nutans, A. senescens, A. sativum* and *A. roylei.* Complete crossability has only been found between *A. roylei* and *A. cepa* (van der Meer and Devries, 1990).

A number of *A. cepa*-allied crops, found only in certain countries or regions, are grown, on a small scale, predominantly in private gardens. A number of them are *A. cepa-A. fistulosum* hybrids, *i.e. A. proliferum* (Germany), *A. wakegi* (Japan), Beltsville's Bunching (United States), and Delta Giant (Louisiana, USA). Three others have an unidentified taxonomical basis: Grise de la Drôme (southern France), Utrechtse Sint Jansui (near Utrecht, the Netherlands) and *A. perutile* (United Kingdom).

All *A. cepa* populations have (2x =) 16 chromosomes. Some of the *cepa* allies have different numbers: Delta Giant (3x =) 24 and Beltsville's Bunching (4x =) 32.

Few genetic analyses have been done, the most important are those on inheritance of colour (El Shafie and Davis, 1967) and on male sterility (Jones and Clarke, 1943). Male sterility is a cytoplasmically inherited trait.

d) *Phytosanitary considerations in movement of germplasm and propagules*

In the seed-propagated onion crop, phytosanitary complications are well-known, as some diseases are seed-borne, namely neck rot (*Botrytis aclada*) and downy mildew (*Peronospora destructor*). Consequently, these diseases are often disseminated from seed growing areas (*e.g.* California, Idaho, France) to bulb growing areas (*e.g.* the subtropics and north-western Europe). It is very probably for this reason that such diseases are very cosmopolitan.

The vegetatively propagated shallot crop is in fact a permanent danger, as it disseminates many pests and diseases, *i.e.* thrips, nematodes, viruses, white rot (*Sclerotium cepivorum*), neck rot, *Fusarium,* downy mildew, etc. Also sets (bulblets, used as propagules) and seed onions (bulbs planted for seed production) represent severe phytosanitary risks – except for viruses – as such progagules are shipped over distances of hundreds or even thousands of kilometres.

Consumption onions, *i.e.* dry bulbs, are shipped over very long distances as well, *e.g.* from Europe to West Africa, the Caribbean area to Singapore, from Chili to Europe, from Egypt to Russia, from India to Malaysia and from New Zealand to Japan. This shipping is also somewhat risky with respect to pests and diseases but undoubtedly less so than shipment of the vegetative propagules which directly enter growing areas.

e) *Current end uses*

Whether fried, cooked or as a salad, the *A. cepa* crops seem to be the most popular and indispensable flavour enhancer in the world.

B. Reproductive mechanisms

a) Modes of reproduction and pollination

At the beginning of domestication, vegetative propagation, which is now common only for shallots, might have been the most natural and dominant way to reproduce onions. Presently, onions are mainly multiplied by seed.

A. cepa is only pollinated by insects like bees, bumble-bees, flies, glider flies and, sometimes, wasps.

All the *A. cepa* allies (see above) with the exception of Beltsville's Bunching, are highly sterile and therefore are propagated vegetatively.

b) Perennial vs annual

A. cepa or *A. cepa*-allied crops never run wild, but their ancestors, *i.e.* the *A. oschanini* alliance, still survive as perennial wild species in the northern border regions of Iran and Afghanistan (Hanelt, 1990). *A. proliferum* is more a perennial than an annual.

c) Dispersal and survival mechanisms

Although seed of commerical cultivars tends to drop considerably in germination power within two years after harvest, onion seed can survive for many years (20-30) when stored under dry and cool conditions. Keepability (*ex situ*) of daughter bulbs of shallots and onions is limited to one or two years.

Dispersal of *A. cepa* is assured by seeds, sets and (for shallots) daughter bulbs, which are often shipped over long distances. Crossings of *A. cepa* with related species, with the exception of *A. roylei,* always result in a high degree of hybrid sterility, as the many existing *A. cepa* allies show. Most of these crops, except Beltsville's Bunching and Delta Giant, only originate from (ancient?) spontaneous crosses. Almost all the more recent species hybrids originating from man-made crossings (with *A. nutans, A. fistulosum, A. senescens* and *A. sativum*) very probably become sterile as well. Thus, such hybrids, as well as the crops allied to *A. cepa,* are maintained vegetatively.

Toxicology

The genus *Allium* seems to contain no toxicants whatsoever. Several species, among them *A. cepa,* have pronounced therapeutic effects on high blood sugar content, high lipid content and platelet aggregation (Augusti, 1990).

Environmental requirements for life cycle

From the beginning of its domestication, the crop has gradually adapted to very diverse environments, either by selection (in generatively and vegetatively propagated strains) or by new growing methods. Today, field growing is initiated from seeds, transplants sets or daughter bulbs and growing conditions – density, soil type, day length and temperature – vary widely.

Longer days and higher temperatures stimulate the bulbing process. Each variety has its own critical day length: it needs a certain minimum day length for proper bulbing and maturing. If the critical day length is short, *e.g.* 12 or 13 hours, a variety is classified as

belonging to the short day group. A long day variety has a critical day length of about 16 hours. Day length and temperature are of course strongly tied to geographical latitudes; at present, varieties are available for almost all latitudes (Table 14.1).

Table 14.1 shows that the latitudinal adaptation amplitude for many varieties is between 5° and 10°. Early Grano and Red Creole are very pronounced exceptions with about 25°. Also remarkable is the wide amplitude (*ca* 18°) for Stuttgarter when using sets.

By following latitudinal zones around the world, one can find some types of onions everywhere, *e.g.* the Grano type in Spain, the United States, Australia, South Africa and also in South America near Mendoza (about 33° SL).

Table 14.1. **Onions and shallots as adapted to specific latitudes**

Type or variety	Propagules	Countries	Approximate latitudal zone
Vegetative propagated onions and shallots	Daughter bulbs	Northern Russia Finland	56-62°NL
Rijnsburger	Seeds	Denmark Netherlands Northern France	48-56°NL
Stuttgarter	Sets	Netherlands Rumania Finland	44-62°NL
Babosa	Seeds	Spain	36-40°NL
Early Grano (= Babosa?)	Seeds	United States Tropical America Spain	8-34°NL
Grano	Seeds	Spain	38-42°NL
Sweet Spanish (= Grano?)	Seeds	United States	30-40°NL
Australian Brown (= Grano?)	Seeds	Australia South Africa	35-40°NL 30-35°NL
Behairy	Seeds	Egypt	30-33°NL
Giza 6 Mohassan	Seeds	Egypt	23-30°NL
Red Kano	Seeds	Nigeria	8-12°NL
Red Creole	Seeds	United States Tropical countries	8-32°NL
Violet de Galmi	Seeds	Tropical countries	8-23°NL
Bombay Red	Seeds	Tropical countries	6-10°NL, SL (Highlands)
Shallots	Daughter bulbs	Indonesia	0-10°SL (Highlands and lowlands)

As the centre of origin of *A. cepa* is thought to be the northern border-region of Iran and Afghanistan, it must have spread north and south from 36°-40° NL. Adaptation efforts were undoubtedly strongly stimulated by its unsurpassed attractiveness as a flavour enhancer. Obviously, adaptation to the most northern and southern latitudes has predominantly been realised by means of vegetative propagation (see Table 14.1). This procedure could receive a generative complement from local breeding procedures that try to tackle the problem of locally grown direct seeded onions and onion seeds.

C. Current breeding practices and variety development research

a) Breeding, genetic basis, strategy, techniques and handicaps

i) Germplasm maintenance

Many local strains are still grown, in, for example, the Netherlands, Egypt, Nigeria and Indonesia. Nevertheless, genetic erosion is proceeding rapidly, for instance, in the United States, the Netherlands, Egypt, Nigeria and Norway.

The Allium Working Group of the IBPGR in Rome stimulates and co-ordinates the collection and conservation of *Allium* germplasm around the world. Base collections, under the aegis of IBPGR, have been founded in Wellesbourne (UK), Wageningen (the Netherlands), Rehovot (Israel), Olomouc (former Czechoslovakia), Fort Collins (Colorado, USA), Tsukuba (Japan) and Tapiozele (Hungary). These gene banks are in charge of collection, conservation, registration, description and distribution (to breeders) of *Allium* accessions, specifically of local strains and wild relatives of onions.

ii) Basic breeding

Onion is a predominantly cross-pollinated crop. Selfing can vary from 0 to more than 50 per cent (van der Meer and van Bennekom, 1968, 1972). Basic breeding includes variety crossings (resulting in composites), breeding of hybrid parents (*i.e.* A, B and C lines) from divergent varieties, species crosses and development of standardised screening methods.

iii) Variety development

Variety development is realised by selection for improved characters in open-pollinated varieties (OPs), hybrid parents and experimental hybrids.

F_1 hybrids are products of complete crossings between two populations, mostly of two lines. A line is a population obtained after selfing of a plant. For the production of an onion hybrid, use is made of the:

- A line, which is completely male-sterile; this is the seed parent;
- B line, which is of a suitable genotype for reproduction of the A line; this is the so-called maintainer line;
- C line, which is the hybrid partner (pollen parent) for the A line.

The A line must be completely and permanently male-sterile, and the B line must be able to maintain the A line. Hybrids can be superior to OPs, with respect to production and uniformity. Superior hybrids are selected from experimental hybrids. Variation in experimental hybrids is mainly achieved by using diverging C lines.

iv) Limiting characteristics

Breeding of onions is limited by its biannual life cycle. Because only one generation can be obtained in two years' time, the development of new varieties can take some ten years or more.

Another handicap of onion breeding is the absence in a number of varieties of the genotype for maintaining total male sterility. This is true for the Indian onion varieties, Giza 6 Mohassan (Egypt), Wolska (Poland), Australian Brown (Australia), and Vsetatska (former Czechoslovakia). Consequently, neither A lines nor hybrids can be bred from such varieties.

The possibilities for better uniformity of the hybrid system are also limited by inbreeding depression. In general, more than two or three successive selfings of a B line have disastrous effects on the seed yield of the A line. A partial solution to this problem might be three-way hybrids, based on male-sterile F_1 hybrids as seed parents (Dowker, 1990).

Breeding of shallots must be done generatively. Growing shallot seed is generally not very difficult. The offspring show a large variation in idiotypes and consequently offer a promising basis for selection. After finding improved clones, it takes at least five years of multiplication before they can be grown on a large scale.

Onions only start bolting and flowering after a cold treatment (vernalisation), *e.g.* six weeks at 10°C. This is a serious handicap for breeding in the tropics, where special measures must be taken to induce flowering, such as planting bulbs at high altitudes (*ca* 1 500 m) or vernalising them in cold storage before planting.

High natural selfing percentages must also be considered a handicap.

b) Main breeding objectives

Main criteria for selection of onions are the following:

- yield;
- keeping quality at ambient temperatures or in cold stores;
- uniformity of maturity, shape and colour;
- shape: the globe shape is the most popular;
- colour: yellow is most popular in Europe and the United States but red is preferred in Africa and India;
- disease resistance;
- early maturity;
- non-bolting, especially in the winter crops;
- winter hardiness;
- higher dry matter content for processing purposes (dehydration);
- lack of bitterness.

Keeping quality mainly entails resistance to sprouting, good skin retention and absence of rotting. This is of paramount importance for onion supply during off seasons (winter, wet or dry season) and for supplying tidial and local wants for onions all over the world. Presently it is a more or less common practice to improve the keeping quality of consumption onions by field spraying with maleic hydrazide, but its effect on human health is still questionable.

For selection purposes, a very diverse pool of genetic resources is available, but adaptation to specific latitudes (*i.e.* to specific temperatures and day lengths) presents a severe handicap and necessitates time-consuming backcross schemes. Moreover, pronounced resistance to several diseases (downy mildew, neck rot, white rot and tip burn) is very rare, and even absent in *A. cepa*. Consequently disease resistance is sought (and found) in other *Allium* species. However, introgression of such characters to *A. cepa* appears to be difficult to achieve in most cases because of lack of crossability and hybrid sterility.

c) Testing for important breeding goals

Testing for the following characters generally takes place in replicated (field) trials using, for example, a randomised block design:

- yield;
- uniformity;
- shape;
- colour;
- earliness;
- non-bolting;
- winter-hardiness;
- keeping quality.

Yield and keeping quality are determined on the basis of weight, whereas the other characters are determined on the basis of field observations (countings, estimations).

Skin retention is tested by increased stress, *i.e.* shaking the bulbs for a certain period of time on a standardised machine, after storage. The dry matter content is determined by using a refractometer (van der Meer, 1984). Up to now lack of bitterness can only be estimated organoleptically. Bitterness seems to be correlated with pungency.

For testing resistance to diseases like downy mildew, neck rot, *Fusarium* and pink root, methods described in the literature are generally used (see *e.g.* van der Meer and van Bennekom, 1970; Kofoet *et al.*, 1990).

d) Assessment of breeding material

For monitoring breeding material, (field) observations "by the eye of the master" are of paramount importance. This is true both for the bulb crop and the seed crop. Skin retention, dry matter content and disease resistance are tested periodically as well. The monitoring is usually done under normal growing conditions. Sometimes specific stress conditions are chosen or simulated in order to check resistance to fluctuations in weather or soil conditions.

D. Seed multiplication for commercial use

a) Stages in seed production

Some practical data for Dutch onions are:

- seed production per ha is about 500 kg (in France and in Italy);

- in practice the multiplication coefficient is about 400;
- the seed requirement per ha is about 6 kg;
- bulb production per ha is about 50 tonnes;
- seed purchase cost is between 5 and 10 per cent of the total commercial bulb growing cost (van der Meer, 1968).

For about 1 000 ha of bulb onions about 6 000 kg of onion seed are needed. This quantity is obtained after multiplication of about 15 kg of onion seed, and, for these 15 kg, multiplication of about 40 g of seed is required. In this case 6 000 kg qualifies as commercial seed, 15 kg as basic seed and 40 g as breeders' seed. Maintenance of breeder seed is the breeder's responsibility. The production of basic seed and commercial seed are generally in the hands of a special seed production unit, which is responsible for production, isolation, roguing, etc., and finally for the quality and purity of the commercial seed.

b) Isolation practices

Onion seeds are grown at least 1.5 km away from other onion seed fields.

c) Certification and registration

In the Netherlands, onion seed can be certified, but this is facultative. Its quality is usually directly guaranteed by the seed firms. Surveillance of variety behaviour in the Netherlands is done in annual field trials under the responsibility of a specific national onion trial committee. Satisfying varieties are registered in a list of recommended varieties, and inferior ones are refused or removed from the list. Adoption of new varieties follows quickly after registration in the variety list.

In most countries, even if variety testing and recommendation are in practice, local breeders and merchants have the final responsibility for variety improvement. However, in a number of countries (*e.g.* Bangladesh, the Sudan, Ghana) only local strains are available; they are maintained (but not improved) by individual farmers.

The number of varieties or strains varies widely among countries and even among regions. In Egypt, south of Cairo, only one strain (Giza 6 Mohassan) of the former Saidi land race is grown, but in the Delta many local strains of Behairy (30 or more) are grown. In the Netherlands, in 1992, 16 recommended strains and hybrids of Rijnsburger were available, whereas in Rumania, in 1990, six varieties were recommended, among them Wolska (from Poland) and Stuttgarter. In Indonesia 10-20 local shallot strains are grown. The planting material of one of them (var. Bangkok) is imported every year from Thailand. The world collection of onions comprises at least 1 000 strains and varieties (personal estimation).

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15. Cassava

by

Kazuo Kawano

A. Characteristics of the crop

a) Geographic origins

Cassava (*Manihot esculenta*), also called mandioca (Brazil, Paraguay and Argentina), yuca (other Spanish-speaking countries), tapioca or manioc (French-speaking Africa), is one of the most important calorie-producing crops and the most important root crop in the tropics. The evolutionary history of cassava, like that of other root crops, has been difficult to trace, and its definite centre of origin has not been located. The most apparent conclusion is that cassava originated in the lowland tropics of America. Multiple sub-centres of diversification in tropical America have been suggested by several researchers.

b) Geographic distribution of use

Cassava was widely distributed throughout the Americas and the Caribbean by the time the European colonists arrived in the 15th century. Cassava is believed to have been first introduced to Africa in the 16th century and later to Asia. Theory and experience confirm that the great majority of genetic variability in cassava exists in the American tropics; yet, a wide range of varietal diversity is observed among the existing cultivars in Africa and, to a somewhat lesser extent, in Asia as well.

c) Main production areas

Until the end of the 1960s, Africa and Latin America were the major producers of cassava; however, during the past two decades the Asian share has increased remarkably and now, on a par with Africa, claims approximately 40 per cent of the world total. Productivity of the crop in Latin America slightly decreased over the past two decades and is now estimated to be around 12 t/ha; in Asia it has steadily improved and is now estimated to be around 13 t/ha. In Africa it has been stagnant at about 7 t/ha. These yields are low, especially compared with the experimental high yield of 70 t/ha/year, or the large-scale commercial high yield of 40 t/ha/year which can be attained by planting improved genotypes in adequate cultural environments.

d) Taxonomic status; genetics and cytogenetics

Genus *Manihot* belongs to the family Euphorbiacae, and the genus is reported to contain approximately 100 species. Cassava is the only important food crop in genus *Manihot* and is known only under cultivation. Clusters of closely related species are distributed in both North and South America, but no specific wild species has been suggested as a possible ancestor. Cassava, with 2n = 36 chromosomes, has been considered as a diploid by some researchers, as a tetraploid by others, and as a segmental allotetraploid by still others.

e) Current phytosanitary considerations in movement of germplasm

With the establishment of a world cassava germplasm centre at Centro Internacional de Agricultura Tropical (CIAT) in Colombia and an African regional germplasm centre at the International Institute of Tropical Agriculture (IITA) in Nigeria in the early 1970s and subsequent establishment and strengthening of national cassava breeding programmes, international germplasm distribution was greatly accelerated. Germplasm exchange is currently conducted through the following three forms: 1) planting stake; 2) meristem culture; and 3) true seed. Stake is the easiest to handle but the risk of accidentally introducing disease and pests is highest; meristem culture is less risky and offers reproduction of identical genotypes, but it is not suitable for transferring a large number of genotypes and requires certain skills and facilities at the receiving end; sexual seed is the least risky and offers easy handling of a large number of genotypes although identical genotypes cannot be obtained.

Phytosanitary measures on germplasm introduction differ widely among countries. Use of stakes is self-restrained between continents and is limited to shipments between neighbouring countries that share by and large the same spectrum of diseases and pests. Meristem culture is generally accepted as a safe method of transferring clonal materials; extra care is taken for trans-continental transfers which may involve the danger of viral disease transmission. Hybrid seed is most widely accepted as a safe and efficient method for the transfer of breeding materials.

f) End uses

Cassava has been used as human food throughout nearly the entire part of its history as a crop. Boiled root must have been the standard form of human consumption and is still in many parts of the world today. A wide variety of root processing techniques are found among the American Indian ethnic groups; and so-called bitter cassava cultivars, which can be consumed only after processing, are widely distributed throughout the American tropics. Consuming cassava roots after some degree of processing may be a procedure as old and important as simple boiling. In this scheme, cassava's most important role is to supply food calories in subsistence agriculture, where cassava is planted in small production fields. Due to cassava's ability to produce high calorie yields under limited availability of water and soil nutrients, new uses of cassava for animal feed and starch production have rapidly gained importance in recent years, especially in southeast Asia. Cassava production for animal feed, starch and other industrial processing has expanded very rapidly in Asia and has far offset the decrease in human consumption.

Reproductive mechanisms

a) Mode of reproduction

In production fields cassava is virtually exclusively propagated by planting stem cuttings (usually 10-30 cm long) vertically, horizontally or diagonally. They are taken from mature plants (more than eight months old). Propagation by seeds is possible in many different environments, but because of the difficulty of gathering large amounts of seeds and the slow initial growth from seed, its use is currently limited to cassava breeders' fields.

b) Perennial vs annual

As a plant, cassava is perennial, but as a crop it is predominantly annual, being harvested from 8 to 18 months after planting.

c) Mode of pollination

Cassava is a monoecious species with the stigma and anther usually separated in different flowers on the same plant. A flower bud is formed every time the plant branches; however, most of the flower buds formed during early growth stages are abortive. Flowers cannot be obtained from non-branching types. The male and female flowers nearly never open simultaneously on the same branch, but it is not rare for female flowers and male flowers on different branches of the same plant to open at the same time. The pollen is relatively large in size and sticky, so that natural pollination by wind is unlikely. Several species of wasps and bees are the main pollinators. Both cross-pollination and self-pollination occur naturally. The proportion of cross-pollination depends on the flowering habit of the genotypes and the physical arrangement of the population. There seems to be no physiological or genetic mechanism to prevent self-pollination and no serious cross-incompatibility has been found. In all, producing a large number of hybrids by artificial pollination is comparatively easy.

d) Survival mechanisms

Strong inbreeding depression has been observed in characters such as root yield and total biomass. This strong inbreeding depression, in addition to the vegetatively propagated nature of the species, is the biological mechanism through which the high heterozygosity of the species is maintained. Male sterility is frequent, and is effective in preventing self-pollination.

e) Ability to cross with related species

Cassava can be crossed relatively easily with a closely related species *M. glaziovii* (ceara rubber) and the genes for cassava mosaic disease from *M. glaziovii* have been extensively used in a backcrossing breeding scheme in Africa. Other wild relatives such as *M. saxicola, M. melanobasis, M. catingae* and *M. dichotoma* have also been included in hybridisations with cassava.

Toxicology

Raw cassava root contains the glycosides linamarin and lotaustralin, which are converted to hydrocyanic or prussic acid, a poison, when they come in contact with linamarase, an enzyme released when the cells of cassava root are ruptured. Although occasional deaths from consuming raw cassava roots have been reported, the traditional processing and cooking procedures, such as boiling, grating and water soaking, drying, or fermenting, usually effectively reduce the cyanide levels. Thus, if normal preparation methods are followed, acute cyanide toxicity does not occur. Chronic cyanide toxicity occurs in some areas of Africa where cassava consumption is high and the consumption of iodine and protein, particularly animal protein, is extremely low. In Nigeria and Zaire, ataxic neuropathy (nervous degeneration) and goitre (which leads to cretinism in severe cases) have been associated with high levels of cassava consumption.

Existing cultivars greatly vary in cyanide level of raw root. Those with high levels are called "bitter cassava" and are used only after some degree of processing, while those considered fit for direct fresh human consumption (after boiling) are called "sweet cassava" and are nearly invariably of low cyanide levels. There is no scientifically convincing proof to indicate a close correlation of high cyanide levels with disease and pest resistance, high-yielding capacity, or tolerance to drought or poor soils, except for a few cases of resistance to root-attacking animals such as bugs, wild boars or human thieves whose habit of feeding on cassava is believed to be of recent origin in the evolution of the crop. The great majority of currently available high-yielding cultivars adapted to large-scale industrial production with a broad spectrum of tolerance to the biotic and abiotic yield constraints are bitter types. One of the greatest challenges to the contemporary cassava breeders is to create so-called dual-purpose cultivars which combine the good eating quality of sweet cultivars with the high yield capacity and robustness of bitter cultivars.

Environmental requirements

Cassava is successfully grown between latitudes 30°N and 30°S, from sea level to about 2 000 m, under annual precipitation of from 600 to 6 000 mm, and with a soil pH between 3.8 and 8.0. This species is tolerant to hot climate, drought and acid soils but highly susceptible to cold temperatures and excessive soil water content. Consequently, no significant cassava production takes place in the temperate areas of developed countries, making cassava the only major food crop largely unknown to the scientific community of the developed world.

B. Breeding practices and varietal development

a) Main breeding schemes/techniques

i) Germplasm maintenance

A worldwide germplasm collection comprising nearly 6 000 accessions from all over the world is maintained at the CIAT headquarters in Colombia, and a regional collection for Africa is maintained at IITA, Nigeria. Each national programme maintains its own collection which mainly comprises the collection from its own area. The size of the collection largely reflects the variability of traditional cultivars in the area and the

strength of the research programme. Most of these collections are maintained as living plants in the field. A duplicate of the CIAT collection is being maintained as slow-growing meristem cultures in a laboratory at CIAT. Also being contemplated is the creation of a working collection, comprising some 10 per cent of accessions but designed to cover 95 per cent or so of effective genetic variability, and of a gene bank by true seeds, in which the identity of each clone or specific gene combination is lost but the genes as a population whole may be maintained.

ii) Basic breeding

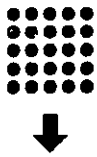
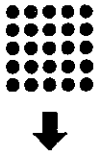
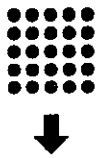
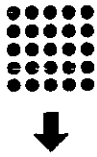
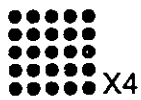
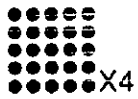
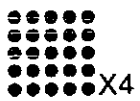
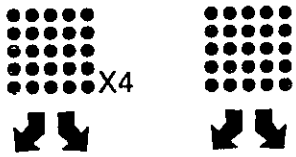
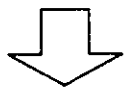
As a crop of vegetative propagation, cassava has great advantages for breeders. In addition, it lacks the usual complications, such as the cross-incompatibility often encountered with other crops. Once a favourable genotype is obtained, it can be multiplied indefinitely. Character expression at the seedling stage is well correlated with that at the later clonal generations. Early studies on cassava breeding presented occasional difficulties, such as scarce or no flowering of some clones, low seed-setting on some female parents and low germination. However, breeders by and large agree that cassava is one of the easiest among the major crops for which to create and handle recombinant genotypes. Hence, a typical cassava breeding programme follows a classic pattern. It starts by creating a large number of recombinants by hybridisations, goes through evaluation steps with a decreasing number of recombinants and increasing degrees of precision per genotype, and ends with the identification of recommendable genotype(s) after repeated multi-locational, multiple-year evaluations (Table 15.1).

Network efforts in the past two decades have resulted in the establishment and strengthening of many cassava breeding programmes. They can be roughly classified into three categories: 1) international basic breeding programmes; 2) comprehensive national breeding programmes; and 3) national varietal development programmes.

Basic breeding programmes aim at the general upgrading of breeding populations and the generation and distribution of advanced breeding materials to national programmes. The basic breeding programme at CIAT headquarters has been successful in upgrading yielding capacity through improved harvest index, improved resistance to major diseases and insects, and adaptation to several major cassava environments, including tolerance to infertile acid soils. The programme at IITA has been successful in improving resistance to cassava mosaic disease and cassava bacterial blight. The Thai-CIAT collaborative breeding programme has been successful in improving biomass production and root dry matter content and in adaptation to the semi-arid climate of the lowland tropics.

Comprehensive breeding programmes have evolved in the stronger national research programmes such as those in Thailand, China, India and Brazil. They take all the necessary steps in breeding and varietal development including hybridisations and on-farm research for varietal release. Varietal development programmes, now operational in many countries, conduct adaptive selections to identify recommendable genotypes for their own conditions. They usually receive advanced breeding materials from one of the international basic breeding programmes.

Table 15.1. **Stages of selection in a typical cassava breeding programme**

Year	Selection stage	Breeding and selection site		
		Breeding HQ	Principal selection site	Other selection sites
1	Hybridisation			
2	Hybrid seedling			
3	Single-row trial			
4	Preliminary trial			
5	Advanced trial			
6	Regional trial			
7	On-farm trial			
8	On-farm trial-2nd year and multiplication			
9	Varietal release			

b) Main breeding objectives

Breeding objectives common to most breeding programmes are:

- high yielding capacity;
- early harvestability;
- high root dry matter (starch) content;
- disease and pest resistance;
- tolerance to adverse soil and climatic conditions;
- low root cyanide content;
- good plant type;

- good stake quality (handling, storage and germination);
- versatility (suitable for both human consumption and industrial production);
- root colour.

Different national programmes accord varying degrees of importance to specific disease and pest resistances and to tolerance to adverse environmental conditions. With the possible exceptions of the search for resistance to cassava mosaic disease and for zero cyanide content, the search for desirable characters in the existing intra-specific germplasm has been largely successful. Many of these characters have been incorporated into the mainstream breeding populations.

c) Testing for important breeding goals and assessment of general performance

Large scale multi-site field testing has proved to be the most reliable and possibly the most efficient and effective method in the long run for identifying truly recommendable cultivars. Reciprocating evaluations between high-yielding environments and high-stress environments have been particularly successful in obtaining widely adapted, robust genotypes. This is true even for resistance to diseases, for which evaluation in greenhouse or disease nursery is usually preferred for other crops.

Cassava is predominantly grown with a minimum of fertiliser and chemical application or irrigation; thus, year-to-year and long-term yield stability are basic criteria for cassava farmers. To assess stability, there is no substitute for evaluations across several sites over several years.

C. Varietal release and adoption

Until recently there has been no official mechanism for varietal release in most countries. Almost all the traditional cultivars have been farmers' selections from the locally available germplasm. Now that national varietal improvement programmes are by and large established in every cassava-growing country, many advanced clones are reaching the farmers through loosely structured semi-official schemes. These consist of on-farm evaluations of promising clones, pre-release of half-finished cultivars, and farmers' selections for their own plantings.

With the gradual strengthening of national programmes, official mechanisms for varietal registration, multiplication and certification are evolving in several countries. In the past, the input from extension services has been minimal for this small farmers' crop; however, recent joint efforts between the research and extension departments in Thailand on the production and distribution of certified planting materials of newly released cultivars may set a good example.

Since the history of official cassava breeding programmes is short in many countries, no national programme as yet offers a means for systematically assessing the socio-economic effects of adoption of new cultivars. A joint survey by the CIAT Economic Section and the Department of Agricultural Extension in Thailand on the adoption of a new cultivar may be the first such attempt.

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16. Potato

by

E. Jacobsen and P. Rousselle

A. Characteristics of the crop

a) Geographic origin; centres of diversity

The potato, of South American origin, was introduced into Europe about 450 years ago. The potato crop expanded greatly in Europe in the mid-18th century and spread to the other continents as a major food crop. It ranks fifth in area and fourth in total yield among crop plants grown for consumption (FAO, 1984). In Europe, the United States and Canada, the area has decreased due to increased yield per ha and changes in the pattern of human consumption. The decrease in human consumption has been stopped by the development of a processing industry that produces French fries, crisps and other consumables.

b) Geographic distribution

The potato is potentially valuable as a major source of carbohydrates in many developing countries. It has great adaptability to different climates, such as highland and lowland tropics. The International Potato Centre (CIP) in Lima (Peru) is involved in the successful introduction of the potato in many tropical countries. The potato is cultivated in all temperate regions and in many tropical and subtropical zones (Ross, 1986).

c) Taxonomic status

The cultivated potato belongs to a species, *S. tuberosum* L., which is considered to be an autotetraploid. The cultivated potato is probably derived from crosses between the cultivated diploid species *S. stenotonum, S. phureja* and the weed diploid species *S. sparsipilum* (Hawkes, 1978). The change from diploid level to tetraploid cultivated species is the result of the functioning of unreduced gametes in both sexes of the species involved, enabling 4x offspring plants in 2x × 2x and 4x × 2x crosses.

To date, about 180 different tuber-bearing wild species are known; they comprise the sub-section *Potatoe* of the section *Petota* and are sub-divided into 18 series. Most series contain only diploid species (2n = 24), but important 3x, 4x and 6x species are available (Ross, 1986). In the series *Tuberosa* are found 68 wild species and eight cultivated species closely related to *S. tuberosum.* These species are distributed from

South Chile up to the southern United States and grow from sea level up to 4 500 m above sea level. These wild species are commonly used in breeding as a source of genetic variation, mainly for resistance to all kinds of diseases and pests.

d) Germplasm movement and maintenance

In the past, several expeditions have been made to South America to collect material not only for its botanical and taxonomic interest but also for breeding purposes and to prevent erosion of genetic resources. Important collections include the Commonwealth Potato Collection, the Collection of the Interregional Potato Project in Sturgeon Bay (USA), the World Potato Germplasm Collection of the International Potato Centre (Lima), and the Dutch-German Potato Collection in Braunschweig (Germany) (Ross, 1986).

e) Phytosanitary considerations

It is important to realise that the use of wild species in potato breeding creates the risk that new or quarantine diseases will be imported. In many countries, a well-developed quarantine system is available to avoid this risk. In general, the gene banks have controlled and certified their material for quarantine diseases, thereby simplifying import of germplasm by researchers and breeders. Generally, imported material has to be controlled by the quarantine authorities before it is released for breeding or breeding research purposes. For the potato, import of the seed-transmissible viroid PSTV (potato spindle tuber viroid) is the main risk. Molecular tests have been developed to detect infected plants easily.

f) Current and developing end uses

Potatoes are used for many different purposes, such as human consumption, animal feed, production of starch and alcohol. Potatoes for consumption can be separated into those used fresh as cooked potato and those that are processed industrially. The percentage of processed potatoes is still increasing, as is the number of consumable products. The most important are French fries, crisps, mashed potatoes and canned potatoes. Characters important for processing are tuber form and size, starch content, low susceptibility to mechanical damage, low content of reducing sugars and resistance to bruising. Potatoes for cooking have been classified, according to the recommendations of a working group of the EAPR (European Association of Potato Research), into four classes (A, B, C and D) based on level of disintegration, consistency (= texture) and mealiness (Keller and Baumgartner, 1982).

Potato starch is also an important raw material used as a thickener in the food industry and as an adhesive in the technical industry. Potato starch granules consist of 20 per cent amylose and 80 per cent amylopectin. Both compounds have different chemical and physical properties and applications. Amylopectin can be produced separately in the plant, as the recently isolated amylose-free potato mutant showed, and thus makes possible a better technical use of starch (Jacobsen *et al.*, 1989).

Reproductive mechanisms

a) Modes of reproduction and pollination

Each variety of cultivated potato is represented by one genotype which is vegetatively reproduced by tubers. Potato plants grown from tubers have to be free or nearly free from viruses. The technology necessary for multiplication of "disease-free" seed potatoes is widely used in the Netherlands but little known in developing countries. Advances in *in vitro* multiplication and virus tests have improved the production of seed potatoes. At present, four multiplication methods are applied:

- growing tubers of selected plants in the field;
- minituber production on stem-cuttings grown in a greenhouse;
- *in vitro* shoot propagation; and
- *in vitro* microtuber propagation.

Many varieties produce flowers and berries containing seeds. Because of the heterozygous nature of this autotetraploidy crop, inferior inbred offspring are obtained that cannot be used for cultivation purposes.

Wild relatives and wild species of potato are propagated both by tubers and seed. Potato is a cross-fertiliser, pollinated mainly by insects. At the diploid level a gametophytic incompatibility system is active. Potato is an annual crop that survives by seed and tubers.

b) Ability to cross with related species

In the Netherlands and most probably throughout Europe, none of the weeds in the ecosystem can cross-hybridise with the cultivated potato (Stiekema and Eijlander, 1991).

Toxicology

An important effect of introducing wild species as source material in potato breeding is the increase in glycoalkaloids in green parts and tubers. In old potato varieties, in which no wild species is involved, total glycoalkaloid content (TGA) is relatively low. TGA of tubers is important to flavour. At present, maximum levels of 60-70 mg solanine glycosides per kg fresh tuber weight are allowed in tubers of consumption and starch potato varieties. The influence of various environmental conditions on the synthesis of these compounds complicates determining the levels of TGA in new varieties. It is highly recommended that the TGA level in new varieties containing wild species germplasm be investigated before registration. From the viewpoint of consumer safety, a low TGA content per kg of fresh tuber is recommended (van Gelder, 1988 and 1989). It is recommended that measuring methods be developed in order to screen TGA level in breeding parents routinely.

Environmental requirements for life cycles

The green parts are susceptible to night frosts (up to –3°C). As the tubers are killed in the soil at –4°C, this crop cannot survive in many areas with relatively cold winters (van Swaay *et al.*, 1987).

B. Current breeding practices and variety development research

a) Main breeding techniques

As mentioned earlier, much germplasm containing important traits is available to the breeder. Basic breeding of all kinds of wild species is needed to develop basic breeding material to create new breeding parents, to be used for variety development.

i) Basic breeding

Ross (1986) described briefly the development of basic breeding material using wild species for the incorporation of resistance and quality genes in existing potato varieties. The species *S. demissun.* (6x), *S. acaule* (4x; 6x), *S. chacoense* (2x), *S. spegazzinii* (2x), *S. stoloniferum* (4x), and *S. vernei* (2x) have been source material in several varieties for hypersensitivity and field resistance to *Phytophthora infestans, Fusarium,* Colorado beetle, pathotypes of wart, potato virus X, Y, A and potato leaf roll virus, the cyst nematodes *Globodera rostochiensis* and *G. pallida,* for frost resistance, and for low content of reducing sugars and high starch content. Species like *S. berthaultii* (2x), *S. gourlayi* (2x; 4x), *S. bulbocastanum* (2x), *S. hertingii* (4x), *S. pinnatisectum* (2x), *S. sparsipilum* (2x), *S. etuberosum* (2x) and *S. brevidens* (2x) are currently being used in breeding research to transfer into the cultivated potato resistance to aphids, *G. pallida, Pseudomonas solanacearum, Synchytrium endobioticum, Erwinia carotovora* and non-susceptibility to bruising.

Apart from the desired traits, wild species contain a high amount of additional genetic variation that make possible the development of breeding parents or varieties with more hybrid vigour. In practice, the breeder always needs to broaden the genetic base of breeding parents.

Most of the mentioned 4x wild species offer no problems for breeding. Hybrids are easily obtained and fertile, and only a few backcross cycles with *S. tuberosum* are needed to create breeding parents with desired traits.

The 2x wild species are more troublesome as source material for 4x *S. tuberosum,* because of a triploid block that prevents triploid hybrid formation. These problems have been overcome by two important factors highlighted in Box 16.1, *i.e.* doubling the chromosome number of the 2x wild species by using 2n gametes in 4x × 2x crosses and haploidisation of the autotetraploid either through parthenogenetic seed development in crosses with *S. phureja* (Hermsen and Verdenius, 1973) or by anther or microspore culture (Jacobsen and Sopory, 1978). In practice both approaches are important. The triploid block in 4x × 2x crosses can be circumvented when unreduced gametes are available in useful genotypes and parthenogenetic dihaploids are basic material for prebreeding of potato at the diploid level.

Breeding of *S. tuberosum* at the diploid level has major advantages, such as:

- simple disomic instead of tetrasomic inheritance, which facilitates the combination of desired traits;
- crossability with many diploid wild species for the introduction of desired traits; and
- simple sexual (meiotic) polyploidisation via 4x × 2x and 2x × 2x crosses, using unreduced eggcells and pollen grains for variety breeding at the tetraploid level.

Box 16.1. **Haploidisation and polyploidisation of potato**

The cross between the autotetraploidy potato and the diploid species *S. phureja* is not expected to deliver offspring because of a triploid block. This block is due to the suboptimal ploidy level of the endosperm (5x), which prevents the normal functioning of this reserve tissue and further development of the embryo. However, seed set is obtained with certain parental genotypes of this diploid species. Ploidy level of the seedlings appears to be 2x and 4x. It is known that development of 3x, 6x, 9x,... endosperm is successful and that two types of pollen grains can be formed containing normal reduced (1x) or unreduced (2x) chromosome number (2n-gamete).

Triploid block

Fertilisation with a normal pollen grain delivers a 3x embryo with 5x endosperm. The 5x endosperm is not formed and is followed by embryo abortion (Figure 16.2A).

Parthenogenetic dihaploids

The second division in the pollen tube is sometimes irregular, so that there is no separation of the two generative nuclei. In this case only the egg cell or the secondary embryo sac nucleus can be fertilised and functional 6x endosperm is formed. Under these circumstances, the unfertilised egg cell is induced to differentiate into a 2x embryo (Figure 16.2B). This so-called prickle pollination is a very efficient way of inducing the formation of parthenogenetic dihaploids (2x) out of the tetraploid varieties which are basic material for breeding at the diploid level.

Meiotic chromosome doubling by unreduced gametes.

The 4x offspring is the outcome of normal double fertilisation with an unreduced pollen grain resulting in a 4x embryo with functional 6x endosperm (Figure 16.2C). This phenomenon is called meiotic doubling and occurs in reciprocal 4x × 2x crosses and 2x × 2x crosses as well if 2n egg cells and/or pollen grains are available. A parthenogenetic dihaploid is developed from an unfertilised egg cell in the presence of normal endosperm.

The production of parthenogenetic dihaploids is common practice all over the world because of the availability of an anthocyanin marker in *S. phureja* that is visible as an embryo spot in ripe seed (Hermsen and Verdenius, 1973). Spotted seeds are hybrids and spotless seeds are potentially of parthenogenetic origin and give rise to dihaploids. The production of 2n pollen is also observed in 2x *S. tuberosum* (Ramanna, 1979) and makes meiotic doubling possible. Selection for this trait is simple because of the presence of large-sized pollen with four germ pores instead of three.

Some diploid species like *S. bulbocastanum* and the non-tuberising species *S. brevidens* and *S. etuberosum* cannot be crossed directly with cultivated potato. Two different ways to solve this problem have been found. One is the use of bridge species crossable with both the wild species of interest and *S. tuberosum* (Hermsen and Taylor, 1979; see Box 16.2). The other is protoplast fusion. This technique is used for several

Box 16.2. **Procedure for making non-tuberous species accessible to potato breeding by the use of bridge species**

			bridge 1
Step 1.	2x-*S. etuberosum* (E)	2x-*S.*	– *pinnatisectum* (P)
		↓	
		2x-EP	(fully sterile)
Step 2.		↓	chromosome doubling
		4x-EP	(fertile)
	bridge 2		
Step 3.	2x-*S. verrucosum* (V)	× 4x-EP	
		↓	
		3x-VEP	(fully sterile)
Step 4.		↓	chromosome doubling
		6x-VEP	(fertile)
Step 5.	6x-VEP (T)	× 4x-*S.*	– *tuberosum* bottleneck (very low seed set)
		↓	
	5x-VEPT	× cultivars	
		↓	
		further crosses with cultivars for introgression of desired characters	

purposes, such as the exchange of extra-chromosomal DNA (mitochondria and/or chloroplasts) in so-called cybrids to create cytoplasmic male sterility and normal nuclear hybrids which are sometimes the first step to a new species, but are mostly used as basic material for the introduction of resistances by backcrosses with the cultivated species.

Recently, protoplasts of the non-tuberising species *S. brevidens,* which carries resistance to potato leaf roll virus (Austin *et al.*, 1985) and to *Erwinia carotovora* have been fused with those of *S. tuberosum* for breeding purposes.

Unfortunately, this interspecific fusion product is, according to the definition, considered to be a genetically modified organism (GMO) both in the Netherlands (Anonymous, 1992) and the EEC because of the absence of reports of direct sexual hybrids in the literature. This is not the case with the same source material obtained by crosses with bridge species. It is expected that *S. tuberosum* and *S. brevidens* can be combined sexually through the use of the embryo rescue technique. As soon as the experiment proves successful, removal of this somatic hybrid from the list of GMOs can be applied for.

ii) Development of breeding parents

Basic breeding material and complicated screening techniques are developed at universities and institutes. The resulting plant material, which is mostly diploid, has to be incorporated into breeding programmes for combination with other agriculturally valuable traits and for removal of undesired characters. This can be complicated when undesired and desired traits are genetically linked. The outcome of this pre-breeding process is the new parental material which can be directly used for variety development. These breeding parents can be either tetraploids or diploids that produce 2n gametes. In one step, the diploids deliver tetraploid offspring in 4x × 2x crosses.

iii) Variety development

As mentioned above, variety development can begin when the genetic variation to be combined is available in a restricted number of breeding parents. These breeding parents have been screened in test-crosses for combining ability. Variety development is finally a matter of selection of individual clones for yield, resistance to (a)biotic stresses and quality in seedling populations from crosses between parents that give the desired heterotic progeny. The basic aim is to develop high yielding varieties with good qualities for several applications, grown with a minimal input of chemicals to protect the crop against biotic factors. Each new variety is, therefore, a compromise of many positive factors and a few negative ones (Parlevliet *et al.*, 1991).

Table 16.1 presents a time scheme for clonal potato selection in the Netherlands using over 4 000 different crosses, including 2 000 test crosses for combining ability (Parlevliet, 1990). One tuber of each tuber-producing seedling is grown in the field. Selection is made for plant appearance, tuber shape, eye depth and uniformity, and the very late maturing genotypes are discarded. The second year clones are grown in a row and selected for some simple quality traits: yield, starch content, diseases and potential resistance to potato cyst nematodes. In the third year, besides resistances, clones are tested at the breeding station for quality traits like cooking type, raw discoloration and suitability for crisps and French fries. In the fifth-year and sixth-year clones, the first tests for resistance to viruses and *Phytophthora infestans* are made. At this stage of the breeding process, clonal multiplication of the most promising genotypes begins. The best clones go into the screening trials, which may last three years. These trials are carried out under the shared responsibility of all the potato breeding firms (ten members). The very best clones enter the official national trials that last another three years, and they are more intensively tested for processing by industry. For the most promising potential varieties, seed potato multiplication for commercialisation is organised separately. At the last stages, the best performing clones are tested in many other countries. Official national trials are organised differently in all countries and take two or three years to complete.

It is clear from the selection scheme that variety development is a matter of selecting the genotypes that perform best, using many existing varieties as a control. Breeding for new varieties is complicated because of the many potential applications and the required resistance, at least to potato cyst nematodes, viruses, wart and *Phytophthora infestans.* Observation of the mean number of seedlings needed for breeding one variety makes this clear: around 1920, 2 000 were required; today, some 200 000 are needed, in combination with intensive pre-breeding at the breeding station (Parlevliet, 1990).

Table 16.1. **Time schedule of clonal selection in potato in the Netherlands**

Year	Phase	Number of selected clones
0	Crossing, harvest seeds. Often many crossings.	10^6
1	Sowing *seed*, 250-1 000 per crossing, one tuber is kept per selected seedling.	600 000
2	*First year clones*. One plant/clone. Early harvest. 6-8 tubers kept per selected plant.	60 000
3	*Second year clones*. Six to eight plants/clone. Early harvest. All tubers kept per selected clone.	15 000
4	*Third year clones*. One plot/clone for seed potatoes, harvested early (*ca* 20 plants). One observation plot, harvested at time of maturity (16-20 plants).	5 000
5	*Fourth year clones*. One seed potato plot/clone, early harvest. Observation plots (16-20 pl) at several locations without replicates, right time of harvest.	1 500
6	*Fifth year clones*. One seed potato plot/clone, early harvest. Observation plots (16-20 pl) at many locations, some of them in other countries, harvested at proper time. No replications. Best clones will enter the screening trials (ST).	500
7	*ST*. As in the preceding year, the best clones of all breeders are compared with one another in observation plots (16-20 pl) on many locations without replications. Seed potato production occurs separately as before with early harvest. The best clones from the first year in the ST go over to the second year test. The best out of this second year test can enter the national trials (NT).	200
9	*NT*. The clones are now compared with several of the recommended clones in yield trials on many locations with replicates. All relevant traits are evaluated. The official list of recommended cultivars gives information about more than 30 traits of importance. Each year clones are dropped from the trials when they appear as no improvement over the recommended ones.	15-20
12	*Recommendation*. Only clones that are improvements over those already on the national list of recommended cultivars are admitted to this list.	3-6
	Note: Until the third year clone assessment is at early harvest to prevent virus infection through aphid vectors. After that, assessment is done at the harvest date fitting the clone to be assessed. Seed potatoes for the next year are produced from separate fields, harvested early.	

Source: Parlevliet (1990), with permission.

b) Main breeding objectives

Before starting a breeding programme, it is important to define the breeding aims, so as to know the genetic variation (germplasm) needed. In a modern variety more than 50 traits are purposely combined. They can be grouped into:

- yield, which is a complicated trait including factors such as adaptation to modern agricultural techniques, harvest and storage;

- resistance to abiotic and biotic stress factors, such as drought, heat, diseases and pests;
- the qualities required for current and developing end uses.

Most of these traits are quantitative and inherited by polygenes. Therefore, a low percentage of seedlings is likely to express this kind of trait to a degree equal to or greater than the parents. A few traits are inherited monogenically, mostly in a dominant fashion that simplifies selection. This is not the case for recessively inherited characters at the tetraploid level, especially when recognition is a problem (Jacobsen *et al.*, 1991). Modern farming is obliged to use germplasm containing important traits of these three categories. For this reason, species crossing has become obligatory in potato crop breeding.

C. Multiplication for commercial use

a) Surveillance of the behaviour of clone material during maintenance and multiplication

Multiplication of potential new varieties and of existing varieties is a very important aspect of commercialisation. A new cultivar on the Dutch recommended list of cultivars (Parlevliet *et al.*, 1991) has to be maintained and multiplied pure and healthy. Thanks to the new *in vitro* propagation techniques, it is possible:

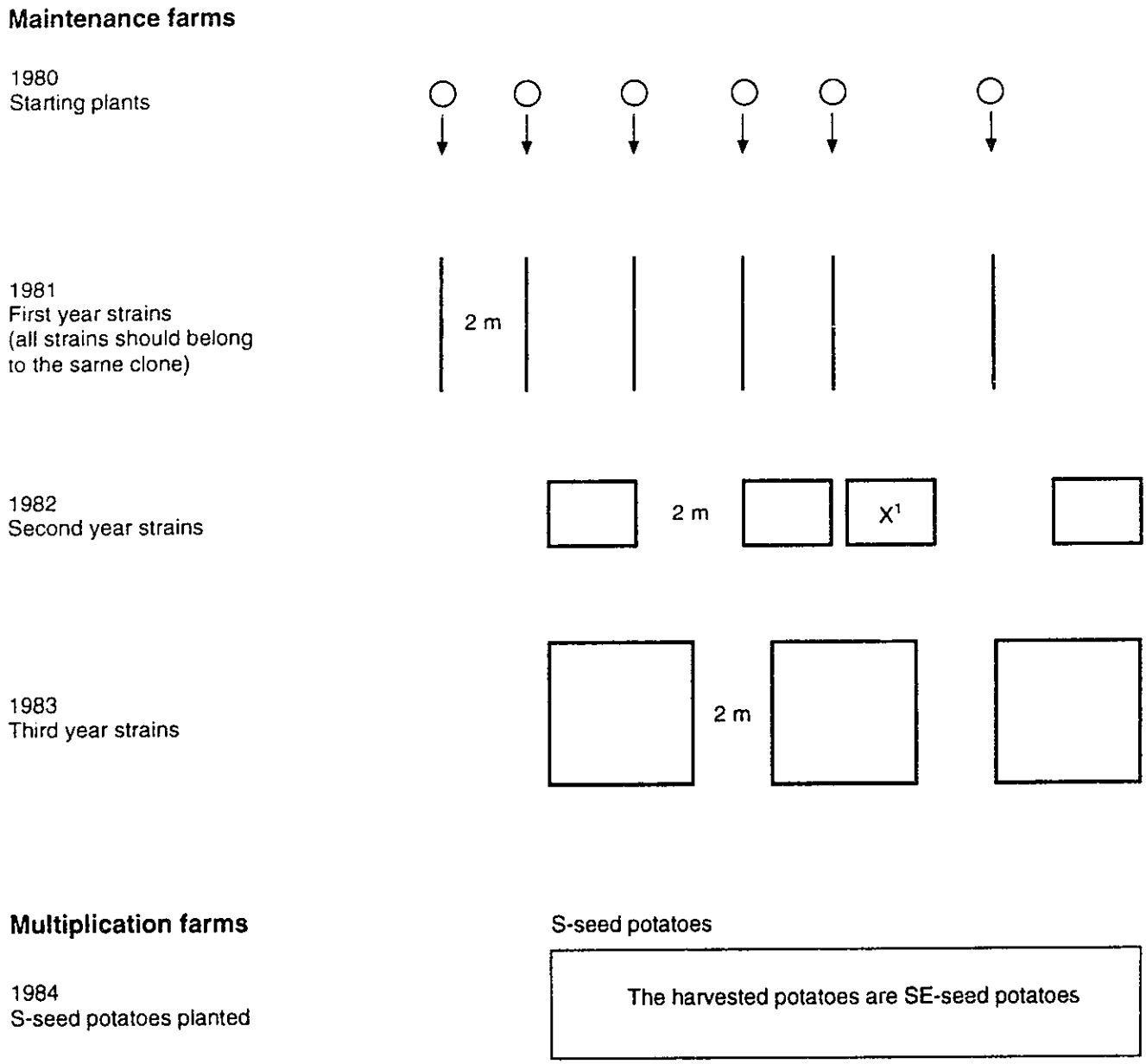
- to speed up the introduction of new varieties on a relatively large scale;
- to maintain basic material of existing varieties virus-free and free of infections by *Erwinia carotovora* and *Corynebacterium scepedonium*; and
- to use meristem culture in order to make infected plants virus-free.

To maintain and multiply a variety, it is important to avoid the dangers of accumulating micro-mutations during maintenance and of contaminating the stock by mixing up cultivars. Both represent real risks. With respect to mutations, it must be said that micro-mutations, those that cause a minor effect, are most important because they create recognition problems. Macro-mutations, instead, are easily recognised. It is equally important to maintain healthy basic plant material. Infection with pathogens, especially with viruses and bacteria, is a risk during maintenance and multiplication. The presence of such pathogens is often not easy to detect in tubers.

In the Netherlands, an efficient multiplication system involving three production groups and the Dutch inspection service for arable crops (NAK) has been developed. The groups are:

- the breeder of the new variety, who provides the initial material to the maintainer;
- the maintenance farmer, who ensures the maintenance of the pure and healthy cultivars and produces high-grade basic seed potatoes of classification S (Figure 16.1, S);
- the multiplication farmer, who multiplies the S-seed potatoes into material with a degree of quality (Figure 16.1, SE, E, A, B and C), which finally will be sold to the potato growers.

Figure 16.1. **The maintenance and multiplication scheme of a potato cultivar in the Netherlands**



1985: The SE-seed potatoes produce E-seed potatoes.
1986: The E-seed potatoes produce A-seed potatoes.
1987: The A-seed potatoes produce B-seed potatoes.
1988: The B-seed potatoes produce C-seed potatoes.

1. Refused if any of the following tests is negative:
   – Field inspection.
   – Serological test (Elisa).
   – Type.
   – Pest control.

Source: Parlevliet (1990) with permission.

b) Potato seed certification

The state agency controls seed potatoes for the absence of disease and for true representation of the variety during all stages of multiplication and certification of seed potatoes. Basic seed potatoes are designated S, SE and E (Anonymous, 1982), and certified seed potatoes are designated A, B and C. Grade C is sold to the normal potato grower for production in the field.

c) Surveillance of variety behaviour during its commercial life

Figure 16.1 shows a scheme for commercial maintenance and multiplication of varieties. The variety is delivered by the breeder to the maintenance farmers. The maintenance farmers undertake the first four years of multiplication in order to produce healthy S-seed potatoes from individual plants (15-20 per variety). If these plants look healthy and true to the cultivar, they are investigated intensively for the absence of infections. (A more modern starting point is from shoots multiplied *in vitro*.) During the following three years multiplication takes place, and some of the stems are discarded because of small deviations or virus infections. A distance of 2 m is maintained between the rows of the different stems to avoid mixing, exchange during harvest, and virus transfer during growth from stems infected by aphids or by mechanical means. Strips of wheat are grown between the rows. The harvest is always early, before aphid invasion. All plants are tested serologically for virus infection the first year, 10 per cent are tested in the second year, and 2 per cent in the third. In the second year, a sample of all strains is grown at the so-called "central strain field trial" of the NAK to control health and to detect small deviations caused by micro-mutations. At this stage individual plants are selected for a new multiplication cycle. The selected second year strains are grown separately again in the third year. After early harvest the most reliable strains are combined. This lot of seed potatoes is sold to the multiplication farmers as S-seed

Figure 16.2. **Fertilisation biology (double fertilisation) in 4x x 2x crosses explaining abortion of 3x embryos (A) and outgrowth of 2x (B) and 4x (C) embryos**

	Pollen tube		
Ploidy level of:			
	A	B	C
Embryo (egg cell + sperm cell)	3x	2x	4x
Endosperm (sec embryo sac nucleus + sperm cell)	5x	6x	6x
	Abortion	Dihaploid	Tetraploid

potatoes, which have a licence from the breeder. It can generally be said that, per cultivar, 8-12 strains survive all selection steps.

During large-scale multiplication, virus-infected plants are discarded; the material is also controlled for homogeneity (absence of micro-mutations). The NAK is much involved as inspector during the process of producing and classifying certified seed potatoes. In classes such as E, A and B, material can remain for one or two multiplications at the same classification level; this mainly depends on the frequency of virus-infected plants. The certified seed potatoes are sold to the regular grower.

It is clear from Figure 16.1 that the maintenance and multiplication of a variety of a vegetatively propagated crop is complicated by the many potential risks. In the Netherlands, the most advanced propagation system involves the breeder, the maintenance farmer, multiplication farmers and the NAK, working together as a team. Its goal is to produce healthy plant material true to the variety. The process takes about ten years.

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17. *Prunus*

by

Françoise Dosba and R. Bernhard

A. Characteristics of the crop

a) Geographic origins; centres of diversity

Almost all the species in the genus *Prunus* originated in the northern hemisphere. The sub-genera *Prunophora* and *Cerasus* appear to have three origins: Europe, eastern Asia and North America. The sub-genus *Amygdalus* apparently has two, *i.e.* western and eastern Asia.

b) Main production areas

Table 17.1 contains information on world production of the principal fruits in the genus *Prunus*.

c) Taxonomic status

Prunus is a very large genus in which botanists have placed between 70 and 400 species. While some are ornamental, three out of the five commonly acknowledged sub-genera are grown for their kernel fruit, namely *Prunophora* (plum and apricot), *Amygdalus* (almond and peach), and *Cerasus* (cherry). A simplified classification of species in the genus *Prunus* is provided in the Annex.

d) Genetic and cytogenetic characteristics

The *Prunus* family has a basic chromosome number of 8, although some forms contain between 16 and 64. Female fertility is lower in tri- and pentaploids.

Cross-breeding, a relatively easy task within a sub-genus, usually produces hybrids with satisfactory, albeit variable, fertility. Hybrids of different sub-genera are generally sterile, but some have a degree of male fertility enabling them to be used as parents.

Table 17.1. **World production (thousand tonnes) of apricots, cherries and peaches for the main producer countries**

(average calculated over three years: 1989-91)

	Total production	Imports	Exports	Fresh consumption	Processed	Withdrawn
Apricot						
European community	550	21	73	354	128	16
Northern hemisphere	1 089	22	81	523	490	17
Southern hemisphere	123		4	30	89	
Subtotal	1 762	43	158	907	707	33
Cherries (sweet and sour)						
European community	474	37	34	365	112	
Northern hemisphere	1 145	54	64	710	425	
Southern hemisphere	19		4	11	4	
Subtotal	1 638	91	102	1 086	541	
Peaches and nectarines						
European community	3 546	73	596	1 799	588	636
Northern hemisphere	5 714	185	667	3 208	1 389	635
Southern hemisphere	643	3	62	270	310	4
Subtotal	9 903	261	1 325	5 277	2 287	1 275
Total	13 303	395	1 585	7 270	3 535	1 308

Source: *Horticultural Products Review*, November, 1991

e) Current end uses and past evolution of end uses

Many *Prunus* species grown for their fruit are intraspecific hybrids of varieties with diverse geographical origins. These hybrids, some older than others, have been improved by mass selection, cloning or hybridisation.

Domestic plum-trees, in some cases at least, are said to have been obtained by cross-breeding *P. spinosa* and *P. cerasifera,* but this remains a controversial point. The sour cherry can be traced back to *P. avium* and *P. fruticosa.* The Japanese plum tree comes from either the species *P. salicina* or hybrids of this and other European species such as *P. cerasifera* or more commonly from North American species (*P. munsoniana, P. angustifolia, P. hortulana*). "Black" apricots can be traced back to *P. armeniaca* and *P. cerasifera,* and plumcots to *P. armeniaca, P. hortulana, P. angustifolia, P. munsoniana* and *P. subcordata.*

The species and varieties grown as rootstock were initially selected from well-adapted spontaneous local forms and propagated using seeds or cuttings. Now, stock cultivars are selected improved lines or clones or, increasingly, interspecific or inter-subgeneric hybrids that have been multiplied vegetatively.

Progress in vegetative propagation techniques, whether horticultural or *in vitro,* offer substantial potential for stock breeders to resolve certain problems relating to:

- adaptation to soil, climate or a given parasitic/phytotoxic environment (soil depletion);
- changes in orchards due to the creation of more or less developed forms of trees (dwarfing or fast-growing stock);
- greater productivity in grafted varieties or higher quality for fruit crops.

Species grown for their blossom are produced from species in the various subgenera, their mutants (*P. persica,* weeping, red-blossom, pink-blossomed almond-tree) or interspecific hybrids (*P. serrulata, P. pseudocerasus, P. cerasifera, P. tenella,* peach/almond hybrids grown in response to demand for "peach blossom" from herbalists).

Reproductive mechanisms

Sexual reproduction is used to propagate certain breeds of stock:

- lines of peach, *P. mahaleb* and apricot (self-fertile);
- F_1 hybrid seed controlled through self-incompatibility or male sterility (hybrid St. Julien, hybrid wild cherry).

Sexual reproduction has virtually been abandoned today as a means of propagating varieties grown for their fruit; for rootstock, the method is used but the selection process is lengthy and expensive.

Vegetative propagation is widespread, now that technical advances have made it possible to muliply intraspecific or interspecific hybrids as soon as they are discovered to be of interest.

Environmental requirements for life cycles

a) Climatic restrictions to extension of the crop

The improvement in fruit species has brought with it definite changes in cultivation areas. Over the past 50 years, for instance, improvements in the peach species have extended its cultivation area northwards (Canada, Poland) as branches but more especially roots have been made more resistant to cold weather, and southwards (Florida, South Africa, southern Mediterranean) now that the chilling requirements for buds have been reduced. Nevertheless, climatic conditions may act as a barrier to further extension.

b) Biological restrictions to extension of the crop

Insufficient light may limit the farming of certain kernel crops (almonds in western France, peaches in Reunion Island). On the Atlantic coast (France, Portugal) high ambient humidity, in which brown rot and bacterial disease thrive, also hampers the growing of several stone fruit crops (apricots, plums, sour cherries). Resistant or less sensitive sources do exist, however.

B. Current breeding practices and variety development research

a) Main breeding techniques

i) Germplasm maintenance

Conserving the genetic resources of *Prunus* is the responsibility of individual countries, or even regions. In France, most of this conservation work is done by the National Institute for Agricultural Research, INRA (Institut National de la Recherche Agronomique) and the National Botanical Conservatory in Porquerolles. A plan for conservation at regional level combined with nationwide data collection has been put forward, but has not yet been fully implemented. Europe-wide co-ordination exists within the IBPGR and a *Prunus* database has been set up for this purpose at the Nordic Gene Bank in Sweden. Management of this database will devolve to INRA in Bordeaux (France) from 1992. It will be co-ordinated for the whole of Europe.

ii) Basic breeding

INRA has undertaken enhancement work, based on ambitious and mostly long-term objectives. Examples include improved methods of peach-tree breeding, greater resistance to a variety of parasites, research into rootstock to achieve better quality and higher productivity, etc. To improve cultivated species of *Prunus,* the right agronomic features are and will be increasingly obtained by using certain ecotypes or mutant forms, or indigenous and exotic wild species.

Substantial success has already been achieved using the late-flowering and self-compatible characteristics of Apulian almond ecotypes, the resistance to *Meloidogyne* nematodes displayed by Indian peach ecotypes and *P. davidiana,* and the tolerance to white root rot found in *P. cerasifera* and its peach hybrids, etc.

There is still a great deal of room for progress, particularly as regards wild species, but this will require long- to very long-term research projects, with a time horizon of up to 20 years.

iii) Variety development

Breeding new fruit varieties usually means cross-breeding those with complementary phenotypes. A breeder with experience will know how to detect parents that can pass on valuable features. Thanks to the development of quantitative genetics for fruit species, the heritability of the main quantitative characters has become a more exact science.

New methods are being envisaged, and variants of a variety resistant to *Bacterium pruni* have been obtained from protoplast cultures that were found to have resisted infection. Controlled mutagenesis of isolated organs or cells may also produce valuable characteristics.

The introduction of genes through genetic engineering is also beginning to be used in *Prunus*. For instance, genes coding for the capsid protein in the Sharka virus have already been introduced into the young tissue of plum and apricot trees. Regenerated embryos were also obtained in 1992.

Finally, the use of molecular biology in genetic enhancement programmes is expected to improve breeding methods – in particular by introducing molecular markers for agronomic characteristics not expressed during the juvenile phase – by searching out interesting QTLs (quantitative trait loci).

Table 17.2. **Important selection objectives for different varieties**

Objectives	Peach Nectarine Pavy	Domestic plum	Japanese plum	Cherry	Apricot	Almond
Fruit size and appearance	X			X		
Late flowering					X	X
Higher quality		X	X	X	X	
New features (fruit)	X					
Fewer branches		X				X
Technical processing problems	X	X		X	X	X
Easy mechanical harvesting	?	?		X	?	X
Resistance to pit splitting; strong skin		X		X	X	
Resistance to parasites	Leaf curl Sharka *Fusicoccum* Mildew	Brown rot Sharka Rust	Bacterial blight ACLR [1]	Bacterial blight Brown rot (sour cherry)	Sharka ACLR [1] Bacterial blight Brown rot	Bitter rot *Fusicoccum* Brown rot

1. ACLR: Apricot chlorotic leaf roll (mycoplasm).

b) Main breeding objectives

The objectives are numerous, some of them broader in focus, others more closely targeted. They may for instance aim to solve a phytosanitary problem in a particular production area or develop a somewhat exotic crop close to a high-consumption centre or under less than suitable soil/climate conditions.

Table 17.2 indicates important cultivar breeding objectives. The broader objectives that remain for improving rootstock, in spite of technical advances, include suitability for vegetative propagation, if possible with no subsequent tendency to produce suckers; characteristics that favour nursery work and grafting (no thorns, few branches, etc.); ability to be used with several different scion species.

More specific, and more or less ambitious, objectives include:

- achieving higher productivity in grafted varieties;
- improving and supplementing the range of vigour induced by rootstock;
- continuing to pursue resistance to chlorosis and asphyxia as a fundamental objective; resistance to asphyxia improves the ability to explore various soil horizons;
- achieving tolerance of, or reducing sensitivity to, the most threatening soil fungi or bacteria, *e.g. Phytophthora*, white root rot, crown gall (*Agrobacterium tumefaciens*);
- obtaining breeding stock which is resistant to or tolerant of graft-transmissible parasites (viruses and especially Sharka) or can limit their multiplication (MLO);
- developing resistance to certain nematodes (*Meloidogyne* and above all *Pratylenchus vulnus*).

Species in the genus *PRUNUS*

Simplified classification of sub-genera

I. *AMYGDALUS*

1. *Amygdalus (2n = 16)*

N.E. Mediterranean	*P. dulcis* (= *P. amygdalus* or almond tree), *P. webbii*
Armenia, Iran	*P. fenzliana, P. kotschyi (P. argentea, P. orientalis)*
Afghanistan, Tajikistan	*P. bucharica, P. kuramica*
China	*P. dehiscens (= P. tangutica), P. triloba*
Siberia	*P. tenella (= P. nana)*

2 *Persica (2n = 16)*

China	*P. davidiana (P. kansuensis), P. persica* (or peach tree), *P. mira*

II. *PRUNOPHORA (2n = 16 to 48)*

Western Asia + Europe (1 to 2 flowers)	*P. cerasifera, P. divaricata* *P. cocomilia, P. spinosa* *P. insititia, P. domestica*	
Eastern Asia (1 to 3 flowers)	*P. simonii, P. salicina (= P. triflora)*	N
North America (2 to 4 flowers)	*P. americana (P. nigra)*	↓
trees	*P. hortulana (P. munsoniana)*	↓
	(P. mexicana, P. umbellata (= P. injucunda)	S
shrubs	*P. maritima (P. alleghaniensis)*	N
	P. subcordata, P. angustifolia	↓
	P. reverchonii, P. gracilis	S

III. *ARMENIACA (2n = 16)*

Southern Alps	*P. brigantiaca (= P. brigantina)*
Central-eastern Asia	*P. mume*

Northeastern Asia	*P. sibirica (P. mandshurica)*
Western Asia	*P. armeniaca* (= apricot tree)

IV. *CERASUS*

1. *Microcerasus (2n = 16): shrubs (1 to 2 flowers)*

Central-northern Asia	*P. tomentosa (P. incana, P. prostata), P. japonica, P. glandulosa*
North America	*P. pumila (P. besseyi)*

2. *Pseudocerasus (Asia): (2n = 16 to 32): 3 to 5 flowers*

China	shrubs	*P. canescens, P. concinna*
Japan	shrubs	*P. incisa (P. nipponica)*
Japan	trees	*P. subhirtella, P. campanulata*
Himalayas	trees	*P. rufa (P. puddum, P. serrula)*
Central China	trees	*P. conradinae*
Eastern Asia	trees	*P. sargentii, P. serrulata (= P. pseudocerasus)*

3. *Lobopetalum: 3 to 6 umbellate flowers – 2-lobed petals*

Central China	*P. dielsiana*

4. *Eucerasus (2n = 16 or 32)*

Eastern Europe	shrubs	*P. fruticosa* (2n = 32)
		P. cerasus (2n = 32) (= sour cherry)
Western Europe	trees	*P. avium* (2n = 16) (= sweet cherry)

5. *Mahaleb (2n = 16): 5 to 12 flowers, umbellate or clusters*

Southern Europe	*P. mahaleb* (= St. Lucie)
Northeastern United States	*P. pennsylvanica*
Western United States	*P. emarginata*
Northeastern Asia	*P. mazimoviczii*

V. *PADUS (2n = 32): clustered flowers*

Western Europe	trees	*P. padus*
United States	trees	*P. serotina (P. alabamensis)*
	shrubs	*P. virginiana*
China	trees	*P. sericea (P. pubigera)*
Himalayas	trees	*P. cornuta*
Northeastern Asia	trees	*P. grayana (P. ssiori), P. maakii*

VI. *LAUROCERASUS clustered flowers – evergreen*

Southwestern Europe	large shrubs	*P. lusitanica*
Mediterranean	large shrubs	*P. laurocerasus*

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Glossary

Accession	Entry in a gene bank collection; accessible sample in a gene bank
Adaptation	Any change in an organism's structure or function that allows it to better cope with conditions in the environment. Adaptation can be achieved as pure phenotype or as genotype adaptation, the latter leading to inherited value or fitness in a certain environment[1]
Additive factors	See additive genes
Additive genes	Genes interacting and showing no dominance (if alleles) or showing no epistasis (if non-alleles)[1]
Allele	Any of the different forms of a gene occupying the same locus on homologous chromosomes, and which undergo meiotic pairing and can mutate one to another. Often a gene consists of more than two alleles (allelic series)[2]
Allogamous	Cross-fertilising
Allotetraploid	Hybrid polyploid derived by the doubling of the chromosome complement of a diploid hybrid[2]
Amphidiploid	A polyploid formed of hybrid diploid parents by doubling of the chromosome set. The diploid hybrids from which amphidiploids originate are generally sterile owing to the significant non-homology between the chromosome sets and the consequent difficulty of chromosome pairing during meiosis. With the doubling of the chromosome the sterility barrier is removed. Amphidiploids commonly possess better viability and competitive ability than the original species and the diploid hybrid[1]
Androecious	Of plants having only male flowers[1]
Androgenesis	Development of a haploid embryo from a male nucleus, *e.g.* by the germination of a pollen grain within an anther[1,2]
Annual	Having a yearly periodicity; living for one year[2]
Anther	The part of the stamen in which the pollen grains are produced[2]

Apomixis	Reproduction without fertilisation, in which meiosis and fusion of gametes are partially or totally repressed. The embryo generally has the same chromosome number and the same genotype as the mother individual[1,2]
Arable	Suitable for plant cultivation
Autogamous	Self-fertilising or self-pollinating
Bank	See gene bank
Basic seed	See foundation seed
Biotype	A group of genetically identical individuals
Bolting	Flower and seed stalk formation
Bract	See sepal
Bran	See pericarp
Breeder seed	Seed population, true to type and uniform, based on individual plant selections and representing the genuine variety characteristics
Budding	Forming leaf or flower buds
Bulk harvesting	See bulking
Bulking	Harvesting, with or without mass selection, of seed from a given population of a self-pollinated crop, followed by single plant selection[3]
Callose	Sediment of carbohydrate composition in vascular tissue. Callose has strong light refraction
Canopy	The uppermost continuous layer of foliage in the vegetation of a crop (after note 2)
Carpel	Fruit wall segment
Castration	Removal of sexual parts of the flower
Centre of diversity	Geographic area where the greatest genetic diversity of a crop is found. This area may, or may not, coincide with the centre of origin
Centre of origin	Geographic area (according to the theory of Vavilov) where the greatest genetic diversity of a crop or species is originally found
Certified seed	Officially approved commercial seed, true to type and sufficiently pure for agricultural use
Chloroplast	Cell organelle limited by a double membrane and containing chlorophyll; a lamelar structure in a protein-rich ground substance, containing a minor portion of the plant genome[1]
Chlorosis	Deficiency in the synthesis of the green substance in the leaves, chlorophyll, the site of photosynthesis

Chromosome	Nuclear body composed largely of DNA and protein, and comprising a linear sequence of genes[2]; chromosomes transmit genetic information from cell to cell and from generation to generation; they release information to control cellular functions. Chromosomes are self-reduplicating structures. In diploid plants each chromosome has a structurally similar, homologous partner
Clone	Assemblage of organisms derived by vegetative multiplication from a single (sexually derived) individual[2]. Individual plants of a clone are genetically identical
CMS	See cytoplasmic male sterility
Contamination	1. Introduction of an undesirable agent such as a pest or pathogen into a previously uninfested/uninfected situation[2] 2. Genetic impurity of a population by involuntary cross-pollination with alien pollen grains
Corolla	Petals and sepals
Cotyledon	First leaf or leaves appearing on seedling after germination
Crop	A domesticated species or subspecies grown for agricultural purposes
Cross-fertilisation	The union of male and female gametes from different individuals of the same species
Cross-pollination	Transfer of pollen from a flower of one plant or population to the stigma of a flower on another plant or population (after note 2)
Culm	Stem of a grain crop
Cultigen	1. See cultivar, variety 2. A group of varieties and various types of lines, such as breeding lines, mutants or marker lines, of cultivated crop species[4]
Cultivar	See variety
Cybrid	The fusion product of a cytoplast from which the nucleus has been removed with an intact, nucleus-containing cell[1]
Cytoplasm	See protoplast
Cytoplasmic male sterility	Maternally inherited inability of a higher plant to produce viable pollen
Dehiscence	Spontaneous opening of ripe plant structures to liberate seeds and spores[2] as well as pollen
Dihaploid	1. Individual produced from a tetraploid form which possesses half the tetraploid number of chromosomes[1] 2. Individual produced by doubling the number of chromosomes from a haploid individual. In this case dihaploids are completely homozygous.

Diploid	Carrying a double set of homologous chromosomes, typical of most organisms derived from fertilised egg cells (2n or 2x)[2]
Domestication	Adaptation of plants for life in intimate association with man[2], adaptation of wild plants to cultivation by man
Dominant	Expression of a gene into a character for which the corresponding alleles are manifest in both homozygous and heterozygous conditions (after note 1)
Dormancy	State in which viable seeds fail to germinate under conditions favourable for germination and vegetative growth[2]
Drift	See genetic drift
Ear	Female inflorescence of maize and grains
Ecotype	Locally adapted population; a race or intraspecific group having distinctive characters which result from the selective pressures of the local environment[2]
Egg cell	The fertile haploid cell in the embryo mother cell that developes into the diploid embryo after fertilisation
Emasculation	Removal of anthers
Emergence	Capacity to germinate in a farmer's field
Endemic	Native to, and restricted to, a particular geographical region[2]
Endosperm	Part of the egg cell that develops into nutrition tissue, distinct from the embryonic tissue
Epidemic	High degree of diffusion of disease in a plant population
Exotic	1. Organism coming from a non-related species 2. A non-indigenous plant
Family	1. Selected group of basic seed populations or selected pollen producing populations in a cross-pollinating species 2. A group of genera phytogenetically related
Female-sterile	Unable to produce viable ovules or seed
Fertilisation	The union of a male and female gamete to form a zygote[2]
Field emergence	See emergence
Filament	Part of male flower supporting the anther
Fixed	Homozygosity of genes having favourable pleiotropic effects; the attainment of homozygosity in a population which thereby becomes monomorphic with respect to a given allele[2]; genetically uniform
Flower initial	The cell division area from which flower bud formation begins
Fodder crop	See forage crop
Forage crop	Animal feed producing crop

Foundation seed	Seed population grown from breeder seed, of guaranteed identity, genetic purity and homogeneity, suitable for multiplication to registered seed
Free-threshing	Seed hull detaches from seed when dry
F_1-hybrid	See hybrid
Gamete	Mature reproductive cell, usually haploid, serving in fertilisation (after note 2)
Gametophyte	Plant stage in haploid sexual generation which produces the gametes[1]
Gene bank	Internationally recognised collection of wild materials, landraces, cultivars, mutants, research materials and breeding lines
Gene pool	The total genetic material of a freely interbreeding population at a given time[2]
Genetic drift	Occurrence of random changes in the gene frequencies of small isolated populations, not due to selection, mutation or immigration
Genetic marker	Any allele used as an experimental probe to identify a nucleus, chromosome, or gene[1]
Genetic variation	That part of phenotypic variance of individuals in a population produced by differences or changes in genetic constitution such as mutation or recombination[2]
Genome	The basic (monoploid) set of chromosomes of a particular species minimally required for the proper functioning of a cell (after note 2)
Genotype	Sum total of the genetic information contained in the plant DNA. The genotype determines not a unique phenotype, but a range of phenotypic capacities referred to as an individual's "norm of reaction" to the environment[1]
Genus	Taxonomic rank between family and species; classification comprising one or more phytogenetically related and morphologically similar species[2]
Germ	Inside part of the seed; embryo and endosperm
Germplasm	The hereditary material transmitted to the offspring via the gametes
Haploid	Carrying only one set of chromosomes (n or x). Haploid plants are unstable, weak and sterile. Normally they do not occur in nature
Herbaceous	Non-woody
Hermaphrodite	Separate male and female organs in one organism (flower, plant)
Heterogeneous	A breeding population in which the majority of genes are heterozygous

Heterozygote	Plant with a heterozygous gene pair
Heterozygous	Having two different alleles at a given locus of a chromosome pair[2]
Hilum	Navel
Homogeneous	A breeding population in which the majority of genes are homozygous
Homozygous	Having two identical alleles at a given locus of a chromosome pair[2]
Horizontal resistance	Resistance depending on many genes, each of which is of low effectiveness
Hulled	Seed to which the seed hull remains attached when seed has dried
Husks	Foliage surrounding the inflorescences or seeds
Hybrid	1. Variety of which the seed is obtained by multiplying the seed of one basic seed population – the female parent line – and restricting pollination to one other basic seed population – the male parent line. Both female and male parent lines are usually obtained by continued self-fertilisation, or inbreeding. 2. Offspring of a cross between genetically dissimilar individuals[2]
Hybridisation	The process of crossing two dissimilar plants
Hybrid variety	See hybrid
Hypocotyl	The stem part under the cotyledon of the seedling
Inbreeding	Continued self-fertilisation over several generations; mating of individuals more closely related than average pairs in the population[2]
Infection	Invasion of a host by a parasite or pathogenic microorganism[2]
Infestation	Invasion by parasites or pests[2]
Inflorescence	The grouping of flowers in a special plant organ
Inoculation	Administering inoculum to a plant or to a plant population
Inoculum	Individual or (concentrated) group of individuals comprising the founders of a colony or a newly established (usually pathogenic) population[2]
Insect vectors	Insects transmitting disease from one organism to another
Introgression	Spread of genes of one species into the gene pool of another by hybridisation and backcrossing[2]
Karyology	The branch of cytology dealing with the study of nuclei, especially the structure of chromosomes[2]
Landrace	(Formerly) cultivated population maintained by farmers without methodological selection

Legume	All pea and bean crops, pulses, belonging to the Leguminosae
Linkage	Presence of specific genes on the same chromosome located so closely that the traits are not independently assorted. The greater the proximity of these genes, the lesser the chance of their separation by crossing over (when gametes are formed) and the stronger the linkage[2]
Locus	The site on the chromosome where a gene is located
Lodging	Inability to stay upright; bending and falling down of culms (stems)
Maintainer line	Pollen parent line carrying restorer gene(s)
Male-sterile	Unable to produce viable pollen grains
Maternal haploid	Individual with a single genome or chromosome set, entirely derived from one mother plant
Meiosis	Reduction division, consisting of two successive divisions of a diploid nucleus, resulting in the formation of the haploid gametes, each of which contains one of each pair of the homologous chromosomes of the parent cell[2]
Micro-mutation	Mutation within a single gene[2], causing a minor effect
Mitochondria	Semi-autonomous higher plant cell organelles having their own genetic system encoded in mitochondrial DNA[1], a minor portion of the genome. They are maternally inherited. Malfunction of mitochondria is probably a general cause of CMS[1]. See pollen restoration
Monoecious	Having complete male flowers and complete female flowers growing on the same plant
Monogenic	Trait depending on only one gene
Multifoliate leaf	Leaf composed of many small leaflets
Mutagenesis	Purposely directed destabilisation of the genome in order to cause mutation
Mutant	Any organism, gene, or character that has undergone a mutational change[2]
Mutation	A sudden heritable change in the genetic material, most often an alteration of a single gene by duplication, replacement or deletion of a number of DNA base pairs[2]
Necrosis	Death of plant tissue
NMS	Nuclear male sterility
Nucleus	Cell organelle enclosed in a membrane containing the bulk of the individual's genetic information[1]
Oligogenic	Trait depending on a small number of genes
Onion set	Small onions grown from seed for transplanting and production of large bulbs
Organelle	Part of a plant organ

Ovary	Organ containing the ovule
Ovule	The flower organ producing the seed. It includes the nucellus and the integuments[1]
Paddy	Rice
Panicle	Inflorescence of rice, oat, sorghum
Parthenogenesis	Induction of an embryo in an unfertilised egg-cell[1]
Partial resistance	A situation in which a pathogen only affects the plant to a certain extent
Pathogen	Microorganism causing disease
Pedigree seed	See breeder seed
Perennial	Plant that persist for several years with a period of growth each year[2]
Pericarp	Outside skin of the seed; tissue originating from the mother plant, not from the fertilised ovule
Petal	The inner circle of flower leaves, usually coloured, surrounding the sexual flower organs
Petalloid anthers	Anthers transformed into petals
Petiolate leaf	Leaf with stem
Phenotype	The product of the interaction between the genotype and the environment – the observable structural and functional properties of an organism[2]
Photoperiod	Day length
Photosynthesis	The biochemical process that uses radiant energy from sunlight to synthesise carbohydrates from carbon dioxide and water in the presence of chlorophyll[2], releasing oxygen to the atmosphere
Pistil	Entire female part of the flower
Plant species	See species
Plant variety	See variety
Ploidy	Number of sets of chromosomes
Pollen	The sum of all pollen grains
Pollen grain	One of four haploid microspores formed in the anther by a pollen mother cell, which germinates to form the male gametophyte (after note 1)
Pollen restoration	Return of male fertility to a cytoplasmic male-sterile plant by a nuclear restorer gene able to override the effects of the cytoplasm, or cytoplasmic reversion to male fertility[1]
Pollen tube	Tube growing from a germinating pollen grain into the stigma, style and ovary of a flower. The pollen tube carries the male gametes

Pollination	The transfer of pollen from the anther to the receptive area of a flower, used loosely to mean fertilisation of a seed plant[2]
Polygenic	Trait controlled by the integrated action of multiple independent genes[2]
Polymorphous	Having several different simultaneous forms; polymorphism is supposed to be a self-regulatory phenomenon in population behaviour
Propagation	Multiplication
Propagule	Any individualised plant tissue able to grow out and produce a complete new plant[2]
Protoplast	Plant cell after removal of cell wall[1]
Race	An intraspecific category characterised by conspicuous physiological, biological, geographical or ecological properties[2]
Ratoon crop	A crop of which the above-ground parts have been harvested and the ground part is allowed to sprout again from auxiliary buds on basal nodes or crown to produce another crop[4]
Recalcitrant	1. (of tissue) tissue resisting genetic modification 2. (of seed) having short longevity, even under artificial favourable influences
Recessive	An allele that is not expressed in the phenotype except when homozygous[2]
Registered seed	Officially approved commercial seed of excellent purity and trueness to type, suited for one, sometimes two further commercial multiplications to certified seed. Registration is made by seed officials or by the producer under licence from the seed office
Resistance	Ability of a plant to cope with attacks by pathogenic organisms, pests or adverse physical conditions, thereby reducing the effect of the attack
Restorer gene	See pollen restoration
Rogueing	Elimination of off-type plants
Scion	Part or structure transplanted from one plant to another during a graft[2]
Sclerenchyma tissue	Hard-walled plant tissue
Seed-borne	Carried by or in the seed
Seed-head	Inflorescence
Seed stock	A certain quantity of qualified seed
Seed-transmitted	See seed-borne

Segregation	The process in sexual organisms by which the two members of an allele pair, or a pair of homologous chromosomes, separate during gamete formation, with each gamete receiving only one member of the pair[2]
Select seed	First multiplication of breeder seed following severe individual plant selection
Self-fertilisation	The union of pollen grains of a plant with the ovules of the same plant
Self-pollination	Transfer of pollen from anthers to stigma of the same flower or to another flower on the same plant[2]
Semi-dominant	Having alleles that are neither completely dominant nor completely recessive so that each is expressed to some extent in the heterozygote[2]
Sepal	The usually green outer circle of flower leaves surrounding the sexual flower organs
Serological test	Disease test using antigens and antibodies
Sessile leaf	Leaf without stem
Set	See onion set
Shattering	Natural detachment of the seed from the fruit or inflorescence at maturity
Short day species	Species dependent on the decline of the photoperiod to trigger the process of flower initiation and flowering. Dependence may vary by variety or group of varieties
Sib pollination	1. Pollination of one plant by another plant derived from the same parents[2] 2. Selection method whereby one basic seed population (family) is outcrossed to many pollen parents (half-sib) or to only one pollen parent (full-sib)
Silique	Seed pod of *Cruciferae*
Silk	Bundle of styles extending from a maize ear
Sink	Physiological term for a process or a plant part serving as a reservoir capable of absorbing or receiving energy or matter without undergoing significant change
Stamen	Anthers and their support organ
Somatic cell	Any non-reproductive cell
Somatic embryogenesis	The production of embryo-like structures (embryoids) from somatic cells of the plant (as opposed to germ cells)[1]
Somatic hybrid	A plant originating from cell protoplast fusion
Species	A taxon ranking in the hierarchy of biological classification as the category below genus. The species is the basic unit of biological classification[2]. Plants within the species limit normally pollinate, fertilise and set seed naturally, unless selected for a form of sterility

Spikelet	Individual part of rice inflorescence
Sporophyte	The diploid, spore producing, asexual generation in the life cycle of a plant[2]
Starting propagule	See propagule
Sterile	Unable to reproduce
Stigma	Receptive part of the female flower organ
Stomata	Pores in the leaves of all plants that regulate gas exchange between the inside of the leaf and the environment
Strain	Selected type of a cultivar; subvariety; a group of similar individuals within a variety[3]
Stubble	Plant parts remaining in the soil after cutting or mowing the above-ground parts of a crop
Style	Flower organ connecting ovary and stigma
Subgenus	A section within a genus
Subspecies	A section within a species
Sucker	Vegetatively developing branch of a tree
Synthetic variety	A variety the seed of which is composed by bulk harvesting a mixture of two or more basic seed populations or families, each population being stabilised for its main characteristics, and tested for combining ability with the other basic population(s)
Tassel	Male inflorescence of maize
Taxon	A taxonomic group of any rank, including all the subordinate groups[2]
Taxonomy	Scientific classification of plants, including systematic description and naming[2]. In addition to morphological classification, gene identification has become an important taxonomic tool
Tillage	Mechanical preparation of the soil to enhance growth of cultivated plants
Tolerance	The range of an environmental factor within which an organism or population can survive[2]. The same applies to pathogens and pests. The tolerant plant will not alter or change the attacking pathogen, pests or adverse environmental conditions
Trait	Hereditary characteristic
Translocation	Movement of a segment of a chromosome to another part of the same chromosome or to a different chromosome[2]
Tri-, tetra-, hexa-, octa-, polyploid	Carrying respectively, 3, 4, 6, 8 or many sets of chromosomes
True breeding	Homozygosity of a plant or homogeneity of a plant population

Unreduced gamete	A complete chromosome (2n) taking part in fertilisation. Two unreduced diploid gametes give rise to a tetraploid plant.
Variety	A subgroup of a plant species, prepared for use in agriculture, sufficiently homogeneous and stable in its hereditary characteristics and distinct from other varieties; cultivar; cultigen
Vernalisation	Cold treatment for plants to enhance their natural need of cold in order to initiate flower formation
Vertical resistance	Resistance depending on one major gene
Winter nursery	Experimental garden in a mild climatic area allowing for cultivation and seed harvest in a counter-season in order to gain a breeding generation
Zygomorphous	Symmetric flower pattern
Zygote	Cell formed by the union of two gametes, and the individual developing from this cell[3]

Notes and References

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PRINTED IN FRANCE
(93 93 06 1) ISBN 92-64-14047-6 - No. 46859 1993

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